Multidisciplinary Remediation: An Analysis of Chlorinated Metabolites in Groundwater Contaminated by Pentachlorophenol Following 15 Years of Air/Biosparging, Phytoremediation, and In-Situ Chemical Oxidation Protocols

Stratton, J.; Stokes, C.

Pentachlorophenol (PCP, Penta) got its foothold as a wood preservative in the United States because it extends the lifetime of wood products up to 40 years, even in adverse conditions. It is also an effective herbicide and biocide. Because of this effective nature against pests, it was applied as a protectant in many areas of agriculture and manufacturing. The site utilized in this study has been a receiver of penta wastewater from a wood product treating facility. To comply with mandated cleanup, injection wells for air injection were installed in 2000. These were used until 2011, when they upgraded the airsparging system and included enhanced biosparging. Shortly after this, 400 hybrid poplar and cottonwood trees were planted in the area for an added aspect of phytoremediation. The latest remediation protocol for the site utilizes in-situ chemical oxidation (ISCO) with dilute hydrogen peroxide, started in 2015. A set of nine wells were sampled monthly following ISCO treatment. Metabolites were extracted from water samples using a novel modified liquid microextraction protocol, followed by analysis on an Agilent GC 6890 to determine the presence of chlorinated compounds resulting from the degradation of penta (ongoing through April 2016). We expect to determine the concentration of chlorinated metabolites, analyze the spatial distribution of these compounds across the site, and make recommendations as to the future of remediation treatments for this location.

Introduction

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]. [2]. Penta got its foothold as a wood preservative because it extends the lifetime of wood products up to 40 years, even in adverse conditions[2]. 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[2, 3]. Penta is now a pollutant of concern worldwide. Its long and widespread usage means that penta can be found in many environments, especially near manufacturing and usage sites. The acute LD-50's for small laboratory animals and domestic livestock are between 27 and 300 mg/kg of body weight[3].

In fact, links between cancer and penta have been well established and cannot be blamed solely on the impurities of the chemical [2-4]. The EPA has even placed limits on the allowable amounts of penta that can be consumed through water in the United States (0.03 µg/L) [5].

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 [6]. Its continued use, despite the adverse environmental and ecological effects, 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?

The site used in this study has been under penta 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. This study's objectives were to determine the general location of any remaining penta contamination

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.

Site Description

The 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 prior to the 1970's, before the current company took over management of the mill [7, 8]. 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. The site also stored used pressure treatment fluid wastewater in a lagoon, which was later filled in with uncontaminated soil [8]. At the time of this study, the mill was not using penta but was producing dimensional lumber [9].

The site has been undergoing remediation for a significant groundwater contamination since 2000 [8]. 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[7]. 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 [7, 8, 10]. 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[10].

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 was 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. 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).

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) [10]. 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 samples were transported to the lab in a cooler filled with ice. 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.

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.

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 screw top 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% potassium 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 (C11H24O) 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.

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.

Gas Chromatography Protocol

Gas chromatographic analysis of the extracted samples was based on the method described in Fattahi et al.[11]. 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.

Statistical Analysis

Identified penta peaks were analyzed with Chemstation Reports, utilizing peak retention time as the identifying factor of the chemicals. The reported limit of detection for the ECD method was 0.010 µg L-1[11]. Statistical analysis was completed by the IBM SPSS program. Friedman's ANOVA was used in the analysis of data.

Results

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.

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, $x^2(7)=27.360$, $p=.000$.

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[12]. 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.

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 [10, 11, 13]. 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.

