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A Novel Microextraction Method for Determining the Presence of Pentachlorophenol in an Ongoing In-Situ Groundwater Remediation

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A novel microextraction method for determining the presence of pentachlorophenol in an ongoing *in-situ* groundwater remediation

By

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Pentachlorophenol (penta) is a pollutant of concern worldwide. This study looks at a site that has been under remediation for the last 16 years due to penta contamination. The site has been air- and bio-sparged, phytoremediated and undergone *in-situ* chemical oxidation treatment in attempts to clean the ground water of penta. This thesis explores a novel microextraction method that has the promise of being more sensitive using less sample and fewer hazardous chemicals than conventional methods. Groundwater samples were extracted for phenolic compounds using a novel modified liquid microextraction protocol. Comparing our results with monitoring information from 2014, our method determined that penta is limited to a single monitoring well. However, our report was unable to determine exact quantitative results of penta concentration, due to loss of extraction solvent during the retrieval process.

DEDICATION

I'd like to dedicate this work to my family, (both near and far) and my friends, all of whom have shown immense patience and support as I worked hard to conclude this project.

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Brad Hodges, for his extensive help in understanding the site's current remediation, and for his allowing access to the site. Without his unwavering cooperation this work would not have been completed.

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Ross McCool, who supports me immensely and who has unending patience.

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CHAPTER I

INTRODUCTION TO PENTACHLOROPHENOL REMEDIATION

1.1 Brief History of Pentachlorophenol

Pentachlorophenol (penta) was first created in 1841[1]. The manufacture of penta on a commercial scale did not occur until 1936, when its properties as a wood preservative became understood[1]. It is an effective herbicide and biocide[1]. Due to this effective nature against many pests, it was applied in the widespread fields of agriculture and manufacturing, including the control of snails to prevent spread of disease in Japan [2-4]. In the US alone it has had registered uses as an herbicide, an insecticide, a postharvest wash for fruit, a preservative for paint, and a fumigant [5]. Penta got its foothold as a wood preservative because it extends the lifetime of wood products up to 40 years, even in adverse conditions[5].

Its long and widespread usage means that penta can be found in many environments, especially near manufacturing and usage site. However, its effective nature in killing pests also creates a hazard to humans and other mammals[6]. The acute LD-50's for small laboratory animals and domestic livestock are between 27 and 300 mg/kg of body weight[6]. Mammals experience a variety of symptoms from a sufficient dose of penta, including increased respiration, cardiology distress, high blood pressure, and elevated body temperature[5]. Plants are also affected by penta, presenting issues to reproduction and growth[6]. The chemical formula of penta is C_6Cl_5OH.The industrial

form of penta has been known to have dangerous impurities such as dibenzofurans. These impurities are part of the danger found with penta[5, 6].

The structure of penta is similar to chemicals known as Persistent Organic Pollutants (POPs). The original POPs were known as the "dirty dozen" because of the way they were persistent in the environment and damaging to the health of different organisms. The nature of penta has resulted in it being restricted by the Stockholm Convention as well. According to the Stockholm Convention, penta and its salts and esters are classified under Annex A, to be eliminated in the production and use as much as possible[7]. This category is also occupied by hexachlorobenzene, which shares a similar structure to penta (see Figure 1.1).

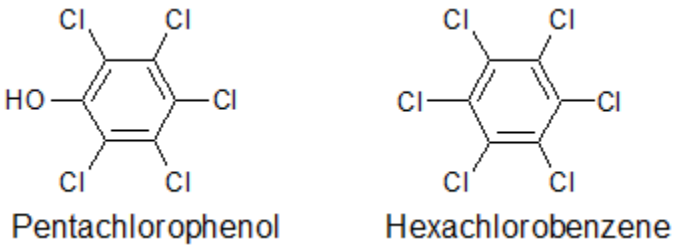

Figure 1.1 Chemical structures of penta and hexachlorobenzene.

Research studies into the effects of penta on humans led to further restrictions on the chemical. Links between cancer and penta have been well established and cannot be blamed solely on the impurities of the chemical [5, 6, 8]. During 1978, the Environmental Protection Agency (EPA) became more aggressive in restricting the permitted uses of penta. By 1981 there were only 13 major uses for the chemical [5]. In 1997, the EPA took action, declaring that the chemical should be regulated even

further[9, 10]. The EPA has even placed limits on the allowable amounts of penta that can be consumed through water in the United States and in 2015 the accepted amount was lowered from 0.27 ug/L to 0.03 ug/L [11].

While penta is still being used for wood treatment in the United States, it can only be used for the treatment of wood utility poles and cross arms [10]. Its continued use, despite the adverse environmental and ecological effects that it causes to vertebrates and invertebrates alike, is a testament to it utility and cost effectiveness. However, due to the harm that this chemical can cause, the handling of wood waste and waste waters are heavily monitored and scrutinized. Another question is, after so many decades of unrestricted usage, how do we go about remediation of the most contaminated sites?

1.2 Remediation Technologies

Many variables come into effect when deciding on a remediation technology to apply to a contaminated site. Often times it is the suitability of the site that determines whether or not a technology can be utilized. General proximity to drinking water sources, soil microbial community, the nature and level of the contaminant, and other details should be considered variables when selecting treatment for a contaminated site.

The most important decision is whether to treat onsite or to remove the contamination completely and treat the contaminated media elsewhere. Both options have their advantages and disadvantages, but it is often this decision that dictates what technology will be used and how. While both onsite and offsite treatment can use the same scientific principals they do so in different methods. The site described in this study uses exclusively onsite treatments; however, it is important to note differences and similarities in the two general categories of remediation.

1.2.1 Offsite versus Onsite treatment.

Remediation technology is varied in the ways that it can effectively clean up an area. Which technology is chosen for the job depends on a large number of factors. These factors can include but are not limited to contaminant type and concentration, type of contaminated media, pH, moisture content, nutrient content in the media, and average ambient temperature. The selection of remediation technology also takes into account non-physical factors, such as public safety, public opinion, and financial capabilities. Generally, remediation technology is categorized under two options: the choice to treat the media on site (in-situ remediation) or to transport and treat the media at another location (ex-situ remediation). While many remediation techniques overlap these categories, each has its advantages and disadvantages. Soil and groundwater contaminations of pentachlorophenol have been successfully remediated by both on site and offsite methods [9, 12]

Offsite treatment, or ex-situ treatment, has several general advantages. One of the main advantages is that the contaminated media is immediately removed and taken to another site for treatment. This is especially helpful if the site of contamination is of immediate threat to a surrounding location. As a general rule, the excavation of media allows the remediation to be done relatively quickly because the conditions that the media is put under can be more easily controlled. It allows for more ideal conditions to be enacted that will make treatment more effective. However, it may be expensive to move and treat contaminated media and may have negative public image. There is also the fact that ex-situ treatment for several different contaminants is not that effective. While it is

often very useful to treat non-halogenated volatile organic chemicals and fuel spills, it is not very effective against many inorganics or halogenated chemicals [13].

Onsite treatment, or in-situ treatment, treats the contamination without removing the media. It requires monitoring and usually requires the installation of specialized equipment. However, many different techniques utilize similar equipment, so it is easy to transition between multiple remediation techniques. While ex-situ remediation can be used to treat penta contamination, in-situ remediation is far more common for this type of contaminant.

