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Characterizing Polychlorinated Biphenyl Degradation Potential in Surface Water Bacteria from Rio Grande Valley, Texas Reservoirs

Alisha M. Janiga
The University of Texas Rio Grande Valley

CHARACTERIZING POLYCHLORINATED BIPHENYL DEGRADATION POTENTIAL IN SURFACE WATER BACTERIA FROM RIO GRANDE VALLEY, TEXAS RESERVOIRS

A Thesis

by

ALISHA M. JANIGA

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ALISHA M. JANIGA

COMMITTEE MEMBERS

Dr. Kristine Lowe
Co-Chair of Committee

Dr. Alexis Racelis
Co-Chair of Committee

Dr. Megan Keniry
Committee Member

Dr. Christopher Gabler
Committee Member

August 2018

ABSTRACT

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The Donna Reservoir in Donna, Texas is contaminated with polychlorinated biphenyl (PCB) pollution. This reviews the current understanding of PCBs and investigate the potential of PCB degradation by native bacteria communities in the Donna Reservoir. The metabolic diversity, biphenyl tolerance, *bphA1* presence were tested on surface water samples from the Donna Reservoir. The Ecoplate data suggest that the communities have a diverse metabolic capacity and the degree of plant life may interact with carbon type metabolism but not the rate of growth. Biphenyl growth curves showed that all the samples from the Donna Reservoir are tolerant to the average PCB concentration in local sediment. The *bphA1* gene was detected in samples with low plant exposure, but not in samples with high plant exposure. It is possible that some bacteria communities in the Donna Reservoir can degrade PCBs, but further testing is need to track transcription rates.

DEDICATION

I started the master's program at the University of Texas Rio Grande Valley to change the direction my life was going. The polychlorinated biphenyl pollution in Donna, Texas, drew me in as a topic for my thesis project because I could design experiments that were purposeful for scientific investigation. Additionally, it felt like I was giving back to the Rio Grande Valley community as a scientist. This thesis is dedicated to anyone that chooses to use the tools of science to change their lives and those around them. If you cannot find inspiration, look for problems around you and how you can use science to address them

ACKNOWLEDGEMENTS

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CHAPTER I

MICROBIAL ECOLOGY AND POLYCHLORINATED BIPHENYLS: A REVIEW

Introduction

Bacteria are often overlooked in the grand scheme of things because they cannot be seen by the naked eye. Without bacteria however, there would be great declines in oxygen levels, organic matter decomposition would slow, and food webs would struggle at foundation levels. Advances in technology have significantly improved out understanding of microbial ecology, but there are still many techniques that have not been applied to imperative situations. For example, polychlorinated biphenyls (PCBs) have been a persistent organic pollutant since the 1930s, but there are still many misunderstandings about how to implement microbial PCB bioremediation to resolve this issue. This noncomprehensive review discusses advances in microbial ecology technology, provides an overview of PCB history and disposal methods, reviews microbial ecology technology applications towards PCB pollution, and address areas for future research.

Microbial Ecology Advances

Microbial communities influence food chains (Amalfitano et al., 2014; Filstrup et al., 2014) biogeochemical cycling (Lahiri et al., 2018), decomposition of organic material (Ruiz-Gonzalez et al., 2015), and bioremediation (Crawford and Crawford, 1999). A strong effort has been made to understand the role of bacteria, archaea, and fungi in ecological systems and how these roles fit classic ecological theory. Advances in DNA sequencing techniques has significantly improved identification of microorganisms and differences in community structure

(Konopka et al., 2015). Additionally, ecological theories such as the intermediate disturbance hypothesis, biodiversity drivers, and resiliency are extending their application from macroorganisms to microbial systems. In marine samples, as turbidity stress increased microbial diversity followed the intermediate diversity hypothesis (inverted u curve). There was an increase in genetic diversity initially, but as the experiment progressed only a handful of phyla dominated (Galand et al., 2016). Dense colonies of bacteria, algae, and fungi, often found in streams and rivers that break down organic matter, are often places to find high biodiversity among microbial communities (Battin et al., 2016; Graham et al., 2016). Due to the adaptive nature of microbes, one could expect that genetic diversity among microbial communities would recover rapidly after a disturbance. However, it seems that genetic diversity does not readily return to the original composition; instead, functional diversity is maintained by the new dominant organisms (Allison & Martiny, 2008; Bottom et al., 2006).

Functionality Diversity

Functionality can be described as the flow of metabolites and energy within and among communities (Rosenfeld, 2002). In microbial communities, functionality can vary as a factor of redundancy or plasticity. Functional redundancy occurs when microbial community composition changes, but the functional chemistry of the community does not. Conversely, functional plasticity occurs when the community composition does not change but the functional potential does; indicating that the present organisms have a wide range of metabolic capabilities (Berga et al., 2012; Comte et al., 2013).

One widely accepted technique to assess functional diversity is BIOLOG Ecoplates (Chen et al., 2017; Comte et al., 2013; Ruiz-Gonzalez et al., 2015) . BIOLOG Ecoplates are microplates that are pre-supplemented with carbon sources in of the following categories:

polymers, carboxylic acids, amines, amino acids, and carbohydrates. Attached to the carbon sources is tetrazolium a dye that changes color from white to purple when the carbon source is metabolized (Garland & Mills, 1991). The degree of the color change is indicative of how much the community used the carbon source and can be quantified via absorbance with a microplate reader. The colonies metabolize the supplement in 1-3 days. The absorbance values can be analyzed by average well color development (AWCD) (Chen et al., 2017; Garland & Mills, 1991; Tiquia et al., 2008), a phenotypic tree of substrate utilization comparisons can be designed with the NTedit V2.0 program, and community level physiological profiles (CLPPs) can be compared among samples. Data from BIOLOG Ecoplates coupled with DNA 16S rRNA sequencing or denaturing gradient gel electrophoresis show that taxonomic diversity varies by environmental conditions, but functional diversity is more dependent on resources available (Chen et al., 2017; Comte et al., 2013; Ruiz-Gonzalez et al., 2015). While the BIOLOG Ecoplate tool is useful because large amounts of data can be collected in a short amount of time, there are some limitations. The design of the plate provides data on cultured microbes, not necessarily in situ metabolisms, and not all organisms can oxidize tetrazolium. Additionally, the plate comes with triplicate carbon source wells, but three replicates may not hold significant statistical power (Preston et al., 2002).

Analyzing functional genes allows scientists to overcome the limitations of BIOLOG Ecoplates and next generation sequencing. Next generation sequencing gives a snap shot of microbial community species diversity, and BIOLOG Ecoplates give insight to the metabolic potential of a sample subset. However, these techniques still do not alleviate the ambiguity that comes with defining which microbes are performing what activity. Polymerase Chain Reaction (PCR) based reactions can bridge this indistinctness. In soil microbiomes, there are many

pathways to metabolize nitrogen such as nitrification, annamox, and fixation (Barton & Northup, 2011). Expression of nitrogen metabolizing genes strongly correlates with the present forms of nitrogen in the soil and organisms that break down that form of nitrogen (Chen et al., 2017; Kapoor et al, 2016). In one experiment (Chen et al., 2017), the team recorded a decrease in $NH4^+$ and an increase in NO_2 and NO_3 concentrations. At the same time, next generation sequencing showed that *Nitrospirae* had a high population at the time. Additionally, quantitative PCR (qPCR) showed that as $NH4^+$ concentrations decreased the expression of *amoA* and *nirS* genes, genes responsible for the conversion of $NH_4^+ \rightarrow NO_2 \rightarrow NO_3$, also decreased. The team concluded that *Nitrospirae* expressed the *amoA* and *nirS* genes to convert the NH_4^+ to nitrate and nitrite, and as the resource declined, other opportunistic populations rose. By combining classic microbiology techniques with progressive molecular biology technology, identifying and utilizing the function of microorganisms can be refined and applied to current environmental issues.

Polychlorinated Biphenyl Overview

Chemical Properties

PCBs are characterized by a set of biphenyl rings with chlorine attachments ($\leq$10). There are 209 possible combination of chlorine attachments, and the quantity and positioning of chlorine atoms determines the toxicity and degradation time. The phenyl rings give the structure hydrophobic properties, making it insoluble in aqueous solutions and readily available to solutions high in hydrocarbons, such as adipose tissue (ATSDR, 2000a; Letz, 1983). When organisms try to metabolize the molecules, the detachment of the chlorine atoms often leads to free radical formation, and the free radicals can have toxic effects on the exposed organism (Klaassen, 2001).

Production History

The German chemical company, Schimdt and Schultz originally synthesized PCBs in 1881 (Cairns & Siegmund, 1981). However, mass production didn't begin until the American Swan Corporation developed cost and time effective methods to produce the chemical (USEPA, 1979). Because PCBs are heat resistant and not readily degradable, they were included in a wide array of materials including: dielectric fluid, adhesives, printing ink, ship paint, chewing gum, sealant, insecticide carrier, insulation, plastics, coolant, and many others (USEPA, 1979; ATSDR, 2000a; Cairns & Siegmund, 1981). Toxic effects were noticed in human exposure throughout the 1930s, but it was not until 1977 that production was prohibited by the US government (USEPA, 1979; ATSDR, 2000a). ATSDR (2000a) estimated a total of 630 million kg of PCBs were synthesized in the United States. Recently, PCBs have been found as a byproduct of electronic waste recycling and in countries using outdated equipment running PCBs (Chakraborty, 2015; Xu, 2015; Zhang 2016).

Toxicology

The effects of PCB exposure in humans is very diverse and depends on the type of congener and duration of exposure. Most of the toxicological affects are understood through animal models, workplace exposure, and the *Yusho* tragedy in Japan. Dermal exposure can lead to chloracne and irritation (ATSDR, 2000a; Jensen, 1972a); PCB exposed hepatic cells showed increased microsomal enzymes, increased cdk2 activity, and increased cellular proliferation (ATSDR, 2000a; Vondráček et al., 2005). Disruption of sperm morphology and menstruation cycles (ATSDR, 2000a) as well as lower FT3 and FT4 thyroid hormones can also be effects (Eguchi et al., 2015; Xu et al., 2015). Children exposed to PCBs show decreased reflexes, memory and learning deficits, and decreased IgA and IgM antibody levels. There is also evidences that suggest PCB exposure is linked to cancer, diabetes, and attention deficit disorder (ATSDR, 2000a;

Carpenter, 2006; Fonnum et al., 2006). Gene regulation is radically affected by PCB exposure. Out of 100 genes, 16 were upregulated and 84 were down-

Table 1.1 Distribution of PCBs in Water and Sediment in Select Regions

Contaminate	*Source*	*Range*	*Date*	*Citation*
Water (ng/l)	US, Texas	0.49-12.49	1999	Howell et al., (2008)
	US, Delaware River Basin	1.2-6.5	1999	Howell et al., (2008)
	US, Michigan	1.1×10^{5}	Before 1999 clean up	Santini et al (2015)
	US, Michigan	2,500 *	After 1999 clean up	Santini et al (2015)
	US, Mississippi River	22.2-163.4	2007	Eremina et al., (2016)
	US, Hudson River	0.2-1.2	2008	Eremina et al., (2016)
	France/Belgium	250-7340	2014	Rabodonirina et al., (2015)
	Southern China	0.91-13.05	2013	Yang et al., (2015)
Sediment (mg/kg)	US, Texas	0.004-100	Not found	Oziolor et al., (2018)
	US, Delaware River Basin	1.1×10^{-7} *	2008-2010	Guo et al., (2014)
	US, Michigan	0-700	Before 1999 clean up	Santini et al (2015)
	US, Michigan	0.27 *	After 1999 clean up	Santini et al (2015)
	France, Durance River	0.03-13	2010	Kanzari et al., (2015)
	France, Berre Lagoon	15-144	2010	Kanzari et al., (2015)
	Argentina, Chubut province	$0\text{-}1.46 \times 10^{-4}$	2010	Commendatore et al., (2015)

*Mean average

regulated (Ghosh et al., 2015). However, there is strong evidence that suggest PCBs interact with the ARNT and AhR genes, linked with tumorigenesis (Carpenter, 2006; Bersten et al., 2013).

Environmental Distribution

Environmental contamination of PCBs was not discovered until the 1960s with the development of gas chromatography and mass spectrophotometry (Jensen, 1972b). When the US government prohibited the use and production of PCBs, chemical company such as Monsanto and General Electric dumped barrels of PCBs in neighboring creeks and rivers (The Associated Press, 2003; USEPA, 2018; Perez-Pena, 1999). Outdated electrical equipment, paper/fabric dyes (Guo et al., 2014), and ship paint (Wang et al., 2016) are other sources of pollution because they can leach and leak PCB oil into the environment. Once the PCBs leave the containment vessel, they can volatize into the atmosphere or bind to sediment and soil particles. Generally, low chlorinated PCBs are found in the atmosphere while highly chlorinated bind to sediment (Chakraborty et al.,2016). Developing countries, such as Vietnam (Wang et al., 2016), are still using transformers with PCB oil, and are a likely source of PCBs entering the atmosphere. (Nisbet, & Sarofimt, 1972). Once in the atmosphere, PCB molecules can travel to locations where they were not used, such as the Alpines and the Arctic (Pavlova et al., 2015; Zhu et al., 2015). Increasing temperatures due to climate change are likely to release PCBs stored in the Arctic ice and increase vaporization rates; it is also suggested that Antarctica will become a sink of PCBs (Nadal et al., 2015).

PCB National Priorities List

Currently, there are 1341 active National Priority Listed (NPL) sites of various contaminants and 55 proposed new sites (https://www.epa.gov/superfund/superfund-national-priorities-list-npl). A report by the Agency of Toxic Substances and Disease Registry (ATSDR)

(2000a) states that PCBs contaminate 500 out of 1598 NPL sites. A key word search for *polychlorinated biphenyl* in the US Superfund contaminant search queued 76 superfund sites; 5 sites have been deleted, 27 were not on the NPL, leaving 44 sites as active on the NPL (Environmental Protection Agency, 2017). However, there are limitations to accuracy of this search because known PCB superfund sites were missing. For example, the superfund site in Donna, Texas was not listed.

According to the EPA (2005), a high occupancy area PCB contaminated site is considered clean when soil concentrations are less than 1 ppm (uncapped) and porous/nonporous surfaces concentrations are less than 10 ng/100 cm^2. In low occupancy areas, soil concentration increases to 25 ppm, but clean surface concentrations remain the same. The drinking water is considered safe to drink at 0.5 ppb and rivers and streams at 0.17 ppt (ATDRS, 2000b). While some areas are well below these levels (Table 1), cleaning sediment and water does not necessarily remove all the PCBs from the environment.