Conclusions

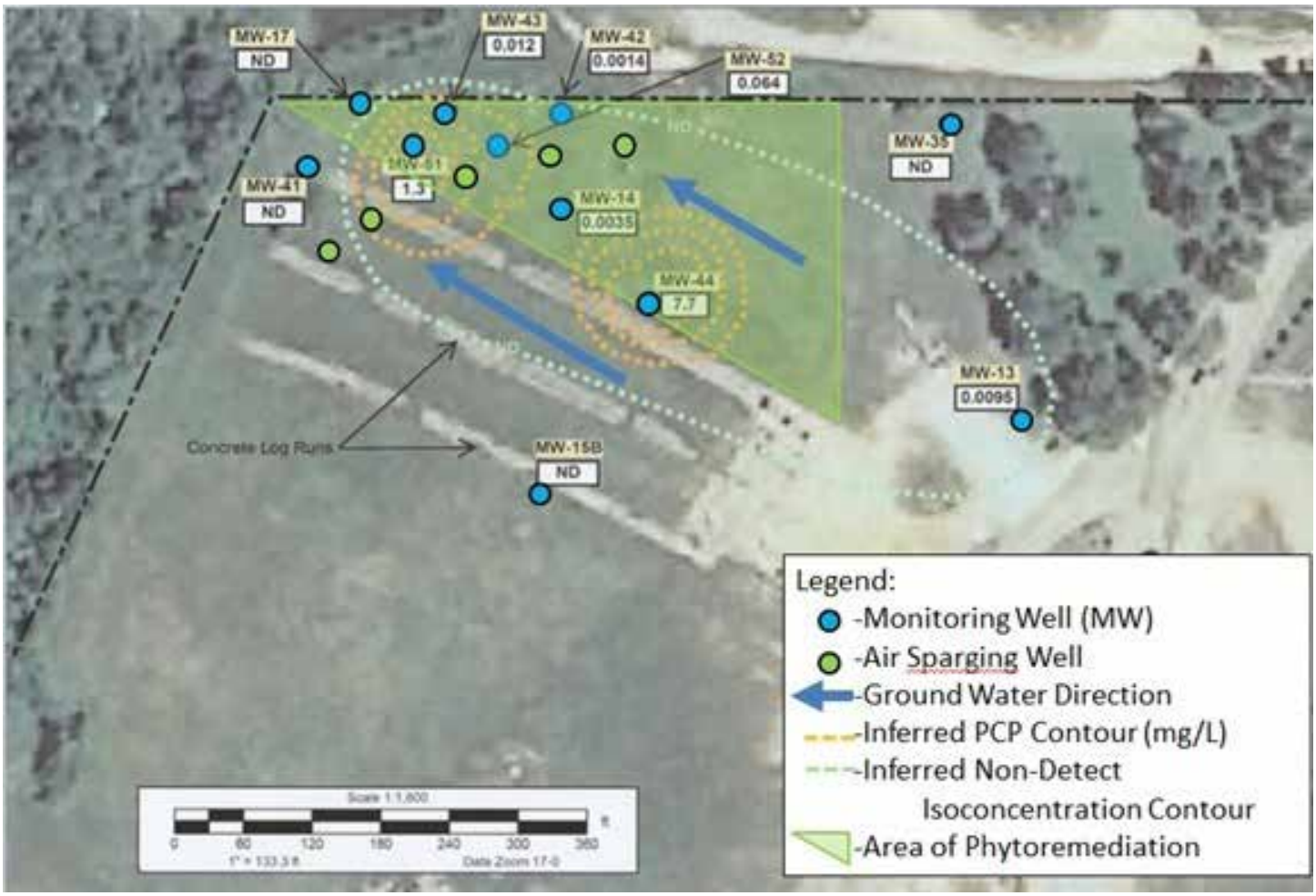
While the method needs refinement to be able to be used quantitatively, it can be used to qualify the data and to determine which monitoring wells were still detectably contaminated. According to our findings, due to years of sequential remediation utilizing bio- and air sparging, phytoremediation, and 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. Crosby, D., Environmental chemistry of pentachlorophenol. Pure and Applied Chemistry, 1981. 53(5): p. 1051-1080.
3. Eisler, R., Pentachlorophenol Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. 1989, DTIC Document.
4. 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.
5. 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.
6. EPA, U., Reregistration Eligibility Descision for Pentachlorophenol. 2008. p. 94.
7. 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.
8. Lybrand, M.S., 2014 Media Monitoring Report. 2014, Lybrand Consulting, LLC. p. 19.
9. Authority, M.D., Weyerhaeuser to invest in Neshoba County Lumber Facility 2014, Mississippi Development Authority: Mississippi.org.
10. 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.
11. 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.
12. Borazjani, H., S.V. Diehl, R. Britto, M. Lybrand. In-Situ biosparging of pentachlorophenol (PCP) contaminated groundwater. in Environmental Science and Technology 2005. 2005.
13. 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.

Multidisciplinary Remediation: An Analysis of Chlorinated Metabolites in Groundwater Contaminated by Pentachlorophenol Following 15 Years of Air/Biosparging, Phytoremediation, and In-Situ Chemical Oxidation Protocols
Stratton, J.; Stokes, C.E.



Multidisciplinary Remediation: An Analysis of Chlorinated Metabolites in Groundwater Contaminated by Pentachlorophenol Following 15 Years of Air/Biosparging, Phytoremediation, and In-Situ Chemical Oxidation Protocols
Stratton, J.; Stokes, C.E.