1.2.2 Injection Based Technologies

Injection based technologies focus on remediating soil and ground water with low disturbances to the surrounding area. Once injection and monitoring wells are created, the site can be treated with the injection of air, nutrients, and chemicals into the ground. Due to the nature of this technology and the guiding principles, these treatments have been used alongside many other in-situ methods [9, 14]. Injection based technology can use biological or chemical processes.

1.2.3 Aerobic Versus Anaerobic Decomposition

Nature has a way of dealing with stray contaminants through the use of microbes that live in different media. The process is slow and often requires multiple steps by the degrading organism, but microbes can eventually break some contaminants down to benign mineralization. There are two ways that nature has evolved to decompose matter on a cellular level: aerobic and anaerobic decomposition.

Anaerobic metabolic pathways utilized by bacteria are performed in an environment with little to no oxygen. These pathways often break down pollutants but are generally not as rapid as aerobic pathways. Aerobic metabolic pathways employ oxygen in chemical decomposition processes. These processes are faster at decomposing chemicals and are generally the preferred biological methods of dealing with pollutants.

Depending on the natural properties of the site, microbes will tend to process nutrients with or without the use of oxygen. A site that has good circulation of air into the soil will most likely go thought an aerobic process. If a site's soil is mainly clay or a similarly impenetrable soil, the process most likely occurring is anaerobic. Penta can be broken down in either anaerobic or aerobic conditions [15, 16].

There are four different pathways specific to the degradation of penta. It can be methylated (gives pentachloroanisole), acylated (pentachlorophenol acetate), dechlorinated (tetrachlorophenols), or go through hydroxylation[17]. After this first step the bacteria can take any number of ways to degrade the resulting byproducts. The following figures describe a few ways that this metabolic breakdown occurs (Figure 1.2 & Figure 1.3)

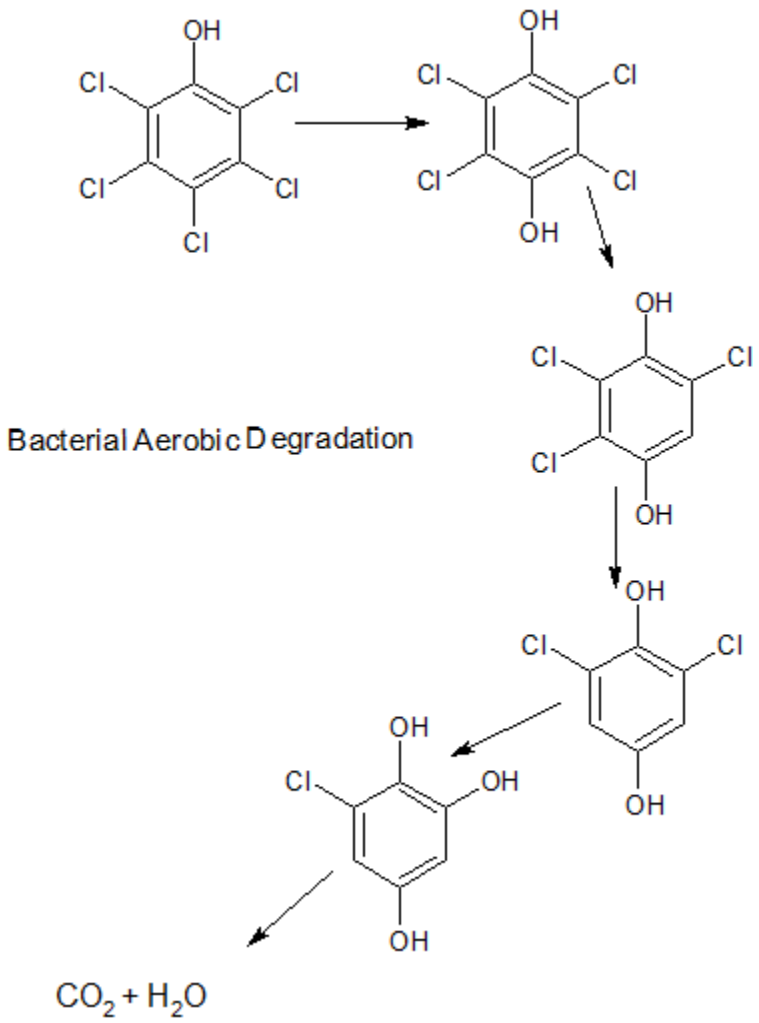

Figure 1.2 An example of biodegradation for bacteria reconstructed through systematic dechlorination from Yu and Shepherd [18].

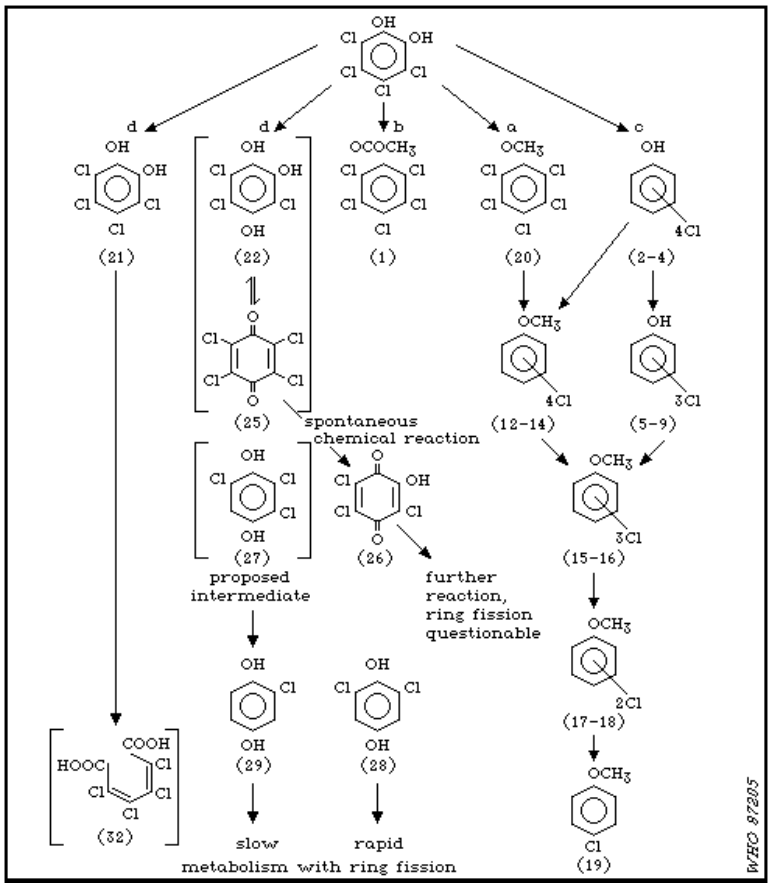

Figure 1.3 Five different metabolic pathways for penta presented with mechanisms from the World Health Organization[17].

1.2.4 Biosparging & Air Sparging

Injection technology can be used to increase the resources available to microbes in the soil. This technology uses a series of injection wells, drilled throughout the site, to pump air and/or nutrients into the soil. When injection consists of only air or oxygen, it is known an air sparging. This injection of air increases the amount of oxygen the bacteria have access to and spurs the organism to go through the aerobic decomposition process.