Bioaccumulation

Because PCBs are hydrophobic, the molecules tend to travel through the food chain attached to cells high in hydrocarbons and fats (Figure 1.1). It is believed that benthic organisms bioconcentrate the PCBs, and as they are eaten by larger organisms, the chemical biomagnifies through the trophic levels (Beyer & Biziuk,2009). PCBs can also be transferred from mother to offspring. Pregnant women who consume high quantities of PCB contaminated fish have high concentrations of PCBs in their breast milk (ATDRS, 2000a; Grandjean et al., 1995). When the child consumes the milk, it is exposed to PCBs. In general, predators near the top of the food chain accumulate higher chlorinated PCBs while lower chlorinated PCBs are found in the sediment, benthic organism, or in low quantity (Oliver & Niimit, 1988; Nakata et al., 1998; van

der Oost et al., 1988). Highly chlorinated PCBs are more hydrophobic than lower chlorinated molecules. As a result, they bind strongly to adipose tissue and are easily transferred through

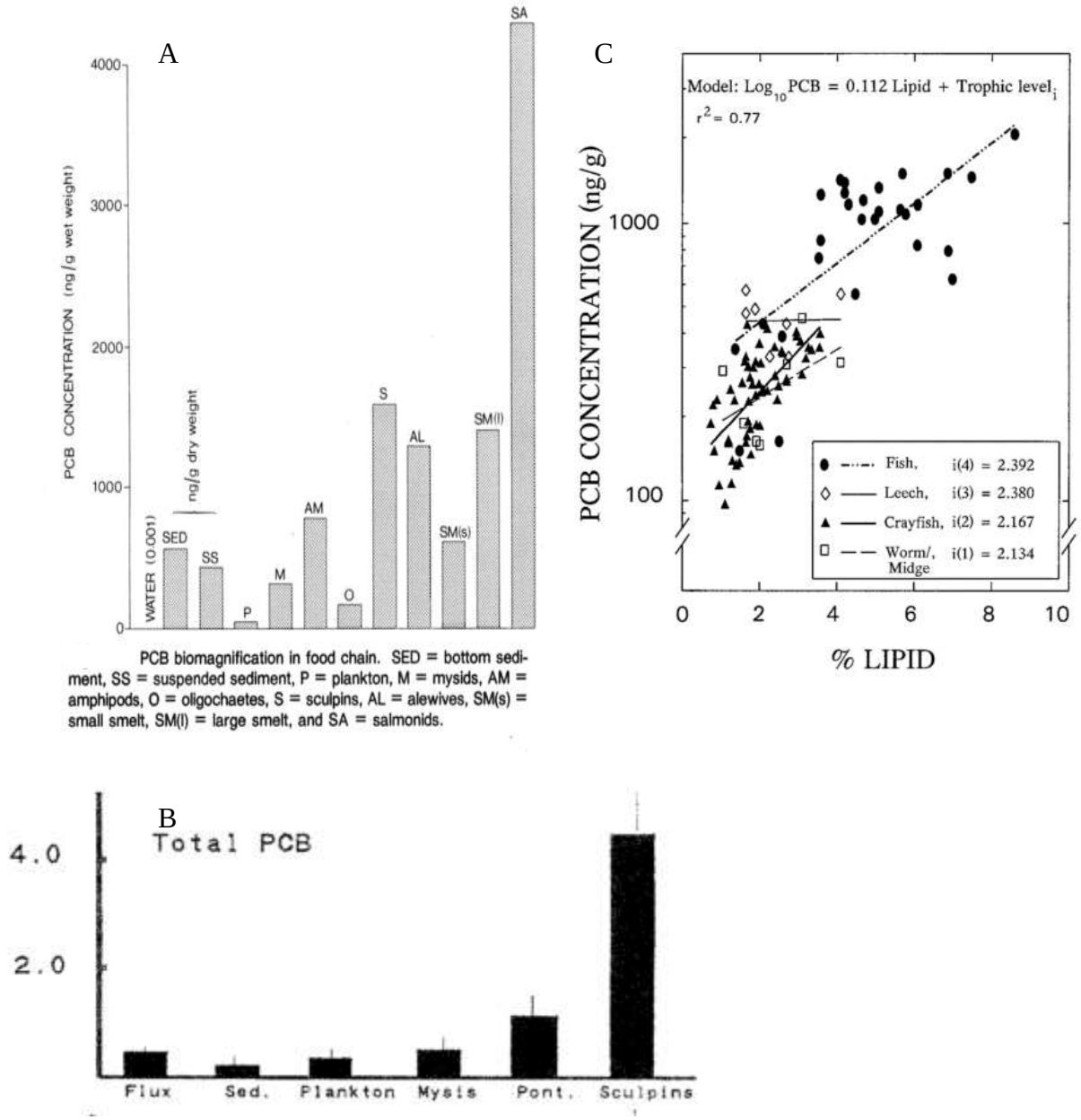

Figure 1.1 Bioaccumulation of PCBs in Aquatic Species *A. Oliver & Niimit (1988)* show that total PCB concentration is higher in predatory salmonids than the sediment, plankton, and other species in Lake Ontario. *B. Evans et al., (1991)* demonstrates biomagnification of PCBs (µg/g) threw particle flux, surficial sediments, plankton, *Mysis relicta*, *Pontoporeia hoyi*, and deepwater sculpins offshore Lake Michigan. *C. Zaranko et al., (1997)* uses samples from Pottersburg Creek to prove as lipid content increases, PCB concentration also increases

consumption (Perga et al., 2017; Pitt et al., 2016). Additionally, lower chlorinated PCBs are easier for biologic species to breakdown into metabolic products (Nakata et al., 1998). The FDA suggests that tissues have less than 2 ppb PCBs to be considered safe for consumption.

PCB Disposal

In the United States, PCBs are disposed by physically heating the product in an incinerator or high efficient boiler to at least 1200°C (U.S. Government Publishing Office, 2017). High heat can break the bonds and oxidize the molecules, but caution is needed when disposing of PCBs this way. If the temperature does not reach sufficient maximums, more harmful dioxincompounds such a tetrachlorodibenzo-p-dioxins form (Kulkarni et al, 2008). Additionally, all the fumes must be contained, or volatile PCBs will enter the atmosphere. Plasma is another energy rich tool used to break down PCB; by exposing the toxicant to high energy electrons, the bonds dissociate into carbon dioxide, water and hydrochloric acid (United Nations Environment Program, 2000). While these methods are efficient, they require large amounts of energy and can be dangerous if safety conditions are not maintained.

Chemical decomposition is another alternative in other countries. Sodium dechlorination is one of the more common techniques. By introducing metallic sodium with a high surface area into PCB oils, the chlorine atoms bind to the sodium, leaving behind biphenyl oil. However, if the reaction does not complete correctly, stable chlorine polymers form, making degradation more difficult (United Nations Environment Program, 2000). Gas phase chemical reduction uses hydrogen gas, high temperatures, and low pressure to break down PCBs into methane, hydrogen chloride gas, and small amounts of hydrocarbons. Companies using this method must take care to capture any emissions or residues that form after processing to prevent contamination (United Nations Environment Program, 2004). Base catalyzed decomposition uses alkaline products such

as sodium bicarbonate and sodium hydroxide in combination with high heat (300°C) to dechlorinate the PCB molecules. Remaining components can be further treated or sent to other companies for reuse (Vijgen & McDowall, 2009; United Nations Environment Program, 2004). This is not a comprehensive list of all the chemical methods to breakdown PCBs but highlights some of the most common one. There is not a consensus on the most appropriate method of PCB disposal, and one poorly understood method is through bioremediation.

Bacteria and PCBs

Genetic Diversity

Shortly after PCBs were detected in the environment, scientist started culturing bacteria with PCBs. Some of the earliest PCB tolerant bacteria identified were *Achromobacter* and *Pseudomonas* (Ahmed Focht, 1973; Wong et al., 1975). As DNA sequencing technology improved, other types of PCB tolerant bacteria were identified. In sites high in PCB pollution, bacteria tolerant to the chemical's toxic properties can usually be found in the soil, sediment, and surface water (Table 1.2). Proteobacteria tend to be the dominant phylum identified in PCB polluted samples, but other common phyla include: Acidobacteria, Actinobacteria, Firmicutes, Bacteriodetes, and Chloroflexi (Begonja et al., 2007; Brito et al., 2015; Dercova et al., 2008; Furukawa et al., 1994; Gentile et al., 2006; Koubek et al., 2013; Li et al., 2017; Liu et al., 2015; Luo et al., 2008; Michaud et al., 2007; Nogales et al., 2001; Qi et al., 2012; Quero et al., 2015; Williams et al., 1997; Kaiser & Wong, 1975; Yoshida et al., 2005). In general, Proteobacteria genera such as: *Pseudomonas*, *Achromobacter*, *Alcaligenes*, and *Acinetobacter*, *Sphingomonas*, as well as members of the Actinobacteria and Firmicute phyla participate in the aerobic oxidative degradation of low (< 3) chlorinated PCBs. \ Highly chlorinated PCB molecules are subject to

reductive dehalogenation by organisms in the Chloroflexi phylum and *Clostridium* genera (Borja et al, 2005; Field & Sierra-Alvarez., 2008; Furukawa & Fujihara, 2008).

Table 1.2. Common Bacteria Phyla Tolerant to PCBs

Phylum	*Sample Source*	*Citation*
Proteobacteria: *Achromobacter, Agrobacterium, Alcaligenes, Burkholderia, Comomonas , Flavimonas, Moraxella, Ochrobactrum, Pseudomonas, Pseudoalteromonas , Rhodanobacter,, Sphingomonadas, Thermomonas, Thiobacillus*	Antarctica, Canada, China, Croatia, Germany, Italy, Japan, Mexico, Ontario, Slovakia, United States	Begonja et al., 2007; Brito et al., 2015; Dercova et al., 2008; Furukawa et al., 1994; Gentile et al., 2006; Koubek et al., 2013; Liu et al., 2015; Luo et al., 2008; Michaud et al., 2007; Nogales et al., 2001; Qi et al., 2012; Quero et al., 2015; Williams et al., 1997; Wong & Kaiser, 1975; Yoshida et al., 2005
Actinobacteria: *Arthrobacter Corynebacterium, Mycobacterium, Nocardia, Rhodococcus, Nocardia,*	Antarctica, Croatia, Japan, Mexico, United States	Begonja et al., 2007; Brito et al., 2015; Gentile et al., 2006; Williams et al., 1997; Yoshida et al., 2005
Acidobacteria	China, Germany, Italy	Li et al., 2017; Liu et al., 2015; Nogales et al., 2001; Quero et al., 2015
Bacteroidetes	Antarctica, China, Italy, Japan, Slovakia,	Gentile et al., 2010; Koubek et al., 2013; Liu et al., 2015; Quero et al., 2015; Yoshida et al., 2005
Chloroflexi: *Dehalococcoides*	China, Italy, Japan	Li et al., 2017; Liu et al., 2015; Quero et al., 2015; Yoshida et al., 2005
Firmicutes: *Bacillus, Desulfitobacterium , Dehalobacter*	China, Italy, Japan, United States	Liu et al., 2015; Luo et al., 2008; Quero et al., 2015; Yoshida et al., 2005
Plantomycetes	Italy, Germany	Nogales et al., 2001; Quero et al., 2015

Reductive Dehalogenation

The simplified mechanism is to decrease the amount of chlorine atoms attached and from there break down the ring for carbon and hydrogen components. However, with co-metabolism

likely occurring this mechanism is expected to be significantly more complicated. The reductive dehalogenation pathway (Figure 1.3A) is primarily utilized by anaerobic bacteria and the rate of dechlorination is strongly correlated with the structure of the PCB molecules. Chlorine atoms attached *para* and *meta* to the central carbon atoms are more likely to detach than those in the *ortho* position (Field & Sierra-Alvarez., 2008; Furukawa, 1994; Wiegel & Wu, 2000). Additionally, PCBs with chlorine attachments on one ring versus both rings degraded at a faster rate (Furukawa, 1994). Some believe that diverse anaerobes are co-metabolizing the PCBs (Fetzner, 1998; Furukawa, 2000) and using the chlorine atoms as a final electron acceptor (Abramowicz, 1990). Unfortunately, the gene pathway for this mechanism has remained somewhat elusive over the years. Recently however, reductive dehalogenase (RDase) genes have been found in Chloroflexi from PCB contaminated sediment (Mattes et al., 2017). The rate of degradation is dependent on the species of bacteria, temperature, pH, and supplemental carbon sources (Wiegel & Wu, 2000), but chlorobenzoate has shown to stimulate the anaerobic pathway (Abraham et al., 2002).

Aerobic oxidative degradation

Aerobic oxidative degradation (Figure 1.3B) involves dihydroxylation of one of the rings, which leads to a series of rearrangements, and ends in the degradation of the two rings into nontoxic products such as, carbon dioxide and acetyl co-enzyme A (Field & Sierra-Alvarez., 2008; Furukawa et al., 1979; Pieper, 2005). This pathway is controlled by *bph* genes that translate into a series of enzymes including dioxygenases, ferrodioxins, and reductases. *BphA1* and *BphA2* begin the gene sequence by coding for the biphenyl dioxygenase (Erickson & Mondello, 1992). This protein adds hydroxyl groups to the biphenyl ring; the position of the addition depends on the species and gene sequence. The *bphA* genes also encode for congener

specificity and can be genetically altered to enhance PCB degradation rates (Erickson & Mondello, 1992; Furukawa et al., 2000). Most species use the 2,3-dioxygenase which adds

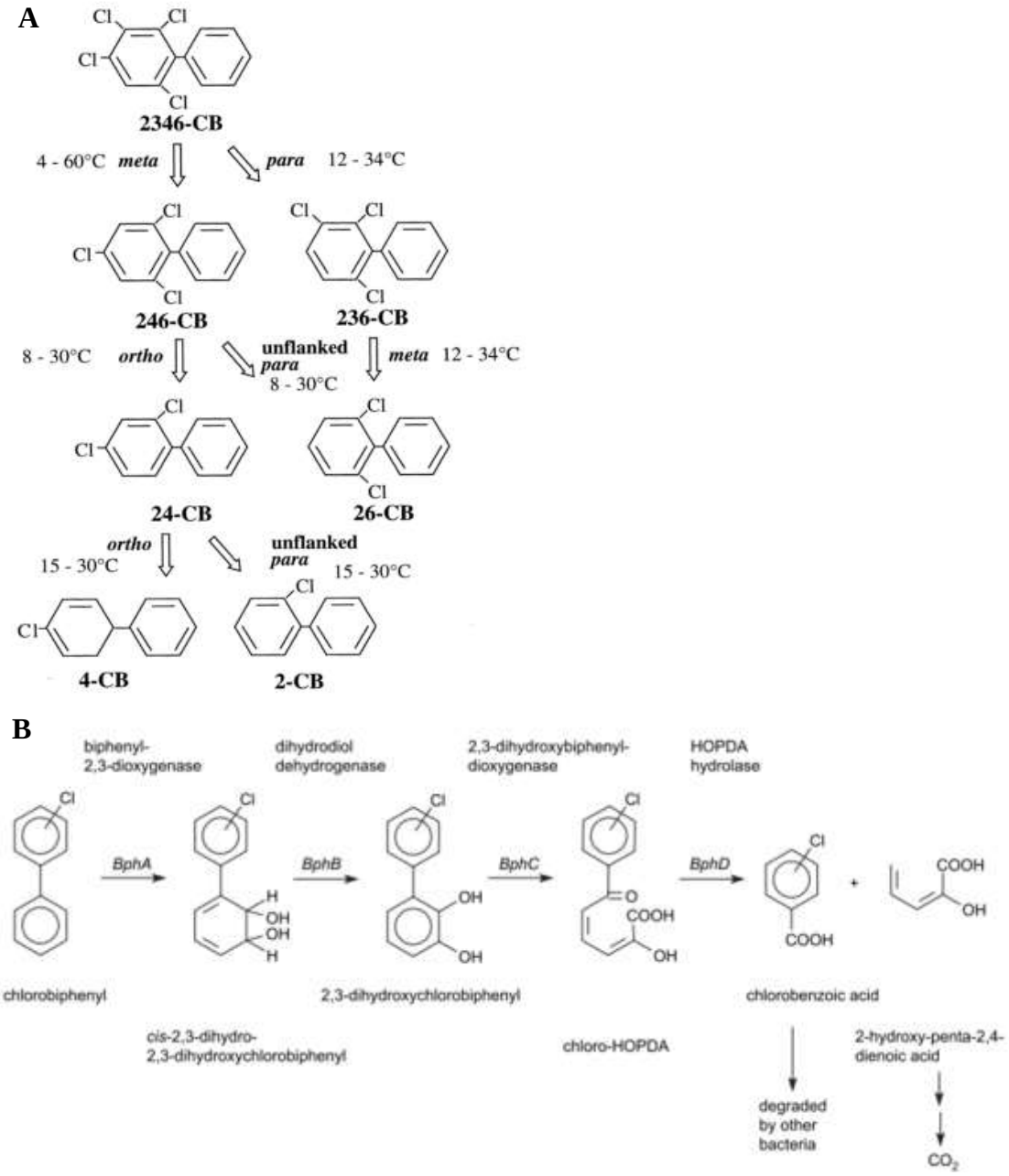

Figure 1.2 Bacteria PCB Degradation Mechanims A. Temperature dependent anaerobic reductive dehalogenation (Wiegel & Wu, 2000). B. Aerobic oxidative degradation of biphenyl molecules (Field & Sierra-Alvarez, 2008).

oxygen to the *meta* and *para* position. However, some organisms, such as *Burkholderia*, have 3,4-dioxygenase that will work with the *ortho* position (Focht, 1995; Furukawa et al., 2004;

Seeger et al., 1997). Operon orf0 will upregulate transcription of the *bph* genes in the presence of biphenyl, and *bphS* will downregulate promotor *bphE* (Pieper, 2005). Additionally, chemicals such as biphenyl, salicylic acid, and plant terpenes have show to upregulate the *bph* pathway (Petrić et al., 2011; Tehrani et al., 2012). Dead end products such as chlorobenzoate, dihydrodiols and dihydroxybiphenyls can form and slow/halt the degradation process (Abraham et al., 2002; Pieper, 2005). Fortunately, the *bph* genes are found in numerous organisms because they can conjugate to other bacteria and transpose into the main chromosome (Furukawa, 2004; Pieper, 2005).