If nutrients are being injected into the ground in addition to air, it is known as biosparging or biostimulation. This technology is specifically designed to aid native or

introduced bacteria in decomposition of chemical contaminants. Injected along with the air, a solution of nutrients is added to support bacterial growth and proliferation. With the added nutrients and increased air flow, bacteria are better able to break down the contaminants. This increases the amount of pollutants decomposed by the bacteria and decreases the time needed for degradation over the natural attenuation alone. However, the treatment may still take years and can plateau over time if a reagent vital to degradation finds itself in short supply.

This injection based technology is mostly used to help native aerobic microbes get a boost in decomposition of contaminants. However, laboratory-created microbes that have been produced to be highly effective at degrading the contaminant have been added to soil as an added remediation effect in some cases[19].

1.2.5 Phytoremediation

Phytoremediation is the use of plants to remediate hazardous chemicals from soil or groundwater, including phenolic compounds [20-22]. Utilizing enzymes and biological responses found naturally in some plants, harmful chemicals are trapped or changed by the plants during their own natural processes. Plants are higher on the evolutionary tree and are more complex organisms than bacteria. Therefore, they can control their environment more than bacteria can, which allows them to better adapt to changes in the soil. The way plants take in nutrients and water helps with degradation of harmful pollutants. These processes allow the pollutants to become integrated into the plants' system, and the plants can ultimately be removed from the site and disposed of. Phytoremediation is relatively inexpensive, effective, and has the added benefit of adding to the landscape of the site [20]. However, phytoremediation takes time for chemical

uptake and allows for less control over how the remediation takes place [20]. These disadvantages often lead it to be a supplementary remediation technique.

Poplar/cottonwood hybrid plants were planted on the study site for this project as a passive means of cleaning penta out of the soil because the trees will degrade the contaminant out enzymatically (see Figure 1.4). Poplar hybrid trees are often used in remediation because they are deeply rooted, take in a large amount of water and are tolerant to high amounts of organic chemicals[21, 22].

Figure 1.4 Phytoremediation with poplar/cottonwood hybrids taking place on the site described in this study.

There were approximately 100 hybrid trees on site, planted between 2011 and 2016.

1.2.6 In-situ Chemical Oxidation

In-situ chemical oxidation (ISCO) utilizes strong chemical oxidants being injected near the plume of the pollutant. As this technology specifically caters to the degradation and treatment of organic contaminants, the oxidants usually chosen are permanganate, ozone, Fenton's reagents, or hydrogen peroxide [23, 24]. The goal of this remediation technology is to oxidize the pollutant to its less harmful base components. This technology utilizes the same injection wells as the air sparging and biosparging. While permanganate and ozone are utilized and will be discussed briefly, this review will focus on the Fenton's reagents because this was the ISCO treatment used at the site.

1.2.6.1 Permanganate & Ozone

ISCO's options for oxidants that can be injected are varied, and selecting one to use depends on many factors. One of the most important considerations is the type of contaminant. Because this technology relies so heavily on the chemical reaction with the contaminant and the oxidant being presented, it is vital to understand the reaction that will take place. Permanganate and ozone are utilized for a few reactions that will be discussed here.

Permanganate is utilized mainly for water remediation when the contaminant is an organic chemical with double bonded carbons, hydroxyl or aldehyde groups and chlorinated alkenes [23, 24]. When decomposing the chemicals, the final products are often free chloride ions, carbon dioxide, and manganese dioxide.

Ozone is another chemical oxidant that can be used in ISCO. Its reactions yield hydroxide molecules, oxygen, and water. However, due to ozone's reactive nature, it often decomposes before it has traveled very far into the media. This requires that the

ozone be directly injected into the plume of pollutant. This is often difficult as many factors determine the location of the plume, and plumes often shift position in response to environmental change. Ozone's reactions with contaminates are short lived because there is not catalyst as there is with Fenton's Reagent and hydrogen peroxide.

1.2.6.2 Fenton's Reagent and Hydrogen Peroxide

Other strong oxidants used commonly in this remediation technology are hydrogen peroxide and a Fenton's reagent. Fenton's reagents are a combination of hydrogen peroxide and an iron catalyst. The hydrogen peroxide reacts with ferrous iron to create a hydroxyl radical using the Fenton reaction. Hydroxyl radicals freely react with almost all organic molecules without preference [24]. The goal of using hydrogen peroxide or a Fenton's reagent is to have a highly reactive species that will be able to react with the contaminants and mineralize them. There are multiple hypotheses for the mechanism that drives the Fenton reaction; however the mechanism proposed by Haber and Willstatter in 1931 is the prevailing theory that is most widely accepted [23, 25-27]. Figure 1.5, shown below, is the general Fenton reaction. The iron is a catalyst in the reaction and will regenerate from iron (III) back into iron (II). Once the iron (II) is regenerated, it is free to react with any remaining hydrogen peroxide.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$
$$OH^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O$$
$$Fe^{3+} + HO_2^\bullet \rightarrow Fe^{2+} + H^+ + O_2$$
$$Fe^{2+} + HO_2^\bullet \rightarrow Fe^{3+} + HO_2^-$$
$$Fe^{2+} + OH^\bullet \rightarrow Fe^{3+} + OH^-$$

Figure 1.5 Full Fenton reaction chain from Barbusinski et al[26].

The iron is regenerated and is available for the next interaction with hydrogen peroxide.

Once the hydroxyl radical (OH·) is created, it can then react with another organic molecule for a variety of oxidizing reactions. Iron (III) can also react with hydrogen peroxide to create a superoxide radical (HO_2·), which can then react with other compounds, and regenerate iron(II) [27]. In a reaction with penta we find that the Fenton reaction dechlorinates and breaks the ring structure so that the final products are CO_2, water, free chlorines, and iron ions [28].

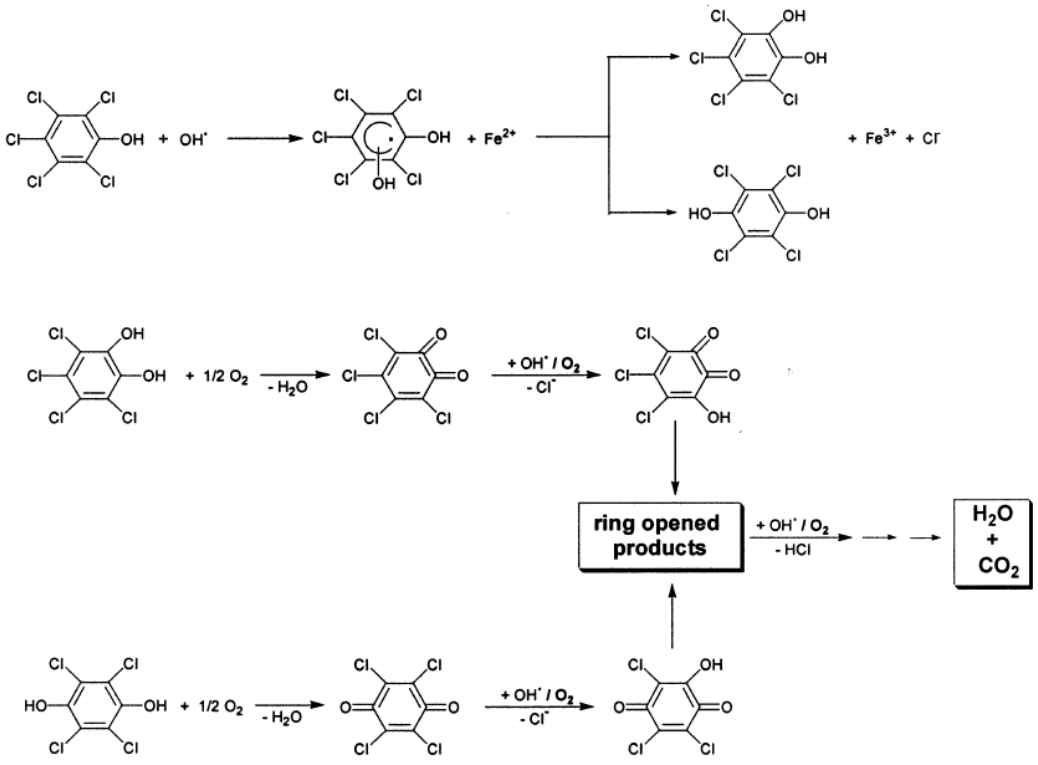

Figure 1.6 A Fenton reaction diagram from Oturan et al.[28], showing the mineralization of penta by radicals produced in Fenton mechanisms.

1.3 Objectives and Importance of Study

The site used in this study has been under remediation for the last 16 years. Thus far it has undergone air sparging, enhanced biosparging, poplar/cottonwood phytoremediation, and ISCO treatment with hydrogen peroxide into iron rich soil. It is important to understand the different types of remediation technologies that have taken place on the site to truly understand the possible byproducts. The degradation pathway from a biological remediation will not follow the same pathway as ISCO. Therefore it is important to have a working understanding of how these products are created. This study's objectives were to determine the general location of any remaining penta

contamination and the location of any breakdown products following subsequent remediation treatments by employing a novel micro-extraction protocol that is sensitive to small amounts of chlorinated phenolic compounds to detect trace levels of products.

CHAPTER II

MATERIALS AND METHODS

2.1 Field Procedures

2.1.1 Site Description

The contaminated site sampled for this study was located in central Mississippi, adjacent to a wood product treatment facility that has used penta for treatment of utility poles in the past. The site was a disposal and storage area for penta waste for a few decades before the 1970's, before the current company took over management of the mill [14, 29]. Before the hazards of penta were completely understood, treated utility poles were allowed to drip dry on concrete log runs with the effluent running into the nearby soil (see Figure 2.1 and **Error! Reference source not found.**). The site also stored used pressure treatment fluid wastewater in a lagoon, which was later filled in with uncontaminated soil [14]. At the time of this study, the mill was not using penta but was producing dimensional lumber [30].

The site has been undergoing remediation for a significant groundwater contamination since 2000 [14]. To clean up the site, 5 air sparging wells were installed on the site to create a "curtain" of air treatment before the plume traveled to a nearby property (Figure 2.1)[29]. The wells were between 40 and 60 ft. (12.2 to 18.3 m) from each other, utilizing 2 in (5.08 cm) diameter, schedule 40 PVC pipes. There is a 5 ft. (1.5 m) mesh screen at the bottom of the well. The wells were between 23 and 29 ft. (7.0-8.8

m) below the surface. Between the wells installation and 2011, the site was air sparged. This original system was used until 2011, when they upgraded the air sparging blower system but left all the original wells in place [9, 14, 29]. This upgraded system was used to do enhanced biosparging with injections of nutrients, such as nitrogen, bio-available phosphate, potash and other micronutrients, during December of 2011[9].

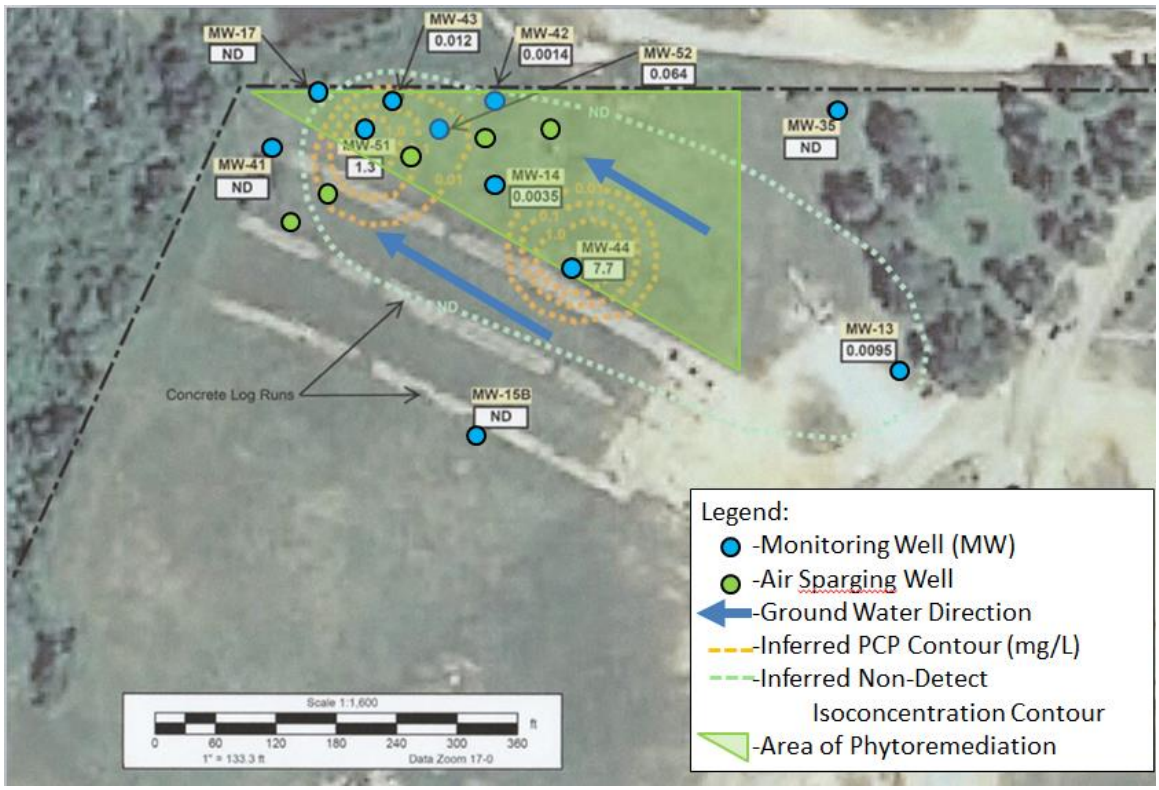

Figure 2.1 Site map, reproduced for this document with permission. [14, 19]

From 2011 to 2012, approximately 100 hybrid poplar and cottonwood trees were planted in the area to add phytoremediation. Some trees were lost due to native wildlife and were replaced in March of 2016. A fence near the border of the property has been added to discourage loss. From 2015 to 2016, ISCO was started by pumping hydrogen

peroxide down into the approximate location of the plume using the sparging set up. Figure 2.2 illustrates the location of the wells and where the penta plume was inferred to be in 2014 [11].

Wells MW35 and MW13 were up the hill from the other wells. They were near the reported old lagoon site. The lagoon portion of the site was cleaned as a separate project and, at the time of this study, there were mature pine trees growing in the area. The ground water in the area flows down the hill, through the site, and into a nearby stream.

The site has also undergone both phytoremediation with the cottonwood/poplar hybrids mentioned in the site description, as well as in-situ chemical oxidation with hydrogen peroxide. The ISCO treatment carried out at the site utilized 55 gallon (approximately 208.2 L) barrel drums of 35% hydrogen peroxide being pumped into the air sparging system at the rate of one barrel every few weeks, weather permitting. This continued from November 2015 to April 2016. The hydrogen peroxide was injected through the air sparging system at monitoring well 43 in a 10:1 ratio until a 55 gallon barrel had been emptied (approximately 3 days).