Future Research Pathways

While there has been thorough research on the mechanisms involved in *bph* gene expression, there is limited data quantifying the presence of *bph* gene in contaminated sites. One study in Germany found *bphC* present in polluted river sediment but not in unpolluted samples. While detection was recorded, quantification of gene expression was not (Erb & Wagner-Doble, 1993). Another study in Lake Michigan found an average of 7.39×10^6 genes/g sediment of the *bphA* gene in sediment 0-1.83 meters deep. This number declined to an average 3.28×10^5 genes/g sediment as depths became anoxygenic. They also detected RDase genes, suggesting co-metabolism between aerobes and anaerobes (Liang et al., 2014). Some studies quantify *bph* genes after inoculating polluted areas with known *bph* carriers or supplementing microcosms with PCBs or biphenyls (Jha et al., 2014; Mattes et al., 2017; Petrić et al., 2011). However, to better understand the dynamics of microbial communities in PCB contaminated areas, it is important to understand the baseline expression before introducing new organisms.

There is also limited data about the CLPPs found from BIOLOG Ecoplates. In general, samples from PCB polluted areas are generalist that can metabolize a high variety of carbon

sources. Amino acids, carboxylic acids, and carbohydrates such as L-serine, D- galacturonic acid and glucose, are commonly metabolized because they are easily broken down for energy. In several papers, there is also an unexplained preference for Tween 80 and Tween 40, unnatural polymers (Cepeda, 2014; Bushaw-Newton et al., 2012; Master & Mohn, 1998; Tiquia et al., 2008).

Although numerous studies have sampled from infamously PCB polluted areas such as the Hudson River, Asian e-waste recycling centers, and ex-manufacturing sites in Europe, there many small towns that also need to address their PCB pollution problem. For example, in Donna, Texas, there is a reservoir that provides water for municipalities and agriculture. Records of PCB pollution in fish tissue go back to the early 1990s, but many residents are still consuming PCB contaminated fish from the reservoir (EA Engineering, Science, and Technology, Inc., PCB, 2016a). The EPA has sent teams to remove fish from the reservoir five times. However, this method of remediation involves high ecological disturbance and does not address PCBs bioaccumulated in piscivores birds and mammals. Ecological assessments for EPA were performed on the effect of PCBs on local benthic invertebrates, fish, avia, mammals, reptiles, amphibia, and plants, but there was no direct investigation of the impact of PCBs on microorganisms (EA Engineering, Science, and Technology, Inc., PCB, 2016b). Because bacteria are foundational species that cycle nutrients, fixate carbon, and breakdown pollutants, it is just as important to this microscopic domain as it is macroscopic organisms.

Conclusions

In nearly 50 years, the understanding of microbial bioremediation of PCBs has made leaps and bounds. There is a good comprehension of the gene pathways bacteria use to oxidatively degrade PCB and what taxonomic groups can degrade PCBs. However, there are still

techniques and technologies that have not been thoroughly applied to PCB polluted areas such as, *in situ* qPCR and community level physiological profiling. Additionally, there are many PCB polluted areas that have not been systematically researched. PCB exposure can cause numerous health issues and is ubiquitously distributed around the planet. Even though there are current methods to dispose of PCBs, there are downsides to these methods. Microbial bioremediation creates a system with low energy input and nontoxic outputs, but further understand about how these microorganisms perform outside laboratory cultures is needed to implement application.

Objectives

The research conducted for thesis was aimed towards the following objectives:

1. Understand the functional diversity of surface water in PCB contaminated areas in comparison to uncontaminated areas through metabolic studies using BIOLOG Ecoplates.

2. Determine if surface water bacteria in the Rio Grande Valley are tolerant to average PCB concentrations in Donna Reservoir and Canal System sediment.

3. Find out if Rio Grande Valley surface water bacteria are capable of oxygenic degradation of biphenyl rings by running real-time PCR for the *bphA1* gene.

CHAPTER II

CHARACTERIZING POLYCHLORINATED BIPHENYL DEGRADATION POTENTIAL IN SURFACE WATER BACTERIA FROM RIO GRANDE VALLEY, TEXAS RESERVOIRS

Introduction

While bacteria are infamous for causing disease, most other species play crucial roles in key biological function such as cycling nutrients, decomposing matter, and bioremediating toxins (Barton & Northup, 2011). Innovative combinations of modern and traditional technology made it possible to understand the mechanisms and relationships of bacteria in novel ecosystems. For example, in the past fifty years, scientists have identified bacteria that are tolerant to polychlorinated biphenyls (PCBs) as well as mapped the mechanisms by which these organisms metabolize PCBs. However, it remains elusive how to make these organisms degrade PCBs *in situ*.

Polychlorinated biphenyls (PCBs) are a type of persistent organic pollutant (POP) mass produced in the United States for their heat resistant and stable properties from the late 1920s to 1977. In 1977, the U.S. Environmental Protection Agency prohibited the production and use of PCBs, and by the end of the 1980s, most countries created similar laws and regulations (USEPA, 1979; ATSDR, 2000). Today, many of the products and structures created in that timeframe are leaching PCBs into the environment, and chemical processes in electronic waste recycling have shown to produce PCBs as a byproduct (Chakraborty, 2015; Xu, 2015; Zhang 2016). Additionally, there are still many areas in the United States that are still on the National Priories

List for PCB pollution. Disreputable areas such as the Hudson River, the Great Lakes, and Snow Creek have received numerous research publications, but many small town with similar pollution issues remain underrepresented in literature searches.

The Donna Reservoir and Canal System in Donna, Texas is a PCB Superfund Site. The reservoir is currently used for municipal and agricultural purposes, and at one point the area was a popular recreational fishing location. However, in 1993, the EPA concluded that the fish in the Donna Reservoir were the source of PCB contamination contributing to a neural tube defect affecting local newborns. Geological surveying suggests that the source of the PCBs in the reservoir is from an inverted siphon constructed to pump water from the Rio Grande River, underneath the Arroyo Colorado, and into the Donna Reservoir. While construction and repair material records could not be located, the siphon was constructed in 1929, a time where PCBs were commonly included in concrete and painting materials. Despite public education efforts, many of the residents continue to consume the contaminated fish (EA Engineering, Science, and Technology, Inc., PCB, 2016). In 2008, the DRIS was added to the National Priorities List as a Superfund Site (EA, 2016a; TDHS, 2010).

The EPA and the city have removed thousands of fish from the reservoir as a cleanup option. Additionally, the EPA feasibility plan in 2016 favored four future options: no action; limited action (signs, public health outreach); slip line siphon, dredge canal, and remove fish; replace siphon, dredge canal, and remove fish. In preparation of the feasibility report the team also organized an ecological risk assessment on small piscivorous birds, piscivorous mammals, benthic invertebrates, interior least tern, reddish egret, Coues rice rate, false spike mussel, Salina mucket, and the Texas hornshell. No federal reports were found investigating microbial ecology nor any impacts of the Donna Reservoir pollution on micro-communities. One paper (Cepeda,

2014) was found isolating PCB degrading bacteria from the reservoir. There is a wealth of literatures on the PCB degradation mechanisms and lab cultures from PCB contaminated areas (in situ and ex situ), but less information is available concerning metabolic preferences of bacteria from PCB polluted site. Additionally, the *bph* gene sequence has been systematically analyzed, but many of these papers work with the presence of the bph genes in samples cultures with high concentrations of PCBs. There is little information of the presence of the gene in the environment nor if the gene is active in low concentrations of PCBs.

The average sediment concentration of PCBs in the Donna Reservoir is 0.41 mg/kg. The purpose of this study was to investigate surface water bacteria from Rio Grande Reservoirs for tolerance and degradation capabilities of the remaining PCBs. This was done using BIOLOG Ecoplates, biphenyl tolerance growth curves, and quantitative polymerase chain reactions (Q-PCR).

Methods

Site Description

Donna, Texas is an active agricultural area adjacent to Reynosa, Mexico in the Lower Rio Grande Valley. In 1928, the city constructed an inverted siphon to pump water from the Arroyo Colorado to the Donna Reservoir and Irrigation System (DRIS) (EA Engineering, Science, and Technology, Inc., 2016a). This water is used for crops, municipalities, and provides a habitat for local wildlife. Samples were taken from the 90^0 bend, slightly downstream from the siphon, an area recorded with high PCB concentrations compared with the rest of the DRIS (Figure 2.1).

Sample Collection

Surface water samples for this study were collected mid-February 2017. Basic physical measurements including temperatures, pH, and salinity were taken for each sample. Three of the

sample sources are from the Donna Reservoir, Donna, Texas; one is from Delta Lake, Edcouch, Texas, and the final sample was taken from deionized tap water, Edinburg, Texas.

Donna Reservoir, Donna, Texas

Sample names (Table 2.1) D.org, D.mix, and D.con are from the Donna Reservoir. D.org was near local plant life; D.mix was from a biofilm formed between an organic wall and a concrete bridge, and D.con was from a concrete lined irrigation canal on the other side of the bridge (Figure 2.3)

Control Samples

C.org was sampled from Delta Lake, Edcouch, Texas, because local scientists believe there are no PCB contaminations in this body of water. This lake is approximately 25 miles from Donna, Texas and had a habitat similar to D.org, surrounded by plant life (Figure 2.3D) and is assumed to experience similar climate changes. A second control (C.tap) was taken from the deionized tap water at the University of Texas Rio Grande Valley Science Building (Edinburg, Texas).

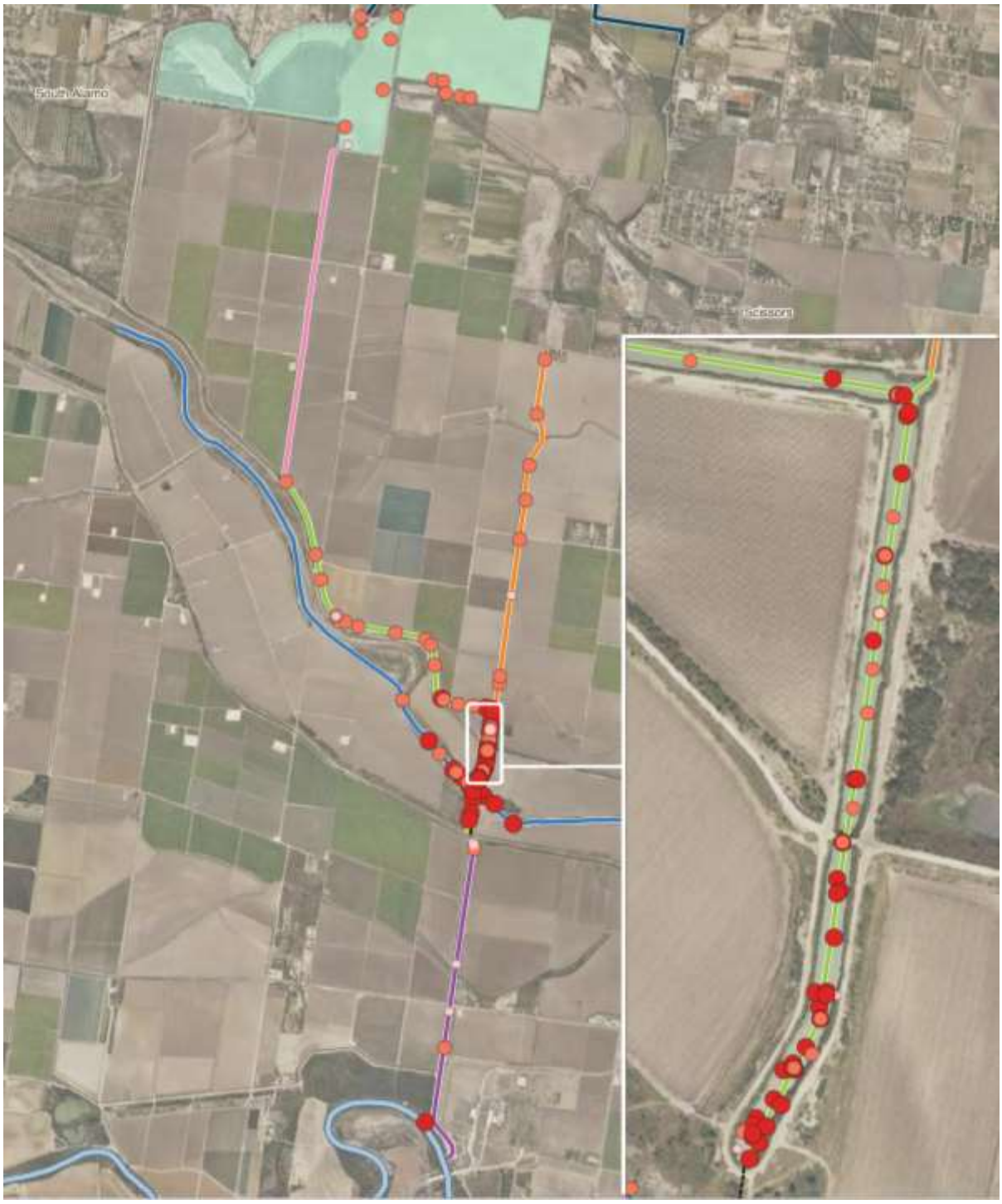

Figure 2.1 Feasibility Report Map This map was used to locate the siphon and sample sources taken upstream from the siphon **(**EA Engineering, Science, and Technology, Inc., PCB., 2016a).

Figure 2.2 Areal Map of Sample Sources (Top) Three samples were taken from the Donna Reservoir. (A) D.org was near plant life, (B) D.mix was from a biofilm near a concrete bridge, and (C) D.con was from a concrete lined irrigation canal (Google, n.d.a). (Bottom) (D) C.org is from an accessible point of Delta Lake, Edcouch, Texas (Google, n.d.b).

Table 2.1 Sample Names and Descriptions

Sample Name	Source	Habitat Description
D.org	Donna Reservoir	High plant life, organic canal
D.mix	Donna Reservoir	Biofilm from area mixed with concrete and plant life
D.con	Donna Reservoir	Concrete lined irrigation ditch
C.org	Delta Lake	High plant life, organic canal
C.tap	Deionized tap water	Municipal reservoir

Figure 2.3 Photographs of Sample Sources A. D.org was upstream from the siphon and bridge and near vegetation. B. D.mix was from murky water near both concrete and plant life. C. D.con from was clear water used for irrigation. D. C.org was from the shore of a lake approximately 25 miles from the Donna Reservoir.

BIOLOG Ecoplates

BIOLOG Ecoplates were used to create a community level physiological profile of the samples. After collecting the second set of samples, 100 µL of sample was pipetted into each of the 96 wells on the Ecoplate. After 72 hours, the Ecoplates were analyzed on a microplate reader,

and absorbance values were recorded. The absorbance values were adjusted to compensate for false positives by subtracting the absorbance of the water well, and these values were used for statistical testing.

A phenotypic tree created using a binomial algorithm to compare community carbon usage base on the type of carbon sources used was created using NTedit V2.0 program. Since the communities displayed well color development for most of the carbon sources, a cut off was set at 0.500 absorbance. Carbon sources with an absorbance value greater than 0.500 were considered utilized and values below 0.500 were considered not used. Additionally, the average of the well color development (absorbance values) were take and compared statistically.

Biphenyl Tolerance Test

Water samples were inoculated into liquid nutrient broth medium, at a ratio of 10 mL sample to 90mL media, for seven days to ensure high growth density. Then the cultures were transferred to liquid broth medium containing biphenyl/methanol solution at concentrations of 0.000 μg/mL, 0.001 μg/mL, or 0.004 μg/mL (control, low, high respectively). Growth recordings were taken at 0, 48 and 96 hours with a spectrophotometer set to 490 nm, and linear regression was used to find the growth rate under each condition.