2.1.2 Groundwater Sampling Protocol

From January through April of 2016, approximately every 2 weeks, 500 mL of groundwater was extracted from existing monitoring wells located throughout the affected area. Groundwater was sampled from wells by use of a hand-operated vacuum pump (Blackstone Laboratories), and ¼ inch polyethylene tubing. Tubing the length of each well remained in place throughout the sampling period (the depth of each monitoring well is between 4.72 m to 9.75 m deep) [9]. Amber glass wide-mouth bottles

(Fisher Scientific) were fitted to the pump assembly via an adapter hose containing a support spring, through which the ¼ in tubing from the well was passed, into the bottle mouth. Hose clamps were used to seal connection points. Vacuum pressure was applied with the hand pump, which raised groundwater through the tubing from inside the well, capturing enough water to fill the 500-mL amber jar. Once the jar was filled, the vacuum was released and the jar was taken off the pump assembly. The jar was then sealed, labeled, and placed into a cooler filled with ice to be transported back to the lab. The tubing and adaptor hose were rinsed with an equal amount of deionized water taken from the lab, before sampling continued. The pH and temperature of the samples were recorded before being stored in the refrigerator until extractions could be done. Below are photos of the sampling and transport process (Figure 2.2 & Figure 2.3).

Figure 2.2 Ground water sampling instrumentation.

Tubing inside the well (left) was connected to the hand pump with attached jar (right).

Figure 2.3 Samples were chilled in a cooler until they could be transported to the lab.

2.2 Laboratory Procedures

2.2.1 Sample Handling

Samples were transported to laboratory on the day of collection, on ice, and stored at 2° C until extraction. Temperature and pH were recorded after collection and before extraction. Samples were allowed to settle any debris by settling overnight in a refrigerator.

2.2.2 Sample Extraction

The novel microextraction procedures used in this thesis were based on those set forth in Faraji et al.[30]. This microextraction method was selected for its ability to concentrate phenolic compounds during extraction from water samples, resulting in reduced extraction time and increased sensitivity from traditional liquid-liquid extraction

methods. Before each extraction, temperature and pH measurements were taken again. Out of each 500 mL of water samples taken, 50 mL total was utilized. Five replicates, each containing 10mL in a screwtop cylindrical vial, were completed at the same time for each well. Then 2.3 µL of 2000 µg/mL (in methanol) 2,4,6-tribromophenol (TBP) (Supelco) were added to each replicate as an internal standard. Half a milliliter of 5% Potasium Carbonate (K2CO3) solution (Sigma-Aldrich, BioXtra ≥99.0%) and 40 µL of acetic anhydride were added along with a small magnetic stir bar, approximately 2mm in size, to derivatize the replicates. The five replicates were then placed on a stir plate together. Samples were allowed to stir at maximum speed for two minutes. After two minutes, each sample was transferred to a hot water bath (approximately 55° C), heated by a stirring hot plate. Once a vortex was created in the vial, 10 µL of 1-undecanol ($C_{11}H_{24}O$) was added to the surface at the bottom of the vortex as the extraction solvent. The vial was then recapped and stirred for 15 mins at a speed that could maintain all 5 vortexes. After this time, vials were transferred to an ice bath until the 1-undecanol solidified (approximately 20 mins). The 1-undecanol was retrieved using a sterile metal spatula and placed into a 2 mL amber glass chromatography vial containing a 0.25 mL clear glass insert. To each extracted sample, 50 µL of methanol was added as a disperser solvent to the 1-undecanol for gas chromatography. The vials were sealed and refrigerated until they could be analyzed for phenolic compounds that had been extracted by the 1-undecanol.

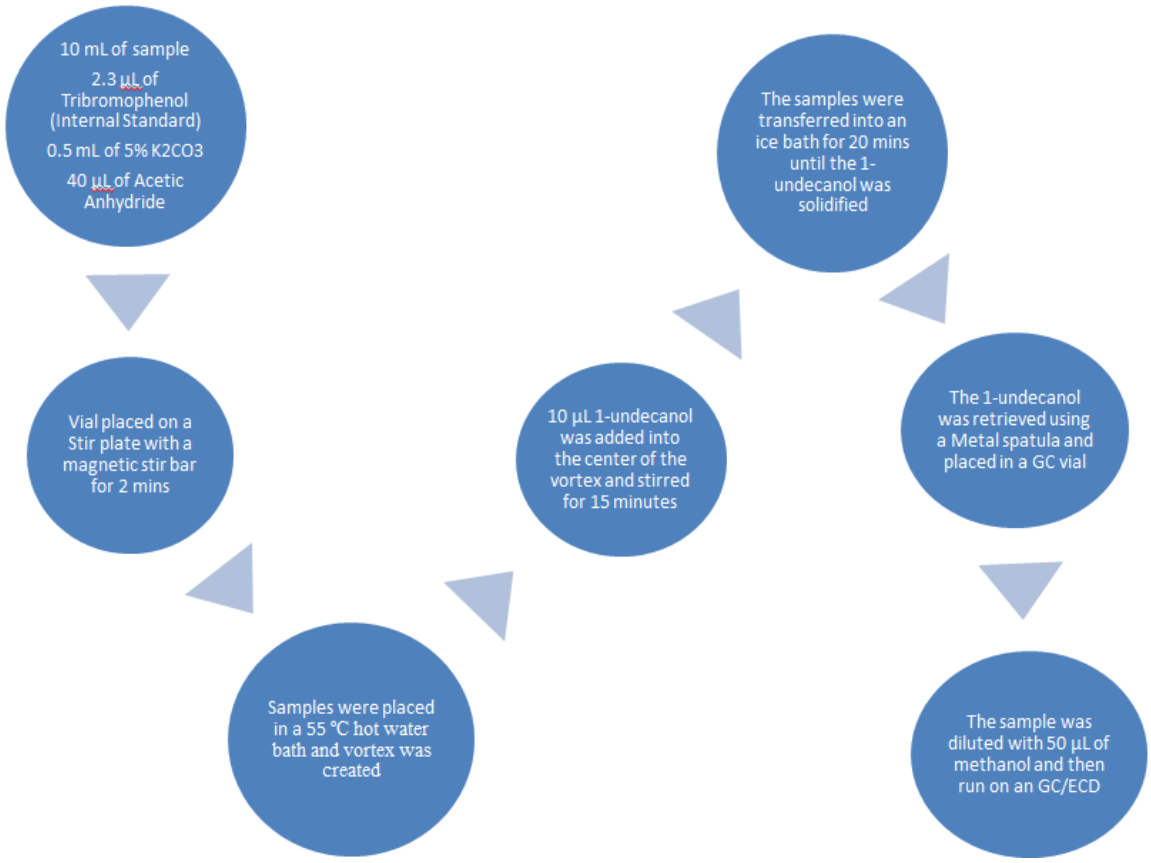

Figure 2.4 Stepwise extraction flow chart.

In addition to water samples, microextractions using the proposed method were performed with penta, 2,4,6-TBP, 1-undecanol, methanol, and EPA phenolic analytical standards. The EPA Standards mix contained 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, penta, phenol, and 2,4,6-trichlorophenol (Supelco). These standards were used for identification of peaks and to test the reliability of the microextraction method.