DNA Extraction

Samples collected before and during the growth curve experiments were selected for DNA extraction. Treatment before DNA extraction included cell lysis with lysozyme and lysis buffer, 37^0 C dry heat, SDS and protein kinase k addition, followed by 60^0 C water bath treatment. After this, sample DNA was extracted with a series of phenol (TRIS buffer pH =8), phenol-chloroform, and phenol-chloroform-isoamyl alcohol extraction phases. DNA was further cleaned by running samples through phase lock tubes and then precipitated out with ethanol,

sodium acetate, and -80^0 C incubation. After incubation, ethanol was removed from samples, and DNA was suspended in TE buffer.

Real-Time Polymerase Chane Reaction (Q-PCR)

Pseudomonas pseudoalcaligenes KF707 was used as a positive control because they are known to contain the *bph* gene set. The set of primers from the *bphA1* sequences were designed from the bipheyl dioxygenase enzyme: BF: TTACTTGGGCACGAGAGTCA, BR: GCACTGGTTCAGGAACACCT (product size: 123 bp). Universal 16S rRNA primers were used as a control and designed from Bacchetti et al. (2011): UF:AAACTCAAAGGAATTGACGG, UR:CTCACGGCACGAGCTGAC. All primers were purchased from Sigma Laboratories. Test were performed on an Illumina model machine with 10 μL per well containing 5 μL *Power*SYBER Green PCR Master Mix (Thermo Fisher Scientific), 0.5 μL RNAase-free water, and 0.25 μL of each primer. Samples were subjected to the following conditions: 2 minutes 50^0 C incubation, 10 minute 95^0 C polymerase activation, 15 second 95^0 C/1 minute 60^0 C cycles for 40 cycles, and 15 second 95^0 C/15 second 55^0 C/15 second 95^0 C melt curve phase. A standard curve was created using *P. pseudoalcaligenes* KF707 DNA diluted serially, and the standard curve was used to quantify the Cq values to ng/μL.

Statistical Testing

IBM SPSS Statistics 25 was used to perform assumption checks and statistic tests. One-way ANOVA was used for the biphenyl tolerance test. Because the Ecoplate data did not meet equal variance assumptions, Kruskal-Wallis test were performed on this data set instead. The phenotypic tree created from the Ecoplate data was derived using NTedit V2.0 program.

Results and Discussion

Properties of Sample Water

Samples included three diverse samples from the Donna Reservoir, and two control samples. The two control samples were chosen from believed PCB free water from both an organic and inorganic water reservoir. At the time of sample collection, the temperature, pH, and salinity were measured with a HANNA meter. The averages for temperature, pH and salinity were 23.4° C, 7.814, and 0.682 ppt (standard deviations: 0.894, 0503, and 0.480; standard error: 0.400, 0.225, 0.215) respectively. As expected, the samples from Donna (D.org, D.mix, and D.con) had similar temperature, pH, and salinity (Table 2.2). The pH of the controls was similar to each other, but lower than the Donna Reservoir samples. Additionally, C.org has a higher salinity level than the other samples. This could be due to low water levels at the access point. Sample C.tap had very low salinity levels because it was sourced from deionized tap water. Additionally, chemical treatments of water samples is not known. These factors could impact the bacteria community composition.

Table 2.2 Properties of Samples

	Coordinates		Temperature	pH	Salinity (ppt)
D.org	N26°0.689	W098°04.306	24° C	8.13	0.64
D.mix	N26°06.090	W098°04.289	24° C	8.20	0.64
D.con	N26°06.090	W098°04.289	23° C	8.21	0.64
C.org	N26°747	W097°56.345	24° C	7.23	1.42
C.tap	-	-	22° C	7.30	0.07
Average			23.4°C	7.814	0.682
Standard Deviation			0.894	0.503	0.480
Standard Error			0.400	0.225	0.215

In addition to the physical properties measured, the samples differed in the amount of plant life surrounding the sample site. D.org and C.org had high surrounding plant life; D.mix was from a biofilm between a concrete bridge and disturbance tolerant plants. D.con was from a concrete lined reservoir, and C.tap is highly filtered and treated.

Carbon Metabolism

The data from the ecoplates describes two concepts: what type of carbon source does the community prefer to metabolize and how much of a carbon source can the community

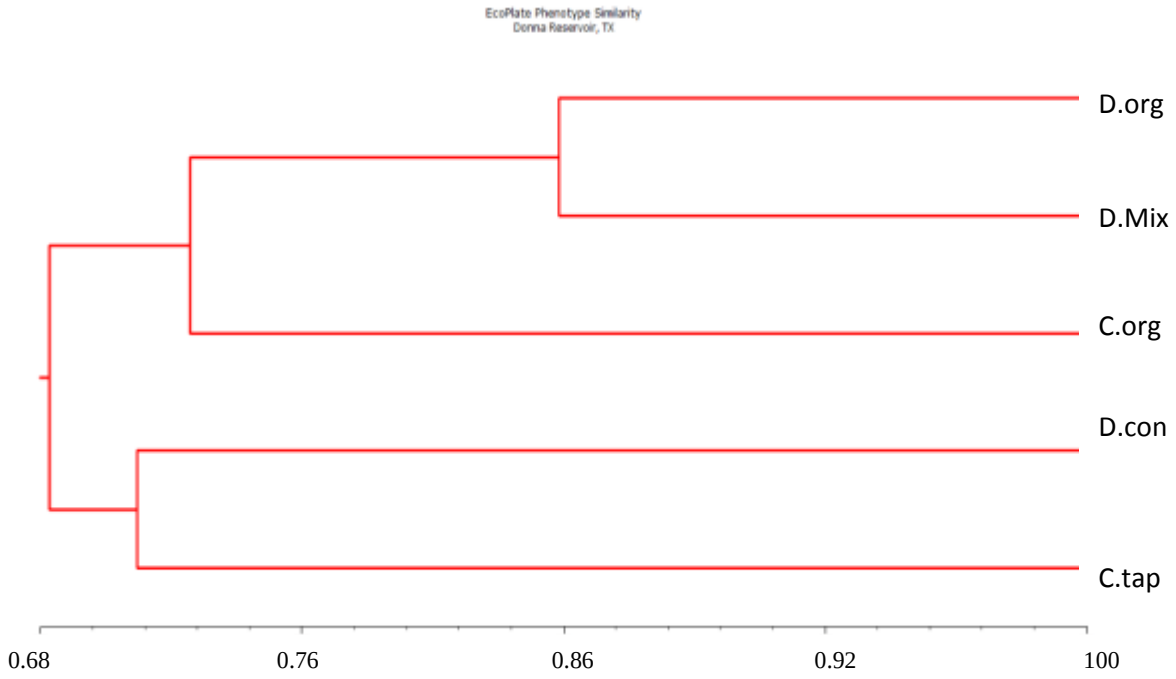

Figure 2.4 Ecoplate Phenotypic Tree Based on the pattern of the Ecoplates, D.org and D.mix are 86% similar, and D.org, D.mix, and C.org are 72% similar. D.con and C.tap are approximately 70.8% percent similar, and all the groups are 68% similar.

metabolize.

The phenotypic tree compares what metabolites were used among the samples. To create the tree, a binary fingerprint of the Ecoplate was created by categorizing less than or equal to

0.500 optical density as 0, and greater than 0.500 optical density as 1. 1 indicates a positive test result, or that the organisms use the carbon source, and 0 suggests that the organisms do not prefer/use the carbon source. Based on the order and frequency of the 1s and 0s, a program determines if the tests are similar or not and determines the coefficient of similarity (Figure 2.4). Where the tree branches split, the test samples are similar to the degree of the coefficient of similarity. D.org and D.mix branch at 0.86, suggesting that their Ecoplate results were 86% similar. D.org and D.mix branch from C.org at 0.72 (72% similarity), and D.con and C.tap branch at approximately 0.708 (70.8% similarity). The two groups (D.org, D.mix, C.org, and D.con, C.tap) branch at 0.68, indicating that all the samples have 68% similarity in their preference for carbon sources despite their geographic source.

Based on this analysis, D.org and D.mix have a similar community level physiological profile. This is expected because they are from the same body of water and have some plant exposure. These two samples are also grouped with C.org, and D.con and C.tap were clustered together in another group. Organic habitat factors may have an influence on the type of carbon sources preferred. Ruiz-Gonzales et al. (2015) present data that suggests that dissolved organic matter (DOM) is a predictor of function with Ecoplates, for this reason it was anticipated that the samples with plant and soil exposure would have high AWCD. The AWCDs indicate how much of a substance the community can potentially use.

High AWCD with high DOM was seen in the the control samples, C.org and C.tap. However, opposing results were shown in the samples from the Donna Reservoir samples. The AWCDs were: 0.481 (D.org), 0.511 (D.mix), 0.729 (D.con), 0.726 (C.org), 0.510 (C.tap) (Table 2.5). A Kruskal Wallis (KW) test showed that the averages of the optical densities are statistically different ($H=31.04$; $\alpha=0.05$) (Figure B1). The completely concrete sample (D.con)

had the greatest AWCD of the Donna Reservoir samples, followed by D.mix and D.org respectively. Post hoc comparisons showed that the AWCDs of D.org, D.mix and C.tap were significantly less from D.mix and C.org. One explanation is that D.org and D.mix are exposed to a higher variety of nutrients because they are near organic matter. This would reduce the need to be specialized in one type of carbon source. Since D.con has a habitat that has limited nutrient exposure, this community needs to have a high rate of metabolism of whatever nutrients to enter the community.

After organizing the substrates into amine, amino acid, carbohydrate, carboxylic acids, polymers, and phenolic compounds, KW test showed that there are significant differences in the optical densities based on carbon source type ($H=40.78$; $\alpha=0.05$) (Figure B3). When the test were performed on individual samples: D.org had significantly greater use of polymers than amino acids ($H=0.006$) and carboxylic acids ($H=0.014$) (Figure B5), D.mix use of polymers was greater than amino acids ($H=0.028$) (Figure B7), and D.con had greater metabolism of amines than carboxylic acids ($H=0.006$)(Figure B9). C.org KW test suggested significant differences ($H=0.02$), but pairwise comparisons did not detect any differences (Figure B10). C.tap had no significant differences among carbon source types ($H=0.668$) (Figure B12). It was expected that there would be a high rate of metabolism of carbohydrates, amino acids, and carboxylic acids because these compounds require little energy to break down. This trend was seen in water samples from Rouge River in Michigan, another area with PCB pollution (Tiquia et al., 2008). However, the trend was not seen in the samples collected (Figure 2.5). It is possible that the bacteria were able to gain more energy from the polymers and amines than the other carbon source types.

All samples had a strong preference for Putrescine (amine), Tween 40, and Tween 80 (polymers) (Table 2.3 and 2.4) and low utilization of L-Theronine, Glycyl-L-Glutamic Acid (amino acids), α-Ketobutyric Acid, and γ-hydroxybutyric acid (carboxylic acids) (Table 2.3 and 2.5). In *Escherichia coli*, putrescine has a high affinity for RNA, and likely has strong roles in gene translation, cell proliferation, and oxygenic detoxification (Igarashi & Kashiwagi, 2000; Wunderlichová et al., 2014). Tween 80 and 40 are man-made surfactants commonly used in food, pharmaceutical, and cosmetic products. Other studies show that Tween 80 can promote cell and biofilm growth, enhance mobility and swarming, and increase lipid production (Nielsen et al., 2016; Niu et al., 2005; Taoka et al., 2011).

Dzantor et al. (2002) found that samples from a PCB inoculated soil struggled to metabolize ecoplate plant exudates (2-hydroxy benzoic acid, 4-hydroxy benzoic acid, D-Xylose, L-Asparagine). D.org and D.mix had low AWCD for 2-hydroxy benzoic acid, D-Xylose, and L-Asparagine. D.con exceled in metabolizing all of plant exudates. D.con was also able to metabolize all but one ringed structure well. C.org ranked next (11/14) followed by D.mix (9/14), D.org (8/14), and C.tap (6/14). If the community can degrade ring structures, it is likely that they can break down PCBs.

Table 2.3 Ecoplate Optical Density Comparison

Supplement	Type	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Phenylethyl-amine	Amine	0.021	0.541	1.367	0.209	0.260
Putrescine	Amine	1.131	0.831	1.021	1.037	1.023
L-Arginine	Amino Acid	0.342	0.268	0.984	1.224	0.503
L-Asparagine	Amino Acid	0.475	0.393	0.929	1.290	1.228
L-Phenylalanine	Amino Acid	0.036	0.050	0.675	0.245	0.440
L-Serine	Amino Acid	0.602	0.560	0.936	1.210	0.574
L-Theronine	Amino Acid	0.000	0.048	0.439	0.327	0.070
Glycyl-L-Glutamic Acid	Amino Acid	0.033	0.201	0.158	0.373	0.130
D-Mannitol	Carbohydrate	0.999	0.990	1.288	1.402	0.732
D-Xylose	Carbohydrate	0.182	0.177	0.620	0.357	0.403
i-erythritol	Carbohydrate	0.095	0.173	0.110	0.521	0.016
n-acetyl-d-glucosamine	Carbohydrate	0.978	0.711	0.565	1.151	0.653
α-D-Lactose	Carbohydrate	0.323	0.440	0.359	0.548	0.053
β Methyl D glucoside	Carbohydrate	0.796	0.657	0.630	1.031	0.706
D-Cellobiose	Carbohydrate	0.784	0.918	1.288	1.382	0.450
D,L-α Glycerol Phosphate	Carbohydrate	0.089	0.208	0.348	0.321	0.273
Glucose-1-phosphate	Carbohydrate	0.197	1.062	0.531	0.846	0.665
D-Galactonic acid γ-lactone	Carbohydrate	0.728	0.540	0.852	0.866	0.949
D-Galaturonic Acid	Carboxylic Acid	0.389	0.648	0.432	0.935	0.627
D-Glucosaminic Acid	Carboxylic Acid	0.453	0.200	0.529	0.396	0.568
D-Malic Acid	Carboxylic Acid	0.320	0.462	1.079	0.492	0.955
Itaconic Acid	Carboxylic Acid	0.159	0.095	0.409	0.751	0.307
α-Ketobutyric Acid	Carboxylic Acid	0.000	0.073	0.419	0.204	0.042
γ-hydroxybutyric acid	Carboxylic Acid	0.000	0.089	0.080	0.394	0.194
Pyruvic acid methyl ester	Carboxylic Acid	0.700	0.761	0.875	0.770	0.587
2-hydroxy benzoic acid	Phenolic compound	0.000	0.000	0.754	0.000	0.064
4-hydroxy benzoic acid	Phenolic compound	0.776	1.153	1.150	0.737	0.450
Glycogen	Polymer	1.256	0.925	1.146	1.115	0.108
Tween 40	Polymer	0.994	0.940	1.009	0.808	1.126
Tween 80	Polymer	1.559	1.218	0.897	0.846	1.138
α-cyclodextrin	Polymer	0.169	0.114	0.578	1.047	0.044
AWDC		0.481[b]	0.511[b]	0.729[a]	0.726[a]	0.510b

a,b statistically significant groups

KEY

0.00-0.29	0.30-0.49	0.50-0.79	0.80-1.19	1.20-1.59

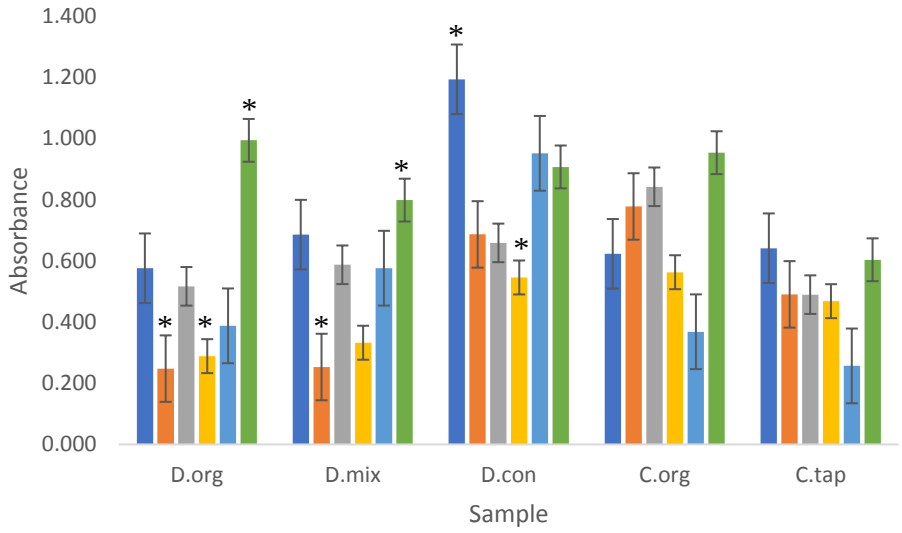

Figure 2.5 AWCD by Carbon Source Type D.org and D.mix have high use of polymers. D.con shows high absorbance for amines. C.org and C.tap displayed no preference. *significantly different

Table 2.4 Sample Substrates with Highest Optical Densities

Sample	Substrate
D.org	Tween 80, Glycogen, Putrescine, D-Mannitol, Tween 40
D.mix	Tween 80, 4-hydroxy benzoic acid, Glucose-1-phosphate, D-Mannitol, Tween 40
D.con	Phenylethyl-amine, Glycyl-L-Glutamic Acid, D-Cellobiose, 4-hydroxy benzoic acid, Glycogen
C.org	D-Mannitol, D-Cellobiose, L-Asparagine, L-Arginine, L-Serine
C.tap	L-Asparagine, Tween 80, Tween 40, Putrescine, D-Malic Acid

Table 2.5 Sample Substrates with Lowest Optical Densities

Sample	Substrate
D.org	L-Theronine, γ-hydroxybutyric acid, 2-hydroxy benzoic acid, α-Ketobutyric Acid, Phenylethyl-amine
D.mix	2-hydroxy benzoic acid, L-Theronine, α-Ketobutyric Acid, γ-hydroxybutyric acid, Itaconic Acid
D.con	γ-hydroxybutyric acid, i-erythritol, L-Theronine, D,L-α Glycerol Phosphate, α-D-Lactose
C.org	2-hydroxy benzoic acid, α-Ketobutyric Acid, Phenylethyl-amine, L-Phenylalanine, L-Theronine
C.tap	i-erythritol, α-Ketobutyric Acid, α-cyclodextrin, α-D-Lactose, 2-hydroxy benzoic acid

Biphenyl Tolerance

The first test conducted on the growth data was a one-way ANOVA among the samples inclusive of all treatments. The results showed that there is a significant difference ($p<0.0001$; $\alpha=0.05$) among the groups total growth (Table B3). A LSD multiple comparison test created three groups that were significantly different: samples D.org and C.org, D.mix and D.con and C.tap was a lone outlier (Table B4).