2.2.3 Gas Chromatography Protocol

Gas chromatographic analysis of the extracted samples was based on the method described in Fattahi et al.[31]. For sample analysis, an Agilent 6890 Plus Gas Chromatograph with a G2397A Electron Capture Detector (GC ECD) was used to obtain the necessary sensitivity for phenolic metabolites. An Ultra 2 capillary column from Agilent Technologies (length 25 meters, internal diameter 0.2 mm, film 0.33 μm) was used. The front inlet was kept at 280 °C, and the detector was held at 300 °C. The temperature programming on the column was set to start at 100 °C and increase every two minutes (at a rate of 5 °C/min) to 210°C. Helium was the carrier gas (50 cm sec^{-1}) and nitrogen (60 mL min^{-1}) was used as the makeup gas.

2.3 Statistical Analysis

Identified penta peaks were analyzed with Chemstation Reports, utilizing peak retention time as the identifying factor of the chemicals. The reports were organized in Excel according to monitoring well number, sampling date, replicates, and peak retention time. Where peak areas were not reported, it was considered to be a zero value rather than missing data. The reported limit of detection for the ECD method was 0.010 μg L^{-1}[31]. Statistical analysis was completed by the IBM SPSS program. Samples were analyzed with multiple statistical tests before Friedman's ANOVA test was chosen, as the data best fit its assumptions.

The data was not normally distributed which was a difficulty when matching up to other tests. Z tests for outliers in detected penta peaks showed that all the data was valid, and therefore all of the data was used for analysis. Appendix A details the results of tests for normality. It was determined that Friedman's ANOVA was acceptable as the

assumptions were met. Friedman's ANOVA utilizes a ranking system for the data. After this data is ranked from 1 (lowest value) to n (highest value), the test statistic is calculated as Equation 1[32].

$$F_r = \left[\frac{12}{Nk(k+1)}\sum_{i=1}^{k} R_i^2\right] - 3N(k+1) \qquad \text{(Eq. 2.1)}$$

R_i = each groups rank sums

N = total sample size

k = number of conditions

Repeated measures analysis was also attempted on the identified penta peak data, however when examined for sphericity, the data failed to meet this assumption, and therefore repeated measures analysis was eliminated as a statistical description. Repeated measures analyses may be especially susceptible to failures of the sphericity assumption, because the Type I error rate is increased to an unacceptable level.

CHAPTER III

RESULTS

3.1 Results

3.1.1 Peak Identification

Peaks of interest were the peaks of penta, 2,4,6-TBP and any other chlorinated peaks that may have been detected. During GC-ECD analysis, it was found that penta eluted at approximately 22.0 mins, 2,4,6-TBP eluted at approximately 21.8 mins, and 1-undecanol eluted at 15.4 mins on the GC-ECD. These times were used to identify the peaks that were found in the extracted ground water samples. In extracted samples trace amounts of other chlorinated compounds were not detected utilizing the ECD across replicates or samples. Because this site has been under remediation treatment for so long, it is postulated that less chlorinated compounds may have been utilized by microorganisms as energy sources. Because of noise generated in the GC-ECD spectrum, it is possible that some trace peaks were not identified. Considering that only one well had detectable amounts of penta, it is also possible that any detectable amounts of chlorinated compounds generated during the breakdown of penta are at such low levels, they cannot be reliably detected with the method described here. However, extracted phenolic EPA standards generated consistent ECD spectra each time. This leads us to believe that the metabolites or breakdown products of penta are in trace and undetectable

amounts in the ground water samples. Of all the wells sampled, the only well with any penta peak detected was MW44.

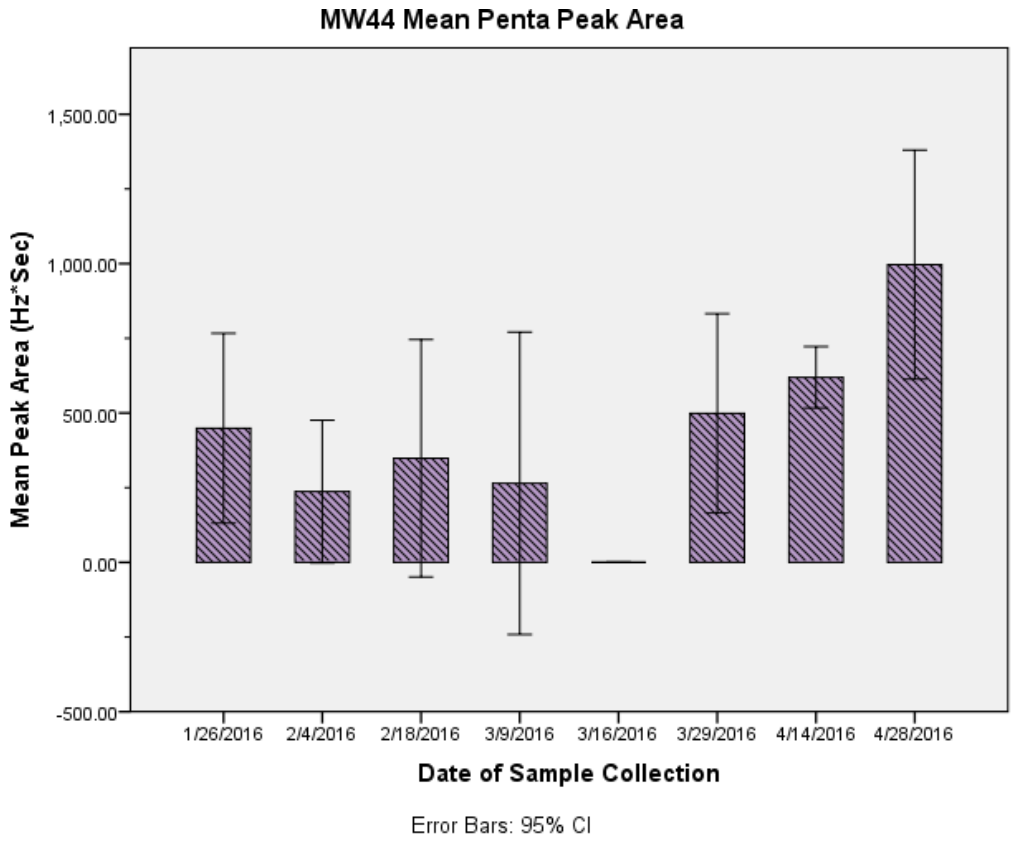

Figure 3.1 MW44 penta peaks separated by sample collection date.

3.2 MW44

The only well containing detectable amounts of penta was MW44. Samples collected during March 16th, 2016 did not report any penta contamination. This was included in the analysis as there were no outliers in the data. Friedman's two-way analysis of variance by ranks found that the mean peak area of penta did significantly change over the sampling dates, $\chi^2(7)=27.360$, $p=.000$.

The sampling dates were compared pairwise with one another. It was found that samples collected from March 16th, 2016 and April 28th,2016 (p=0.001) were significantly different as were February 4th, 2016 and April 28th, 2016 (p=0.017).