Another ANOVA was performed to test for differences among treatment groups. The control ($p<0.0001$; Table B5), low ($p<0.0001$; Table B7), and high treatments ($p<0.0001$; Table B9) all showed significant differences among the groups. When the multiple comparison test were analyzed, the control (Table B6) and the low treatments (Table B8) showed the same grouping trend as the previous ANOVA test: D.org and C.org were groups as not significantly different from each other, as were D.mix and D.con; C.tap was not similar to the other samples. However, in the high treatment (Table B10), D.org, D.mix, D.con, and C.org were all grouped significantly different from C.tap. Based on the sample optical means (Table 2.6), samples D.org and C.org grew better than all the treatments than the other samples, followed by samples D.mix

and D.con. Sample C.tap had the lowest average optical density over time in all treatments. DOM exposure may have a positive effect in average growth when exposed to methanol and biphenyl.

Table 2.6 Mean Average Optical Density in Biphenyl Treatments

Sample	Control	Low	High
D.org	0.110[a]	0.149[a]	0.118[a]
D.mix	0.083[a]	0.096 [a]	0.098 [a]
D.con	0.081 [a]	0.095 [b]	0.098 [b]
C.org	0.111[a]	0.148[a]	0.112[a]
C.tap	0.052[a]	0.066 [b]	0.058 [a]

a, b, c statistically significant groups within treatment group

When the individual samples were tested for significant differences due to the treatment, samples D.org, D.mix, and C.org showed no statistical impact (p=0.340, 0.165, 0.246 respectively) due to biphenyl treatments (Table B11, B12 and B15 respectively). However, samples D.con (Table B13, B14) showed a significantly improved average growth with the biphenyl supplements compared to the control (p_{low}=0.029, p_{high}=0.039), and C.tap had more growth with the low treatment (p=0.009) than the control (Table B16, B17). All samples showed an increased average growth from control to low treatment, but that value decrease when comparing the high treatment samples (Table 2.6). Samples with DOM exposure were not affected by the biphenyl supplements while samples with low DOM exposure showed increased average growth with biphenyl supplements.

The growth rate (Table 2.7) show a similar trend as the average growth. Samples D.org, D.mix, D.con, and C.org show and increased growth rate from control to low treatment, but the rate decreases with high biphenyl supplements. There is a larger decrease in growth rate in

D.org, D.mix, and C.org than in D.con as biphenyl concentrations increase. In C.tap, the rate of growth increases with increased biphenyl concentrations.

Table 2.7 Rate of Growth in Treatment groups

	Control	Low	High
D.org	0.0004	0.0014	0.0008
D.mix	0.0001	0.0002	6E-05x
D.con	5E-06	0.0002	0.0001

The groups created by the ANOVA tests are clearly shown when absorbance values were plotted against time (Figure 2.6). The samples from the Donna Reservoir and Delta Lake are tolerant to biphenyl concentrations equal to the PCB concentrations in the reservoir sediment. Because C.tap is highly filtered and chemically treated, the low growth rates could be due to low biodiversity and microbial populations. Contrarily, the samples at Delta Lake (C.org) likely have high biodiversity and microbial populations because the sample location had high plant life and low pollution. This could be why C.org grew as well as or better the samples from the Donna Reservoir.

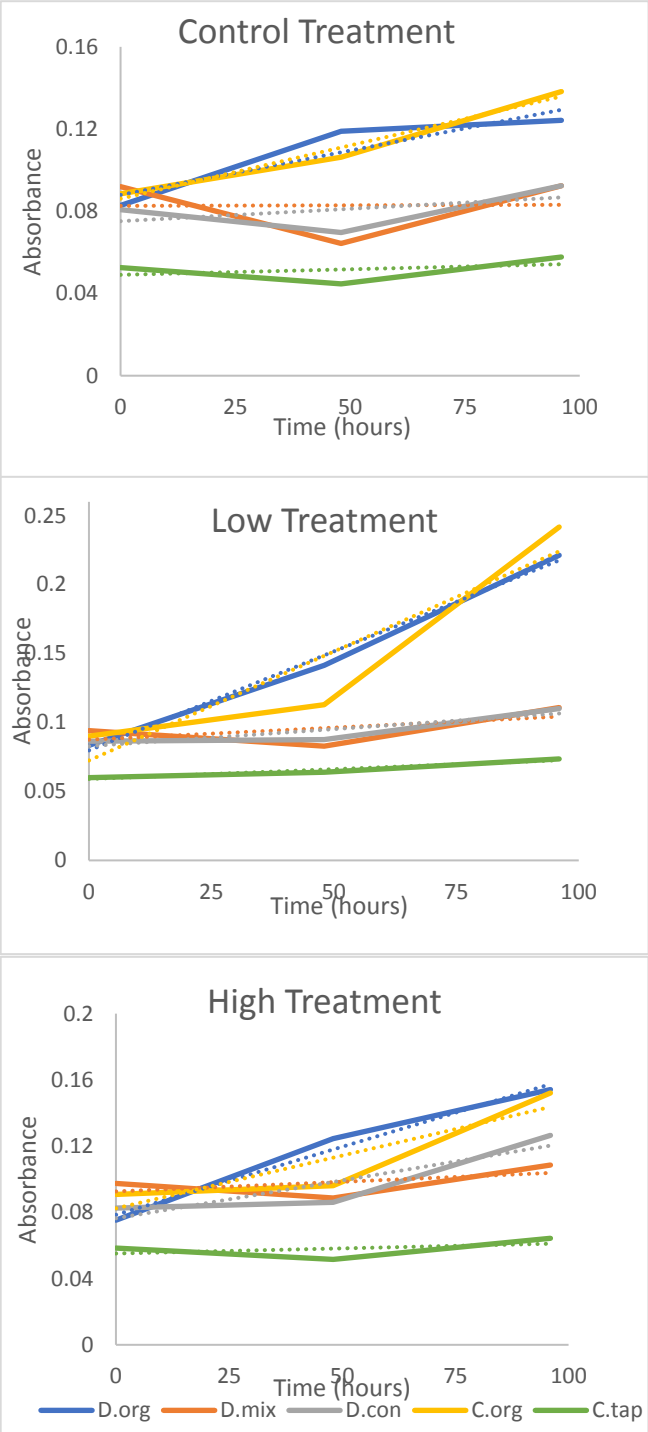

Figure 2.6 Biphenyl Tolerance Average Growth Rates *Control Treatment* shows D.org and C.org with similar rates (0.0004, 0.0005), and D.mix and D.con (0.0001, 5E-06), with C.tap at the lowest (5E-05). *Low Treatment* displays a similar trend with D.org and C.org (0.0014, 0.0016) grouped, D.mix and D.con (0.0002) paired and C.tap the lowest (0.0001). *High Treatment* groups D.org, C.org, D.con, and D.mix (0.0008, 0.0006, 0.0005, 0.0001) away from C.tap (6E-05)0.

Quantification of bphA1

The *bphA1* gene was detected in D.mix before culturing with biphenyl, and in D.con after 96 hours of culturing with 0.000 µg/mL biphenyl/methanol solution. Samples D.org, D.mix,

C.org, and C.tap cultured 96 hours with 0.000 μg/mL biphenyl/methanol (Table A7) struggled to amplify with both the 16S universal rRNA primers and *bphA1* primers. Another set of samples from the same locations but collected four months prior were also tested for the presence of the *bphA1* gene. Only the samples before culturing were tested from this data set. D.con and C.tap tested positive for the *bphA1* (Figure 2.7). The *bphA1* Cq values of D.mix, D.con, C.tap were converted to ng/μL of DNA (Table 2.8) using the linear equation from the standard curve (Figure A2). The *bphA1* gene was found in higher concentration in D.con in October than after culturing in February. Also, the gene was detected in D.mix in February but not October. This data suggests that there are organisms in the Donna Reservoir that carry the *bphA1* gene, and ring structures such as biphenyl are not necessary to select for the gene pathway. Additionally, there may be a seasonality effect on the growth of organisms with the bphA1 gene.

Table 2.8 Conversion of *bphA1* Cq to DNA (ng/μL)

Sample	Sample Time	Cq	DNA (ng/μL)
D.con	Oct	33.19685	1.559849
C.tap	Oct	35.19773	1.212142
D.mix	Feb	35.12964	1.223975
D.con	Feb	38.13054	0.702486
Control	-	19.73338	3.899491

Because the samples are tolerant to methanol, it is possible that methylotrophs are carrying the *bphA1* gene. While common methlyogen species are not common PCB degraders (Bratina et al., 1992), the *bph* genes can behave like conjugate transposons (Nishi et al., 2000). The gene was not detected in D.org nor C.org. However, previous studies suggest that plants and bacteria work symbiotically to degrade PCBs. Flavonoids, terpenes, and salicylic acids have shown to activate the *bph* pathways just as well, if not better than biphenyl (Jha et a., 2014; Zoradova-Murinova et al., 2012). It is possible that the *bphA1* gene was not present in this

microcosm because it was not required anymore. D.mix and D.con do not have the plant resources to increase the efficacy of degradation and require the *bphA1* to continue biodegradation of PCBs.

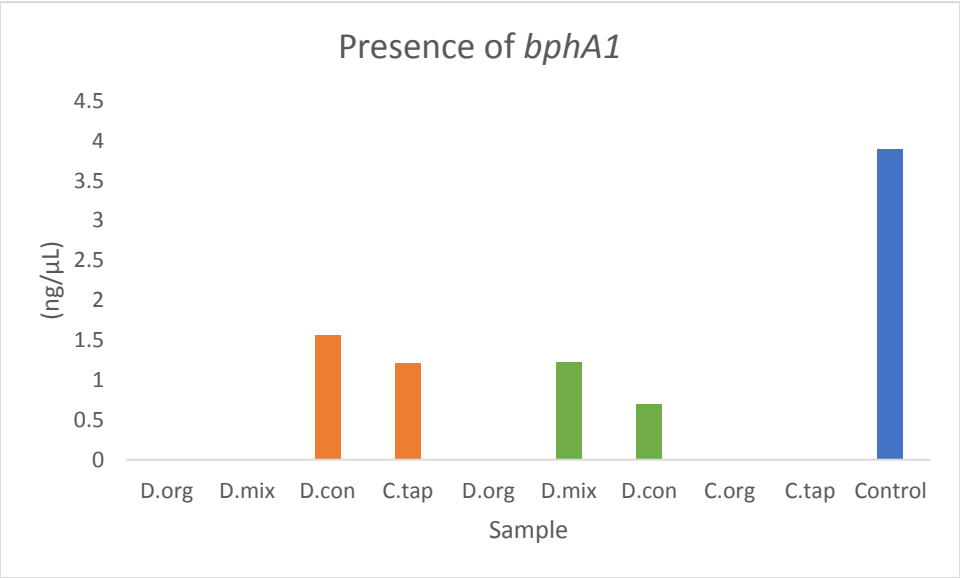

Figure 2.7 Presence of *bphA1* D.con (Feb) was cultured with methanol, then other samples were detected from

Temperature, dissolved oxygen, predation, and resource availability are all other factors that could influence the population of bacteria that carry the *bphA1* gene at a given time. While these tests suggest that the *bphA1* gene is present in the community genome, it does not tell if the gene area actively transcribed. This could be an area of future research for the Donna Reservoir and Irrigation System.

Conclusions

Based on the results from the ecoplates, DOM seemed to explain the type of carbon sources metabolized by the samples because organic samples were grouped away from less organic samples. However, this is not the case for the AWCD since D.con had one of the highest AWCD but one of the lowest assumed DOM exposure. The data suggest that bacteria in the organic canal are generalist while those from the concrete lined canal are specialized. It was expected that there would be high use of amino acids and carboxylic acids, but polymers and

amines were degraded more. It is possible that the molecules were providing more energy and contributing to other important functions for cellular growth.

D.org, D.mix, D.con, and C.org are more tolerant to biphenyl than C.tap because they have higher absorbance values in the biphenyl tolerance tests. These samples likely have more diverse and populated microbial communities than C.tap; biodiversity usually increases resistance to disturbance. The growth in D.org, D.mix and C.org was not affected by increasing biphenyl concentrations, and D.con showed increased growth with increased biphenyl concentrations. All the samples from the 90^0 bend of the Donna Reservoir and Canal System are tolerant to biphenyl concentrations equal to the average PCB concentrations in local sediment.

The *bphA1* gene was detected in D.mix and D.con, but not D.org or C.org. It is possible that symbiotic relationships with plants increased initial biphenyl degradation, reduced the later need for *bphA1* gene, and fewer populations of bacteria in this mesocosm carry the gene. The gene was also unexpectedly detected in C.tap. These results show that the *bphA1* gene is present in *in situ* communities, but further research is needed to determine if the gene is actively transcribed.

While this study focuses on plant exposure as a driver of the results, other elements such as temperature, oxygen concentration, predation, and nutrient availability could also be confounding factors. However, the results from these experiments suggest that some organisms in the Donna Reservoir are tolerant to and capable of degrading PCBs (Table 2.9). Further experiments could include sediment samples, *bph* transcription activity, and longer-term comparisons.

Table 2.9 Summary Table

Sample	Metabolism	Tolerance	*bphA1*
D.org	• Similar preferences to D.mix • Generalized • Polymer preference over amino acids and carboxylic acids	• Highest growth in all supplements • Growth not affected by biphenyl addition	• Not detected
D.mix	• Similar preferences to D.org • Generalized • Polymer preference over amino acids	• Increased growth with biphenyl addition	• Detected in February but not October
D.con	• Least similar preferences to D.org and D.mix • Specialized • Amine preference over carboxylic acids	• Increased growth with biphenyl addition	• Detected in October and in February after cultured in methanol

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CHAPTER I

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CHAPTER II

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APPENDIX A

RAW DATA

To simplify statistical computing, samples D.org, D.mix, D.con, C.org, C.tap are labeled as samples 1, 2, 3, 4, 5, respectively.

Ecoplate Reference Table, Raw, Water Compensated, and Binary Data

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

Figure A1 BIOLOG Ecoplate Carbons Source Layout The location of the well is in the

Table A1. Sample Site 1 BIOLOG Ecoplate Data

Raw Data										Reading time=	5	sec
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.363	1.339	0.762	0.664	0.224	1.103	1.451	0.698	0.196	0.727	0.754	0.444
B	1.030	0.440	0.569	0.700	1.117	0.464	0.740	0.844	0.737	0.424	0.640	0.664
C	1.135	0.321	0.193	0.344	1.310	0.449	0.193	0.296	1.317	0.296	0.174	0.248
D	1.768	0.549	0.873	0.514	1.905	1.926	0.704	0.656	1.787	1.304	1.531	1.419
E	0.557	1.623	0.280	0.239	0.393	1.437	0.228	0.208	0.339	0.654	0.243	0.227

F	1.601	0.976	0.571	0.260	1.330	0.744	0.499	0.340	1.620	0.450	0.190	0.281
G	1.563	0.757	0.217	0.418	0.384	0.255	0.246	0.242	1.188	0.361	0.232	0.187
H	0.251	0.367	0.206	1.391	0.222	0.269	1.004	1.531	1.278	0.413	0.534	1.252
AV.	1.034	0.796	0.459	0.566	0.860	0.831	0.633	0.602	1.058	0.579	0.537	0.590
SD	0.591	0.479	0.271	0.375	0.637	0.607	0.439	0.444	0.578	0.327	0.459	0.487
%CV	57.15	60.19	59.05	66.18	74.02	73.03	69.32	73.82	54.70	56.52	85.52	82.50
Water Compensated												
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.000	0.977	0.399	0.302	0.000	0.879	1.227	0.475	0.000	0.532	0.558	0.248
B	0.667	0.078	0.206	0.338	0.893	0.240	0.517	0.620	0.541	0.228	0.444	0.468
C	0.773	-0.041	-0.170	-0.018	1.087	0.225	-0.031	0.073	1.121	0.100	-0.022	0.052
D	1.406	0.186	0.511	0.152	1.681	1.703	0.481	0.432	1.591	1.108	1.335	1.223
E	0.194	1.260	-0.082	-0.124	0.169	1.214	0.005	-0.015	0.143	0.459	0.047	0.031
F	1.238	0.613	0.208	-0.102	1.106	0.521	0.275	0.116	1.424	0.255	-0.006	0.085
G	1.201	0.394	-0.146	0.055	0.160	0.032	0.022	0.018	0.992	0.165	0.036	-0.009
H	-0.111	0.005	-0.157	1.029	-0.002	0.045	0.780	1.308	1.082	0.217	0.338	1.056
AV.	0.671	0.434	0.096	0.204	0.637	0.607	0.409	0.378	0.862	0.383	0.341	0.394
SD	0.591	0.479	0.271	0.375	0.637	0.607	0.439	0.444	0.578	0.327	0.459	0.487
%CV	88.03	110.50	281.88	183.94	100.01	99.92	107.18	117.46	67.12	85.41	134.57	123.47

Binary	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0	1	0	0	0	1	1	0	0	1	1	0
B	1	0	0	0	1	0	1	1	1	0	0	0
C	1	0	0	0	1	0	0	0	1	0	0	0
D	1	0	1	0	1	1	0	0	1	1	1	1
E	0	1	0	0	0	1	0	0	0	0	0	0
F	1	1	0	0	1	1	0	0	1	0	0	0
G	1	0	0	0	0	0	0	0	1	0	0	0
H	0	0	0	1	0	0	1	1	1	0	0	1

Table A2. Sample Site 2 BIOLOG Ecoplate Data												
Raw Data										Reading time=	5	sec
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.221	0.309	0.345	0.495	0.219	1.132	0.802	0.305	0.199	1.170	1.110	0.669
B	0.986	0.411	0.978	0.540	0.863	0.386	0.942	0.479	1.071	0.372	0.663	0.798
C	1.249	0.320	0.208	0.263	0.919	0.356	0.197	0.287	1.290	0.481	0.173	0.239
D	1.246	1.454	1.382	0.685	1.469	0.668	1.176	0.979	1.577	1.486	1.539	0.653
E	0.412	0.552	0.406	0.320	0.232	1.201	0.237	0.245	0.337	1.018	0.262	0.216
F	1.278	0.272	0.299	0.306	0.668	0.422	0.374	0.340	1.417	0.545	0.252	0.597
G	1.024	1.213	0.288	1.222	0.931	1.480	0.274	0.825	1.436	1.129	0.297	0.213
H	1.248	0.617	0.274	0.996	0.301	0.375	0.309	0.733	0.408	0.272	1.442	1.403
AV.	0.958	0.644	0.523	0.604	0.700	0.753	0.539	0.524	0.967	0.809	0.717	0.598
SD	0.414	0.447	0.424	0.347	0.436	0.451	0.377	0.283	0.562	0.446	0.567	0.400
%CV	43.22	69.49	81.10	57.55	62.28	59.94	70.02	53.94	58.12	55.11	79.12	66.83
Water Compensated												

%Conc.	0	5	10	20	30	40	50	60	70	80	90	100
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.088	0.124	0.274	0.000	0.913	0.584	0.086	0.000	0.971	0.911	0.470
B	0.765	0.190	0.756	0.319	0.645	0.168	0.724	0.261	0.872	0.173	0.464	0.599
C	1.028	0.099	-0.013	0.042	0.700	0.138	-0.021	0.068	1.091	0.282	-0.025	0.040
D	1.024	1.233	1.161	0.464	1.250	0.450	0.957	0.761	1.379	1.287	1.340	0.454
E	0.191	0.331	0.185	0.099	0.013	0.982	0.018	0.027	0.138	0.820	0.063	0.017
F	1.057	0.051	0.078	0.084	0.449	0.204	0.155	0.121	1.218	0.346	0.053	0.398
G	0.803	0.992	0.067	1.001	0.713	1.262	0.055	0.607	1.237	0.931	0.098	0.014
H	1.027	0.396	0.053	0.775	0.083	0.156	0.090	0.515	0.209	0.073	1.243	1.204
AV.	0.737	0.422	0.301	0.382	0.482	0.534	0.320	0.306	0.768	0.610	0.518	0.400
SD	0.414	0.447	0.424	0.347	0.436	0.451	0.377	0.283	0.562	0.446	0.567	0.400
%CV	56.19	105.86	140.59	90.82	90.53	84.46	117.79	92.50	73.16	73.05	109.44	100.06
Binary	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	1	1	0	0	1	1	0
B	1	0	1	0	1	0	1	0	1	0	0	1
C	1	0	0	0	1	0	0	0	1	0	0	0
D	1	1	1	0	1	0	1	1	1	1	1	0
E	0	0	0	0	0	1	0	0	0	1	0	0
F	1	0	0	0	0	0	0	0	1	0	0	0
G	1	1	0	1	1	1	0	1	1	1	0	0
H	1	0	0	1	0	0	0	1	0	0	1	1

Table A3. Sample Site 3 BIOLOG Ecoplate Data

Raw Data										Reading time=	5	sec
%Conc.	0	5	10	20	30	40	50	60	70	80	90	100
	1	2	3	4	5	6	7	8	9	10	11	12

A	0.235	0.976	1.009	0.789	0.215	1.205	0.858	1.822	0.235	1.484	1.373	1.026
B	0.996	0.408	0.609	0.958	1.224	1.193	0.363	1.127	1.020	0.944	1.010	1.385
C	0.660	0.436	0.267	0.495	1.293	0.322	1.300	0.401	1.153	0.258	1.379	0.353
D	1.767	1.717	1.415	2.234	1.374	1.868	1.390	1.486	1.919	0.963	1.331	1.569
E	0.382	1.309	0.340	0.334	0.517	0.576	0.374	0.356	0.582	1.311	0.288	0.438
F	1.321	0.478	1.062	0.312	1.213	1.451	0.281	0.371	1.587	0.344	0.568	0.475
G	1.136	0.445	0.256	1.956	1.606	0.961	0.227	1.576	1.807	0.872	0.230	1.754
H	0.396	0.580	1.347	1.457	1.110	0.482	1.208	1.337	1.520	0.636	1.366	1.166
AV.	0.862	0.793	0.788	1.067	1.069	1.007	0.750	1.059	1.228	0.851	0.943	1.021
SD	0.535	0.492	0.481	0.740	0.465	0.527	0.495	0.599	0.595	0.429	0.505	0.545
%CV	62.11	62.00	60.99	69.32	43.48	52.34	66.03	56.56	48.47	50.41	53.58	53.36
Water Compensated												
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.000	0.741	0.774	0.554	0.000	0.990	0.643	1.607	0.000	1.250	1.138	0.791
B	0.761	0.173	0.374	0.723	1.009	0.978	0.148	0.912	0.785	0.710	0.775	1.151
C	0.425	0.201	0.032	0.259	1.078	0.107	1.085	0.186	0.918	0.023	1.144	0.118
D	1.532	1.482	1.180	1.999	1.159	1.653	1.175	1.271	1.684	0.729	1.096	1.335
E	0.147	1.074	0.105	0.099	0.302	0.361	0.159	0.142	0.348	1.076	0.054	0.203
F	1.086	0.243	0.827	0.077	0.998	1.236	0.066	0.156	1.353	0.109	0.333	0.240
G	0.901	0.210	0.020	1.721	1.391	0.746	0.013	1.361	1.573	0.637	-0.005	1.520
H	0.161	0.345	1.112	1.221	0.895	0.267	0.993	1.122	1.286	0.401	1.131	0.931

AV.	0.627	0.558	0.553	0.832	0.854	0.792	0.535	0.844	0.993	0.617	0.708	0.786
SD	0.535	0.492	0.481	0.740	0.465	0.527	0.495	0.599	0.595	0.429	0.505	0.545
%CV	85.42	88.11	86.92	88.92	54.43	66.54	92.54	70.95	59.92	69.59	71.33	69.29
Binary	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0	1	1	1	0	1	1	1	0	1	1	1
B	1	0	0	1	1	1	0	1	1	1	1	1
C	0	0	0	0	1	0	1	0	1	0	1	0
D	1	1	1	1	1	1	1	1	1	1	1	1
E	0	1	0	0	0	0	0	0	0	1	0	0
F	1	0	1	0	1	1	0	0	1	0	0	0
G	1	0	0	1	1	1	0	1	1	1	0	1
H	0	0	1	1	1	0	1	1	1	0	1	1

Table A4. Sample Site 4 BIOLOG Ecoplate Data

Raw Data										Reading time=	5	sec
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.252	1.445	1.068	0.994	0.286	1.301	1.183	1.279	0.264	1.149	1.149	2.201
B	0.890	0.773	1.279	1.498	1.036	0.573	1.266	1.646	1.184	0.526	1.063	1.528
C	1.063	0.564	0.265	0.687	0.811	0.794	0.224	0.454	1.353	1.006	0.064	0.396
D	1.175	1.621	0.944	1.177	1.120	1.803	0.449	1.355	1.043	1.585	1.620	1.899
E	1.411	1.356	0.720	0.464	1.533	1.573	0.641	0.673	0.998	1.327	0.624	0.648
F	1.169	0.727	0.719	0.892	1.654	0.620	0.609	0.410	1.324	0.643	1.729	0.619
G	1.683	1.155	0.355	0.350	1.605	1.208	0.659	0.887	1.660	0.977	0.400	0.194
H	0.722	0.603	1.028	1.312	1.043	0.551	0.696	1.517	0.683	0.611	0.554	1.084
AV.	1.046	1.031	0.797	0.922	1.136	1.053	0.716	1.028	1.064	0.978	0.900	1.071
SD	0.436	0.414	0.353	0.405	0.462	0.486	0.349	0.486	0.433	0.373	0.590	0.735

%CV	41.71	40.21	44.27	43.92	40.66	46.18	48.81	47.25	40.68	38.10	65.55	68.60
Water Compensated												
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.000	1.193	0.816	0.742	0.000	1.016	0.897	0.993	0.000	0.884	0.884	1.936
B	0.638	0.521	1.027	1.246	0.751	0.287	0.980	1.361	0.920	0.262	0.798	1.264
C	0.810	0.312	0.012	0.435	0.526	0.509	-0.062	0.168	1.089	0.741	-0.200	0.132
D	0.923	1.369	0.692	0.925	0.835	1.518	0.164	1.070	0.779	1.320	1.355	1.635
E	1.159	1.104	0.468	0.212	1.248	1.287	0.355	0.387	0.734	1.063	0.360	0.383
F	0.917	0.475	0.467	0.640	1.369	0.335	0.323	0.124	1.060	0.378	1.464	0.354
G	1.431	0.903	0.103	0.098	1.320	0.923	0.374	0.601	1.396	0.712	-0.135	-0.071
H	0.470	0.351	0.776	1.060	0.757	0.266	0.411	1.232	0.418	0.346	0.290	0.820
AV.	0.794	0.779	0.545	0.670	0.851	0.768	0.430	0.742	0.799	0.713	0.636	0.807
SD	0.436	0.414	0.353	0.405	0.462	0.486	0.349	0.486	0.433	0.373	0.590	0.735
%CV	54.95	53.22	64.73	60.45	54.31	63.36	81.20	65.43	54.14	52.22	92.80	91.09
Binary	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0	1	1	1	0	1	1	1	0	1	1	1
B	1	1	1	1	1	0	1	1	1	0	1	1
C	1	0	0	0	1	1	0	0	1	1	0	0
D	1	1	1	1	1	1	0	1	1	1	1	1
E	1	1	0	0	1	1	0	0	1	1	0	0
F	1	0	0	1	1	0	0	0	1	0	1	0
G	1	1	0	0	1	1	0	1	1	1	0	0
H	0	0	1	1	1	0	0	1	0	0	0	1

Table A5. Sample Site 5 BIOLOG Ecoplate Data												
Raw Data										Reading time=	5	sec
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.148	0.813	0.869	0.764	0.140	0.937	1.299	0.538	0.138	0.793	1.104	0.632
B	0.724	0.507	0.678	0.996	0.901	0.520	0.928	1.708	0.564	0.608	0.701	1.405
C	1.477	0.152	0.241	0.695	1.299	0.145	0.204	0.552	1.026	0.176	0.173	0.498
D	1.407	1.111	0.615	0.773	1.056	0.743	0.469	0.530	1.377	0.769	0.691	0.847
E	0.200	0.784	0.379	0.253	0.198	0.920	0.418	0.221	0.159	0.681	0.212	0.164
F	0.260	0.542	0.484	0.266	0.260	0.706	0.505	0.324	0.230	0.881	0.357	0.225
G	0.891	0.782	0.199	0.795	0.681	0.853	0.204	0.235	0.835	0.785	0.148	0.175
H	0.219	0.421	1.312	1.610	0.181	0.386	1.195	0.613	0.184	0.438	0.782	1.273
AV.	0.666	0.639	0.597	0.769	0.589	0.651	0.653	0.590	0.564	0.641	0.521	0.652
SD	0.549	0.294	0.366	0.428	0.456	0.280	0.431	0.477	0.470	0.232	0.349	0.487
%CV	82.51	46.05	61.31	55.69	77.38	43.06	66.03	80.82	83.40	36.24	67.03	74.74
Water Compensated												
%Conc.	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.000	0.665	0.721	0.616	0.000	0.797	1.159	0.398	0.000	0.655	0.966	0.494
B	0.576	0.359	0.530	0.848	0.761	0.380	0.788	1.568	0.425	0.469	0.562	1.267
C	1.330	0.004	0.093	0.548	1.159	0.005	0.064	0.412	0.888	0.038	0.035	0.360
D	1.259	0.963	0.468	0.625	0.916	0.603	0.329	0.390	1.239	0.631	0.552	0.708
E	0.053	0.637	0.231	0.105	0.059	0.780	0.278	0.081	0.021	0.543	0.074	0.025
F	0.112	0.394	0.337	0.118	0.120	0.566	0.365	0.184	0.092	0.743	0.218	0.087