The main sources of contamination at this site were the wastewater holding pond (lagoon) and the concrete drip pads. Much of the subsequent remediation efforts have been dedicated to the mobility concerns of the penta located near the old holding pond, as this area was of initially significantly higher concentration. However, MW44 is considered an "up gradient monitoring well" and therefore is upstream of the "curtain" of the injection wells in the ground water flow of the site[19]. MW44 is understandably the only well with detectable penta chemicals still in the soil because it is the only up gradient monitoring well that is close enough to the concrete drip pads and was also in line for drifting penta from other sources. Metabolites of penta were also scarce and in low enough concentrations that our method did not detect them. This is most likely due to the last 16 years of remediation that was conducted at the site. According to the quarterly reports from 2014, 7 out of the 11 wells tested were at a detectable limit when attempting to locate penta alone with EPA standard extraction methods [13]. This indicates to us that our method is sensitive and that the discrepancies from the 2014 monitoring report to our 2016 study are generally due to the sites successful remediation.

3.2.1 Discussion of Application of Microextraction Protocol

This method needs refinement before it can be used for quantification with environmental groundwater samples. However, for qualification, this method seems to be effective for heavily chlorinated phenols. To improve the method, a more sophisticated approach to retrieving the 1-undecanol from the sample is required or way to

offset/calculate the loss of 1-undecanol, and the use of GCMS in addition to GC-ECD would be strongly recommended.

First, the largest obstacle for quantification of data was the retrieval of the 1-undecanol after it had solidified. The 1-undecanol contains the chemicals of interest. However, due to the chemical properties of 1-undecanol (i.e. its freezing point of 2-4°C) the removal of it from the rest of the sample is an intricate process. If the 1-undecanol broke from a single 10µL solid droplet, it became nearly impossible to regain the smallest bits. This may prove a problem for quantification of chlorinated phenolic compounds and could explain, in part, the large variance that was experienced in the peak height. With no way to know exactly how much 1-undecanol was lost in each replicate it is unlikely that one can quantify using this exact protocol without an egregious amount of error. If there were a better method of retrieving the 1-undecanol, it could be highly useful for the quantification of data.

Finally, an ECD was selected because of its sensitivity to chlorinated and phenolic compounds. However, it would have been better had the samples been analyzed on a Gas Chromatograph with Mass Spectrometer (GCMS) concurrently with the ECD analysis. This comparison could have found many other factors that might have affected retention times, and given us a better idea of what else was inside of our environmental samples. Using GCMS a running in tandem with the GC ECD, would have been a more effective method of detecting exactly what can be found in each well.

In addition to adding GCMS, a tighter resolution for the small chlorinated compounds on the ECD would have been useful. As was noted previously, the retention time between the internal standard of TBP and penta in the sample were very close

together, improved resolution would have allowed separation of the compounds of interest. TBP was chosen as the internal standard, as it is a common choice of internal standard from the literature, and is not known to have an issue with elution timing when used with penta [9, 31, 33]. Fattahi et al, utilized acetone as their disperser solvent to where as we chose methanol. This could have made it so that the GC ECD temperature programing was not better attuned to our process.

3.3 Conclusions

While the method needs refinement to be able to be used quantitatively, it can be used to qualify the data and to determine which monitoring wells were still detectably contaminated. According to our findings, due to years of sequential remediation utilizing bio- and air sparging, phytoremediation, and ISCO treatment, the study site is nearing EPA acceptable standards for groundwater across the entire site. The levels of chlorinated phenolic compounds produced from penta degradation appear to be below detection levels for the method described here.

It can be understood from these results that the penta plume is localized in a detectable amount around MW44, perhaps under the concrete drying pads. However, under current method limitations, exact quantification cannot be determined. With revision, the method could still be useable for quantification for future ventures. Future work will include a direct comparison of the standard EPA 3510C method in analyzing trace compounds from this site versus the method described here, optimized for detection of small chlorinated phenols. It is believed that further optimization of this method will provide a useful analysis alternative to the EPA standard when only small quantities of groundwater are available to analyze.

REFERENCES

1. Carswell, T. and H. Nason, *Properties and uses of pentachlorophenol.* Industrial & Engineering Chemistry, 1938. **30**(6): p. 622-626.

2. Hunter, G.W., et al., *Studies on Schistosomiasis: VI. Control of the Snail Host of Schistosomiasis in Japan with Sodium Pentachlorophenate (Santobrite).* The American Journal of Tropical Medicine and Hygiene, 1952. **1**(5): p. 831-847.

3. Young, H. and J. Carroll, *The decomposition of pentachlorophenol when applied as a residual pre-emergence herbicide.* Agronomy Journal, 1951. **43**(10): p. 504-507.

4. Halawani, A., N. Latif, and A. Taha, *On pentabromophenol and pentachloro phenol as molluscacides in the prevention of bilharziasis.* Journal of the Royal Egyptian Medical Association, 1951. **34**(3): p. 163-170.

5. Crosby, D., *Environmental chemistry of pentachlorophenol.* Pure and Applied Chemistry, 1981. **53**(5): p. 1051-1080.

6. Eisler, R., *Pentachlorophenol Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review*. 1989, DTIC Document.

7. Convention, S. *Listing of POPs in the Stockholm Convention*. 2016 [cited 2016 May 4th]; Available from: http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx.

8. Cooper, G.S. and S. Jones, *Pentachlorophenol and cancer risk: focusing the lens on specific chlorophenols and contaminants.* Environmental health perspectives, 2008. **116**(8): p. 1001.

9. Stokes, C.E., *Effects of In-situ Biosparging on Pentachlorophenol (PCP) Degradation and Bacterial Communities in PCP Contaminated Groundwater*, in *Department of Forest Products*. 2011, Mississippi State University p. 98.

10. EPA, U., *Reregistration Eligibility Descision for Pentachlorophenol*. 2008. p. 94.

11. EPA, U., *Update of Human Health Ambient Water Quality Criteria: PEntachlorophenol 87-86-5*, O.o.W. Office of Science and Technology, Editor. 2015: Washington, DC.

12. EPA, *Handbook on in situ treatment of hazardous waste- contaminated soils*, EPA, Editor. 1990.

13. EPA, *In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites*, in *Engineering Issues*, O.o.R.a.D.N.R.M.R. Laboratory, Editor. 2006: Cincinnati, OH. p. 21.

14. Lybrand, M.S., *2014 Media Monitoring Report*. 2014, Lybrand Consulting, LLC. p. 19.

15. Kuwatsuka, S. and M. Igarashi, *Degradation of PCP in soils: II. The relationship between the degradation of PCP and the properties of soils, and the identification of the degradation products of PCP*.* Soil science and plant nutrition, 1975. **21**(4): p. 405-414.

16. Hurst, C.J., et al., *Soil gas oxygen tension and pentachlorophenol biodegradation.* Journal of environmental engineering, 1997. **123**(4): p. 364-370.

17. (IPCS), W.H.O.W.T.I.P.o.C.S., *Environmental Health Criteria for Pentachlorophenol*. 1987, World Health Orgnization.

18. Yu, P. and J. Shepherd, *Pentachlorophenol in New Zealand-Biological treatment option.* AUSTRALASIAN BIOTECHNOLOGY, 1997. **7**(6): p. 340-344.

19. Borazjani, H., S.V. Diehl, R. Britto, M. Lybrand. *In-Situ biosparging of pentachlorophenol (PCP) contaminated groundwater*. in *Environmental Science and Technology 2005*. 2005.

20. McCutcheon, S.C., et al., *Phytoremediation of hazardous wastes*. 1995, DTIC Document.

21. Burken, J.G. and J.L. Schnoor, *Predictive Relationships for Uptake of Organic Contaminants by Hybrid Poplar Trees.* Environmental Science & Technology, 1998. **32**(21): p. 3379-3385.