G	0.743	0.634	0.051	0.648	0.541	0.713	0.064	0.095	0.697	0.647	0.010	0.037
H	0.071	0.273	1.165	1.462	0.041	0.246	1.055	0.473	0.046	0.299	0.644	1.135
AV.	0.518	0.491	0.449	0.621	0.450	0.511	0.513	0.450	0.426	0.503	0.383	0.514
SD	0.549	0.294	0.366	0.428	0.456	0.280	0.431	0.477	0.470	0.232	0.349	0.487
%CV	106.02	59.89	81.44	68.92	101.46	54.84	84.04	105.93	110.43	46.18	91.22	94.81
Binary	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0	1	1	1	0	1	1	0	0	1	1	0
B	1	0	1	1	1	0	1	1	0	0	1	1
C	1	0	0	1	1	0	0	0	1	0	0	0
D	1	1	0	1	1	1	0	0	1	1	1	1
E	0	1	0	0	0	1	0	0	0	1	0	0
F	0	0	0	0	0	1	0	0	0	1	0	0
G	1	1	0	1	1	1	0	0	1	1	0	0
H	0	0	1	1	0	0	1	0	0	0	1	1

Biphenyl Tolerance Test Absorbance

Table A6. Biphenyl Tolerance Test Absorbance			
Sample	Treatment	Absorbance	Recording
1	C	0.071	1
1	C	0.062	1
1	C	0.081	1
1	C	0.088	1
1	C	0.1	1
1	C	0.056	1
1	C	0.084	1
1	C	0.146	1
1	C	0.076	1
1	C	0.062	1
1	C	0.083	1
1	C	0.085	1
1	H	0.065	1
1	H	0.058	1
1	H	0.094	1
1	H	0.077	1
1	H	0.073	1
1	H	0.063	1
1	H	0.094	1
1	H	0.077	1
1	H	0.066	1
1	H	0.058	1

1	H	0.101	1
1	H	0.077	1
1	L	0.071	1
1	L	0.068	1
1	L	0.093	1
1	L	0.093	1
1	L	0.073	1
1	L	0.069	1
1	L	0.093	1
1	L	0.102	1
1	L	0.069	1
1	L	0.074	1
1	L	0.13	1
1	L	0.066	1
1	C	0.118	2
1	C	0.119	2
1	C	0.119	2
1	C	0.176	2
1	C	0.188	2
1	C	0.111	2
1	C	0.064	2
1	C	0.123	2
1	C	0.096	2
1	C	0.124	2
1	C	0.081	2
1	C	0.108	2

1	H	0.058	2
1	H	0.123	2
1	H	0.121	2
1	H	0.194	2
1	H	0.119	2
1	H	0.109	2
1	H	0.08	2
1	H	0.169	2
1	H	0.116	2
1	H	0.102	2
1	H	0.111	2
1	H	0.192	2
1	L	0.112	2
1	L	0.115	2
1	L	0.117	2
1	L	0.177	2
1	L	0.043	2
1	L	0.109	2
1	L	0.12	2
1	L	0.167	2
1	L	0.143	2
1	L	0.106	2
1	L	0.303	2
1	L	0.184	2
1	C	0.129	3
1	C	0.135	3

1	C	0.127	3
1	C	0.282	3
1	C	0.106	3
1	C	0.131	3
1	C	0.105	3
1	C	0.112	3
1	C	0.076	3
1	C	0.127	3
1	C	0.097	3
1	C	0.111	3
1	H	0.082	3
1	H	0.145	3
1	H	0.141	3
1	H	0.335	3
1	H	0.101	3
1	H	0.163	3
1	H	0.128	3
1	H	0.265	3
1	H	0.095	3
1	H	0.155	3
1	H	0.107	3
1	H	0.133	3
1	L	0.132	3
1	L	0.129	3
1	L	0.149	3
1	L	0.297	3

1	L	0.037	3
1	L	0.117	3
1	L	0.155	3
1	L	0.286	3
1	L	0.147	3
1	L	0.126	3
1	L	0.864	3
1	L	0.216	3
2	C	0.077	1
2	C	0.143	1
2	C	0.108	1
2	C	0.091	1
2	C	0.108	1
2	C	0.059	1
2	C	0.083	1
2	C	0.074	1
2	C	0.067	1
2	C	0.09	1
2	C	0.114	1
2	C	0.089	1
2	H	0.145	1
2	H	0.078	1
2	H	0.087	1
2	H	0.073	1
2	H	0.112	1
2	H	0.111	1

2	H	0.085	1
2	H	0.094	1
2	H	0.089	1
2	H	0.124	1
2	H	0.093	1
2	H	0.078	1
2	L	0.08	1
2	L	0.119	1
2	L	0.092	1
2	L		1
2	L	0.082	1
2	L	0.103	1
2	L	0.123	1
2	L	0.063	1
2	L	0.077	1
2	L	0.131	1
2	L	0.056	1
2	L	0.109	1
2	C	0.104	2
2	C	0.073	2
2	C	0.078	2
2	C	0.063	2
2	C	0.036	2
2	C	0.043	2
2	C	0.044	2
2	C	0.089	2

2	C	0.033	2
2	C	0.058	2
2	C	0.046	2
2	C	0.105	2
2	H	0.101	2
2	H	0.045	2
2	H	0.059	2
2	H	0.138	2
2	H	0.141	2
2	H	0.044	2
2	H	0.07	2
2	H	0.136	2
2	H	0.099	2
2	H	0.04	2
2	H	0.058	2
2	H	0.133	2
2	L	0.128	2
2	L	0.059	2
2	L	0.028	2
2	L	0.148	2
2	L	0.072	2
2	L	0.044	2
2	L	0.074	2
2	L	0.151	2
2	L	0.036	2
2	L	0.044	2

2	L	0.059	2
2	L	0.151	2
2	C	0.122	3
2	C	0.067	3
2	C	0.163	3
2	C	0.113	3
2	C	0.032	3
2	C	0.051	3
2	C	0.086	3
2	C	0.111	3
2	C	0.028	3
2	C	0.082	3
2	C	0.147	3
2	C	0.107	3
2	H	0.105	3
2	H	0.085	3
2	H	0.13	3
2	H	0.109	3
2	H	0.143	3
2	H	0.07	3
2	H	0.172	3
2	H	0.107	3
2	H	0.036	3
2	H	0.067	3
2	H	0.166	3
2	H	0.113	3

2	L	0.101	3
2	L	0.055	3
2	L	0.1	3
2	L	0.139	3
2	L	0.105	3
2	L	0.076	3
2	L	0.186	3
2	L	0.161	3
2	L	0.1	3
2	L	0.083	3
2	L	0.097	3
2	L	0.127	3
3	C	0.069	1
3	C	0.074	1
3	C	0.089	1
3	C	0.076	1
3	C	0.063	1
3	C	0.052	1
3	C	0.102	1
3	C	0.1	1
3	C	0.069	1
3	C	0.07	1
3	C	0.108	1
3	C	0.098	1
3	H	0.062	1
3	H	0.07	1

3	H	0.114	1
3	H	0.095	1
3	H	0.079	1
3	H	0.089	1
3	H	0.113	1
3	H	0.087	1
3	H	0.074	1
3	H	0.079	1
3	H	0.059	1
3	H	0.07	1
3	L	0.066	1
3	L	0.108	1
3	L	0.074	1
3	L	0.073	1
3	L	0.073	1
3	L	0.095	1
3	L	0.108	1
3	L	0.088	1
3	L	0.064	1
3	L	0.106	1
3	L	0.111	1
3	L	0.071	1
3	C	0.036	2
3	C	0.119	2
3	C	0.058	2
3	C	0.075	2

3	C	0.045	2
3	C	0.086	2
3	C	0.036	2
3	C	0.073	2
3	C	0.14	2
3	C	0.07	2
3	C	0.032	2
3	C	0.066	2
3	H	0.116	2
3	H	0.121	2
3	H	0.066	2
3	H	0.069	2
3	H	0.118	2
3	H	0.065	2
3	H	0.037	2
3	H	0.059	2
3	H	0.128	2
3	H	0.101	2
3	H	0.068	2
3	H	0.084	2
3	L	0.119	2
3	L	0.129	2
3	L	0.056	2
3	L	0.068	2
3	L	0.105	2
3	L	0.063	2

3	L	0.063	2
3	L	0.072	2
3	L	0.16	2
3	L	0.082	2
3	L	0.062	2
3	L	0.072	2
3	C	0.03	3
3	C	0.139	3
3	C	0.068	3
3	C	0.191	3
3	C	0.11	3
3	C	0.137	3
3	C	0.036	3
3	C	0.047	3
3	C	0.126	3
3	C	0.135	3
3	C	0.04	3
3	C	0.05	3
3	H	0.12	3
3	H	0.155	3
3	H	0.065	3
3	H	0.158	3
3	H	0.136	3
3	H	0.128	3
3	H	0.029	3
3	H	0.05	3

3	H	0.151	3
3	H	0.138	3
3	H	0.09	3
3	H	0.298	3
3	L	0.132	3
3	L	0.154	3
3	L	0.074	3
3	L	0.087	3
3	L	0.129	3
3	L	0.124	3
3	L	0.076	3
3	L	0.091	3
3	L	0.162	3
3	L	0.138	3
3	L	0.081	3
3	L	0.073	3
4	C	0.079	1
4	C	0.096	1
4	C	0.066	1
4	C	0.072	1
4	C	0.079	1
4	C	0.096	1
4	C	0.141	1
4	C	0.069	1
4	C	0.089	1
4	C	0.077	1

4	C	0.123	1
4	C	0.075	1
4	H	0.077	1
4	H	0.096	1
4	H	0.118	1
4	H	0.068	1
4	H	0.076	1
4	H	0.083	1
4	H	0.14	1
4	H	0.078	1
4	H	0.069	1
4	H	0.09	1
4	H	0.118	1
4	H	0.077	1
4	L	0.064	1
4	L	0.083	1
4	L	0.106	1
4	L	0.074	1
4	L	0.084	1
4	L	0.094	1
4	L	0.117	1
4	L	0.073	1
4	L	0.056	1
4	L	0.101	1
4	L	0.159	1
4	L	0.07	1

4	C	0.147	2
4	C	0.13	2
4	C	0.094	2
4	C	0.15	2
4	C	0.121	2
4	C	0.099	2
4	C	0.053	2
4	C	0.144	2
4	C	0.038	2
4	C	0.128	2
4	C	0.029	2
4	C	0.142	2
4	H	0.116	2
4	H	0.125	2
4	H	0.084	2
4	H	0.138	2
4	H	0.045	2
4	H	0.062	2
4	H	0.07	2
4	H	0.139	2
4	H	0.093	2
4	H	0.083	2
4	H	0.044	2
4	H	0.155	2
4	L	0.152	2
4	L	0.118	2

4	L	0.103	2
4	L	0.155	2
4	L	0.044	2
4	L	0.076	2
4	L	0.12	2
4	L	0.165	2
4	L	0.116	2
4	L	0.134	2
4	L	0.056	2
4	L	0.117	2
4	C	0.191	3
4	C	0.141	3
4	C	0.105	3
4	C	0.195	3
4	C	0.128	3
4	C	0.136	3
4	C	0.068	3
4	C	0.153	3
4	C	0.096	3
4	C	0.163	3
4	C	0.128	3
4	C	0.155	3
4	H	0.165	3
4	H	0.129	3
4	H	0.097	3
4	H	0.193	3

4	H		3
4	H	0.119	3
4	H	0.217	3
4	H	0.174	3
4	H	0.125	3
4	H	0.112	3
4	H	0.136	3
4	H	0.206	3
4	L	0.146	3
4	L	0.111	3
4	L	0.308	3
4	L	0.233	3
4	L	0.9	3
4	L	0.112	3
4	L	0.209	3
4	L	0.315	3
4	L	0.147	3
4	L	0.129	3
4	L	0.138	3
4	L	0.153	3
5	C	0.039	1
5	C	0.067	1
5	C	0.04	1
5	C	0.029	1
5	C	0.044	1
5	C	0.046	1

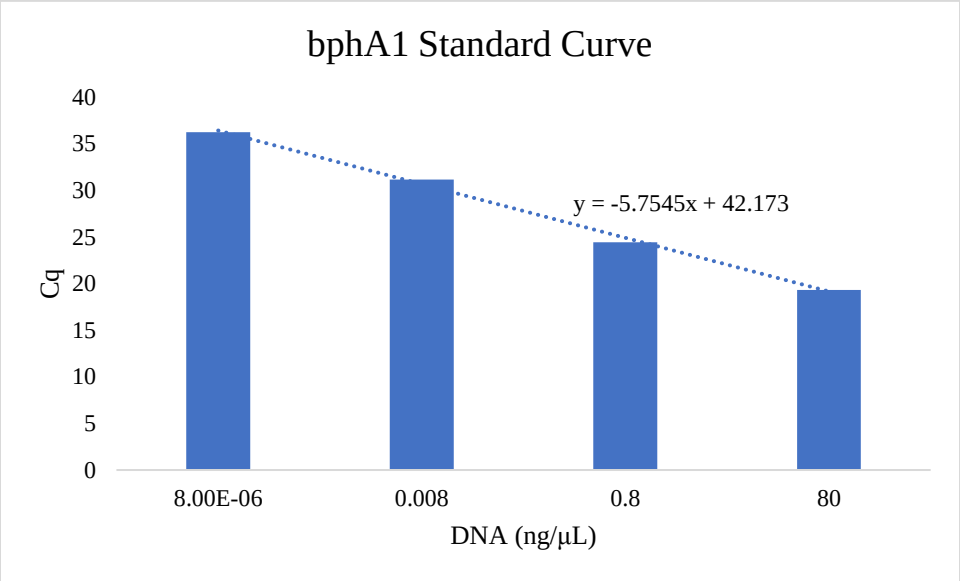
5	C	0.047	1
5	C	0.086	1
5	C	0.059	1
5	C	0.044	1
5	C	0.049	1
5	C	0.081	1
5	H	0.047	1
5	H	0.039	1
5	H	0.091	1
5	H	0.087	1
5	H	0.05	1
5	H	0.041	1
5	H	0.031	1
5	H	0.074	1
5	H	0.046	1
5	H	0.038	1
5	H	0.08	1
5	H	0.077	1
5	L	0.046	1
5	L	0.042	1
5	L	0.093	1
5	L	0.07	1
5	L	0.042	1
5	L	0.045	1
5	L	0.084	1
5	L	0.067	1

5	L	0.054	1
5	L	0.036	1
5	L	0.089	1
5	L	0.052	1
5	C	0.036	2
5	C	0.136	2
5	C	0.022	2
5	C	0.02	2
5	C	0.015	2
5	C	0.046	2
5	C	0.017	2
5	C	0.047	2
5	C	0.077	2
5	C	0.04	2
5	C	0.026	2
5	C	0.054	2
5	H	0.044	2
5	H	0.055	2
5	H	0.069	2
5	H	0.04	2
5	H	0.039	2
5	H	0.037	2
5	H	0.019	2
5	H	0.078	2
5	H	0.069	2
5	H	0.046	2

5	H	0.021	2
5	H	0.102	2
5	L	0.026	2
5	L	0.061	2
5	L	0.1	2
5	L	0.048	2
5	L	0.029	2
5	L	0.027	2
5	L	0.097	2
5	L	0.099	2
5	L	0.065	2
5	L	0.046	2
5	L	0.089	2
5	L	0.081	2
5	C	0.052	3
5	C	0.149	3
5	C	0.068	3
5	C	0.07	3
5	C	0.025	3
5	C	0.065	3
5	C	0.003	3
5	C	0.049	3
5	C	0.096	3
5	C	0.043	3
5	C	0.017	3
5	C	0.056	3

5	H	0.05	3
5	H	0.072	3
5	H	0.086	3
5	H	0.064	3
5	H	0.033	3
5	H	0.037	3
5	H	0.026	3
5	H	0.115	3
5	H	0.093	3
5	H	0.077	3
5	H	0.021	3
5	H	0.098	3
5	L	0.025	3
5	L	0.088	3
5	L	0.065	3
5	L	0.084	3
5	L	0.03	3
5	L	0.052	3
5	L	0.119	3
5	L	0.08	3
5	L	0.086	3
5	L	0.066	3
5	L	0.091	3
5	L	0.097	3

Quantitative Polymerase Chain Reaction Standard Curve and Raw Data



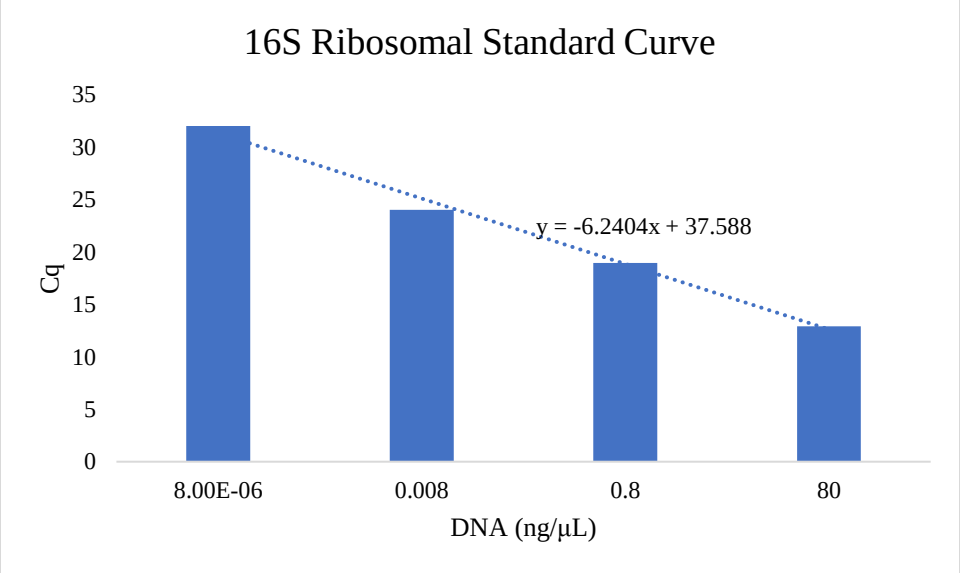


Figure A2 Standard Real-Time PCR Curves (Top) *BphA1* standard curve with linear regression y = 5.7545x + 13.4. (Bottom) 16S rRNA standard curve with linear regression y = 6.2404x + 6.386. DNA (ng/µL) was estimated with a NanoDrop Spectrophotometer.