22. Strand, S.E., L. Newman, and M. Ruszaj, *Removal of trichloroethylene from aquifers using trees*. 1995, American Society of Civil Engineers, New York, NY (United States).

23. Watts, R.J. and A.L. Teel, *Treatment of contaminated soils and groundwater using ISCO.* Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management, 2006. **10**(1): p. 2-9.

24. Seol, Y., H. Zhang, and F.W. Schwartz, *A Review of In Situ Chemical Oxidation and Heterogeneity.* Environmental & Engineering Geoscience, 2003. **9**(1): p. 37-49.

25. Chemizmu, K. and R. Fentona, *Fenton reaction-controversy concerning the chemistry.* Ecological chemistry and engineering, 2009. **16**: p. 347-358.

26. Barbusiński, K., K. Chemizmu, and R. Fentona, *Fenton reaction - controversy concerning the chemistry.* Ecological Chemistry and Engineering. S, 2009. **Vol. 16, nr 3**: p. 347-358.

27. Watts, R.J., et al., *Treatment of Pentachlorophenol-Contaminated Soils Using Fenton's Reagent.* Hazardous Waste and Hazardous Materials, 1990. **7**(4): p. 335-345.

28. Oturan, M.A., et al., *Production of hydroxyl radicals by electrochemically assisted Fenton's reagent: Application to the mineralization of an organic micropollutant, pentachlorophenol.* Journal of Electroanalytical Chemistry, 2001. **507**(1–2): p. 96-102.

29. Borazjani, H., D. Wiltcher, and S. Diehl, *Bioremediation of polychlorinated biphenyl and Petroleum contaminated soil.* Proceedings of Environmental Science and Technology, 2005. **2**: p. 502-507.

30. Authority, M.D., *Weyerhaeuser to invest in Neshoba County Lumber Facility* 2014, Mississippi Development Authority: Mississippi.org.

31. Fattahi, N., et al., *Determination of chlorophenols in water samples using simultaneous dispersive liquid–liquid microextraction and derivatization followed by gas chromatography-electron-capture detection.* Journal of Chromatography A, 2007. **1157**(1): p. 23-29.

32. Field, A., *Discovering Statistics using IBM SPSS Statistics*. 4th ed. 2013: SAGE Publications Ltd. 916.

33. Faraji, H., M.S. Tehrani, and S.W. Husain, *Pre-concentration of phenolic compounds in water samples by novel liquid–liquid microextraction and determination by gas chromatography–mass spectrometry.* Journal of Chromatography A, 2009. **1216**(49): p. 8569-8574.

APPENDIX A

STATISICAL OUTPUT

A.1 Tests of Normality for each sampled month.

Table A.1 Tests of Normality for each sampled month.

Tests of Normality[c]

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Jan26_2016	.181	5	.200*	.983	5	.950
Feb4_2016	.265	5	.200*	.890	5	.359
Feb18_2016	.322	5	.098	.764	5	.040
March9_2016	.342	5	.056	.761	5	.038
March29_2016	.209	5	.200*	.969	5	.867
April14_2016	.158	5	.200*	.993	5	.990
April28_2016	.212	5	.200*	.896	5	.386

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

c. March16_2016 is constant. It has been omitted.

March 16th, 2016's sampling gave no penta results and was omitted by the program because it gave the constant response of zero.

A.2 Full Results of the Friedman's ANOVA

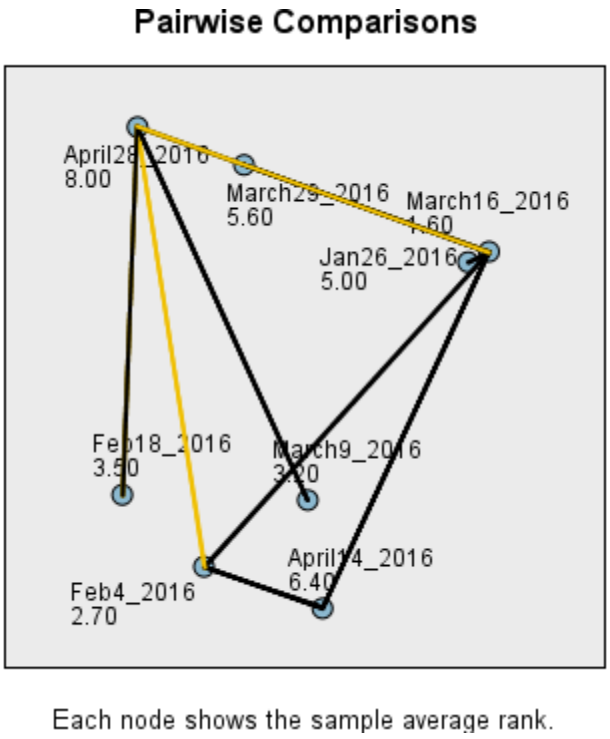

Figure A.1 Pairwise comparisons in a graphical representation.

Table A.2 Pairwise comparison of all sample dates.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
March16_2016-Feb4_2016	1.100	1.549	.710	.478	1.000
March16_2016-March9_2016	1.600	1.549	1.033	.302	1.000
March16_2016-Feb18_2016	1.900	1.549	1.226	.220	1.000
March16_2016-Jan26_2016	3.400	1.549	2.195	.028	.789
March16_2016-March29_2016	-4.000	1.549	-2.582	.010	.275
March16_2016-April14_2016	-4.800	1.549	-3.098	.002	.054
March16_2016-April28_2016	-6.400	1.549	-4.131	.000	.001
Feb4_2016-March9_2016	-.500	1.549	-.323	.747	1.000
Feb4_2016-Feb18_2016	-.800	1.549	-.516	.606	1.000
Feb4_2016-Jan26_2016	2.300	1.549	1.485	.138	1.000
Feb4_2016-March29_2016	-2.900	1.549	-1.872	.061	1.000
Feb4_2016-April14_2016	-3.700	1.549	-2.388	.017	.474
Feb4_2016-April28_2016	-5.300	1.549	-3.421	.001	.017
March9_2016-Feb18_2016	.300	1.549	.194	.846	1.000
March9_2016-Jan26_2016	1.800	1.549	1.162	.245	1.000
March9_2016-March29_2016	-2.400	1.549	-1.549	.121	1.000
March9_2016-April14_2016	-3.200	1.549	-2.066	.039	1.000
March9_2016-April28_2016	-4.800	1.549	-3.098	.002	.054
Feb18_2016-Jan26_2016	1.500	1.549	.968	.333	1.000
Feb18_2016-March29_2016	-2.100	1.549	-1.356	.175	1.000
Feb18_2016-April14_2016	-2.900	1.549	-1.872	.061	1.000
Feb18_2016-April28_2016	-4.500	1.549	-2.905	.004	.103
Jan26_2016-March29_2016	-.600	1.549	-.387	.699	1.000
Jan26_2016-April14_2016	-1.400	1.549	-.904	.366	1.000
Jan26_2016-April28_2016	-3.000	1.549	-1.936	.053	1.000
March29_2016-April14_2016	-.800	1.549	-.516	.606	1.000
March29_2016-April28_2016	-2.400	1.549	-1.549	.121	1.000
April14_2016-April28_2016	-1.600	1.549	-1.033	.302	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.