Table A7. Cq Values of Samples Over Treatments

Primer	Sample Time	Sample	Before Culture	High 0 hours	High 96 hours	Control 96 hours
bph	Feb	C.org	0	0	0	0
bph	Feb	C.org	0	0	0	0
bph	Feb	C.org	0	0	0	0
bph	Feb	C.org	0	0	0	0
bph	Feb	C.tap	0	0	0	0
bph	Feb	C.tap	0	0	0	0
bph	Feb	C.tap	0	0	0	0
bph	Feb	C.tap	0	0	0	0
bph	Feb	D.con	0	0	0	0
bph	Feb	D.con	0	0	0	39.4682
bph	Feb	D.con	0	0	0	37.81457
bph	Feb	D.con	0	0	0	37.92274
bph	Feb	D.con	-	-	-	0
bph	Feb	D.con	-	-	-	0
bph	Feb	D.con	-	-	-	37.31666
bph	Feb	D.mix	35.77806	0	0	0
bph	Feb	D.mix	37.41201	0	0	0
bph	Feb	D.mix	33.84459	0	0	0
bph	Feb	D.mix	33.48388	0	0	0
bph	Feb	D.org	0	0	0	0
bph	Feb	D.org	0	0	0	0
bph	Feb	D.org	0	0	0	0
bph	Feb	D.org	0	0	0	0
bph	Oct	C.tap	35.17177	-	-	-
bph	Oct	C.tap	34.88632	-	-	-
bph	Oct	C.tap	35.5351	-	-	-
bph	Oct	D.con	0	-	-	-
bph	Oct	D.con	33.0434	-	-	-
bph	Oct	D.con	33.3503	-	-	-
bph	Oct	D.mix	0	-	-	-
bph	Oct	D.mix	0	-	-	-
bph	Oct	D.mix	0	-	-	-
bph	Oct	D.org	0	-	-	-
bph	Oct	D.org	0	-	-	-
bph	Oct	D.org	0	-	-	-
uni	Feb	C.org	0	0	28.76434	0
uni	Feb	C.org	29.14521	23.46401	20.01221	0
uni	Feb	C.org	28.39653	24.16661	20.11998	0

uni	Feb	C.org	26.98986	24.60427	24.1665	0
uni	Feb	C.tap	35.71632	32.64962	20.43878	0
uni	Feb	C.tap	21.29373	27.00889	20.98325	0
uni	Feb	C.tap	20.80298	26.61555	20.1876	0
uni	Feb	C.tap	20.8013	39.08338	0	0
uni	Feb	D.con	0	36.72085	38.6854	30.80767
uni	Feb	D.con	29.22715	22.26709	28.56728	21.53095
uni	Feb	D.con	24.09516	20.8943	26.86578	20.4019
uni	Feb	D.con	26.3138	21.16261	28.41351	20.63752
uni	Feb	D.con	-	-	-	22.06697
uni	Feb	D.con	-	-	-	0
uni	Feb	D.con	-	-	-	19.02477
uni	Feb	D.mix		0	0	0
uni	Feb	D.mix	24.4887	0	32.80529	0
uni	Feb	D.mix	20.84502	0	29.9075	0
uni	Feb	D.mix	20.83395	0	34.72334	0
uni	Feb	D.org	27.88869	25.05029	0	0
uni	Feb	D.org	25.63585	24.00209	0	0
uni	Feb	D.org	20.63024	21.91486	0	0
uni	Feb	D.org	20.35858	20.52073	0	0
uni	Oct	C.tap	21.6652	-	-	-
uni	Oct	C.tap	0	-	-	-
uni	Oct	C.tap	35.22092	-	-	-
uni	Oct	D.con	20.60106	-	-	-
uni	Oct	D.con	20.49019	-	-	-
uni	Oct	D.con	20.3289	-	-	-
uni	Oct	D.mix	20.85644	-	-	-
uni	Oct	D.mix	20.02369	-	-	-
uni	Oct	D.mix	21.70855	-	-	-
uni	Oct	D.org	0	-	-	-
uni	Oct	D.org	0	-	-	-
uni	Oct	D.org	0	-	-	-
bph	-	Control	19.82456	24.67506	27.30041	18.01572
bph	-	Control	31.99648	28.13814	14.8858	17.77612
bph	-	Control	18.5383	16.24902	18.50807	18.13046
bph	-	Control	0	0	0	0
bph	-	Control	19.62775	-	-	-
bph	-	Control	19.49643	-	-	-
bph	-	Control	22.57174	-	-	-
uni	-	Control	19.6462	13.80462	16.42591	11.84447
uni	-	Control	12.30466	15.81133	12.07333	11.48686

uni	-	Control	13.45433	12.76085	11.99407	11.39463
uni	-	Control	0	0	0	0
uni	-	Control	14.77123	-	-	-
uni	-	Control	14.7619	-	-	-
uni	-	Control	0	-	-	-

Values of 0 indicate that Cq values are below detection, and – symbols mean that test were not run on that sample of treatment. In addition to the samples collected in February, another set of samples was previously collected in October. This data set was also analyzed to see in the bph gene presence varies by season. Uni stands for 16S universal ribosomal primers and the bph stands for *bphA1* primers.

APPENDIX B

STATISTICAL TABLES

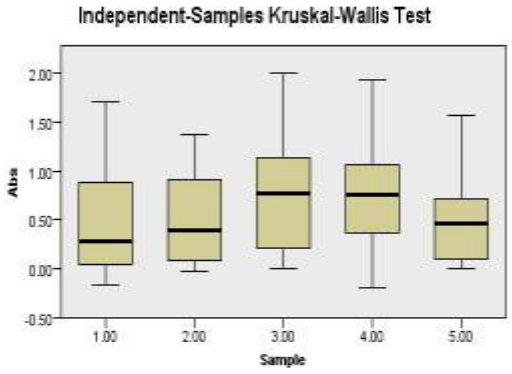
Ecoplate Assumption Tests

Table B1. Ecoplate Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Abosrbance	Based on Mean	4.187	4	476	.002
	Based on Median	2.985	4	476	.019
	Based on Median and with adjusted df	2.985	4	415.515	.019
	Based on trimmed mean	4.147	4	476	.003

ANOVA comparisons could not be performed on the Ecoplate data set because the homoscedastic assumption was not met. Kruskal-Wallis test were performed instead. Because this test does not require normality, that data is not presented here.

Among Samples Ecoplate Kruskal-Wallis and Pairwise Comparison Tests

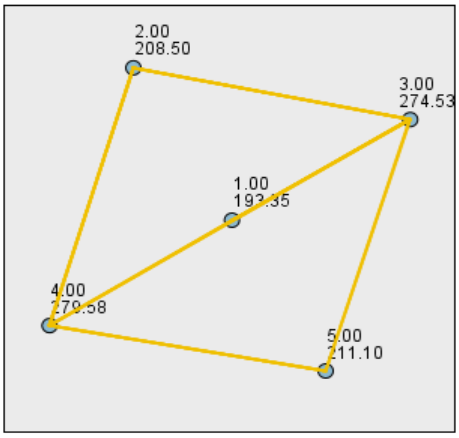


Total N	466
Test Statistic	33.658
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Figure B1. Ecoplate Optical Density versus Sample Group Kruskal Wallis Results (Top) Absorbance versus sample category boxplot. (Bottom) Kruskal Wallis test result table.

Pairwise Comparisons of Sample



Each node shows the sample average rank of Sample.

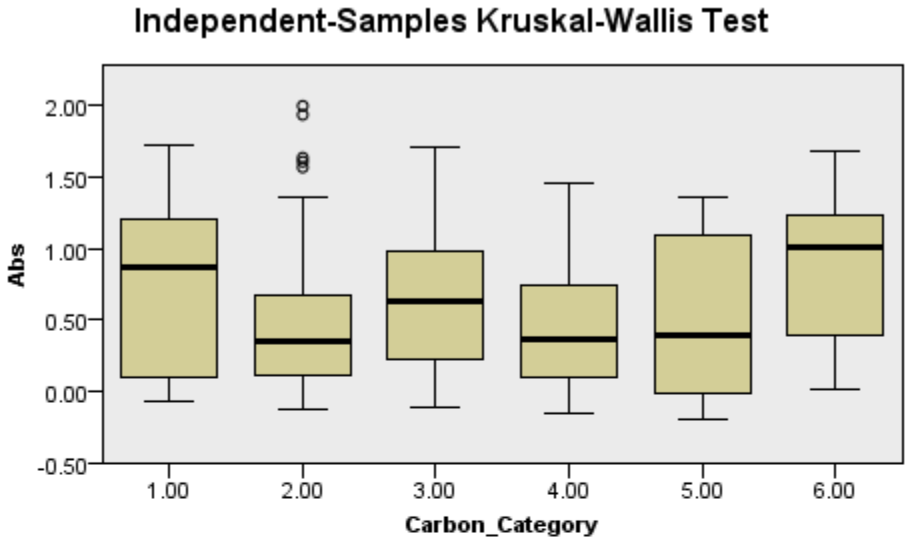
Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
1.00-2.00	-15.151	19.748	-.767	.443	1.000
1.00-5.00	-17.753	19.748	-.899	.369	1.000
1.00-3.00	-81.182	19.696	-4.122	.000	.000
1.00-4.00	-86.226	19.748	-4.366	.000	.000
2.00-5.00	-2.602	19.748	-.132	.895	1.000
2.00-3.00	-66.032	19.696	-3.353	.001	.008
2.00-4.00	-71.075	19.748	-3.599	.000	.003
5.00-3.00	63.430	19.696	3.220	.001	.013
5.00-4.00	68.473	19.748	3.467	.001	.005
3.00-4.00	-5.043	19.696	-.256	.798	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.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure B2. Ecoplate Pairwise Comparison Among Samples (Top) Visual representation of significant differences among sample groups. Connecting lines represent a significant difference and samples that are not connected by lines are not significantly different. (Bottom) D.org, D.mix, and C.tap are significantly different than D.con and C.org.

Among Carbon Sources Ecoplate Kruskal-Wallis and Pairwise Comparison Tests

Carbon sources are coded as such: amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6).

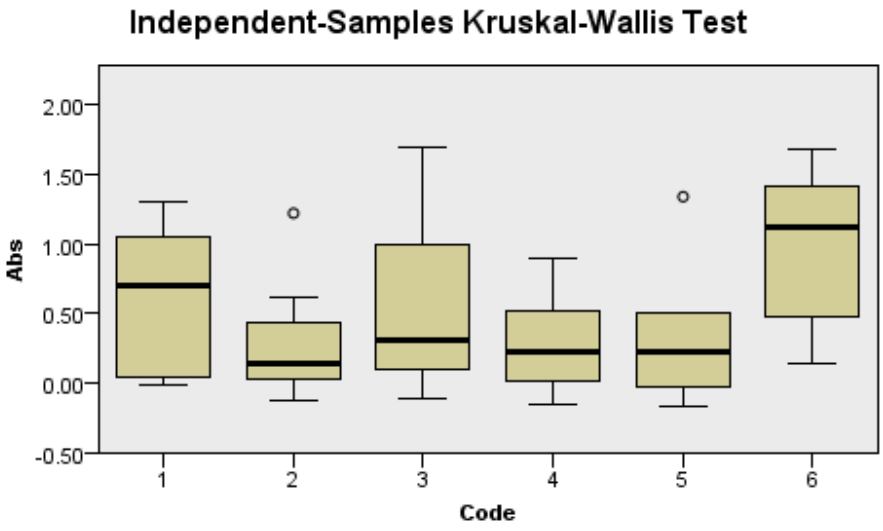


Total N	466
Test Statistic	40.778
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Figure B3. Ecoplate Total Optical Density verus Carbon Source Type Kruskal Wallis Results (Top) Carbon category (amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6) (grouping factor) versus mean optical density box plot. (Bottom) Kruskal Wallis test result table comparing total optical density means by carbon source. There is a significant difference in carbon source type use among samples

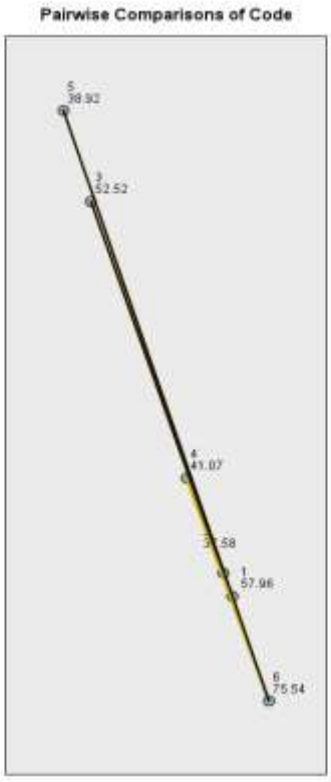
Carbon Source within Sample Ecoplate Kruskal-Wallis and Pairwise Comparison Tests



Total N	99
Test Statistic	16.930
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.005

1. The test statistic is adjusted for ties.

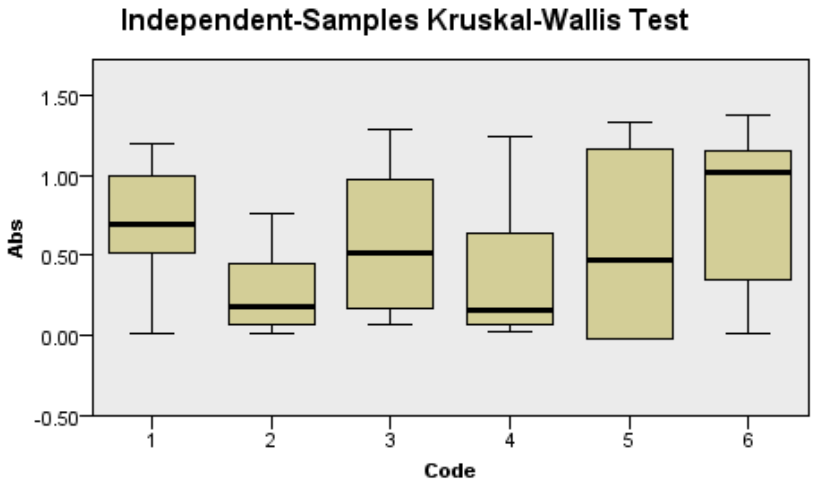
Figure B4. Ecoplate D.org Kruskal Wallis Results (Top) Carbon category (amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6) (grouping factor) versus optical density box plot. (Bottom) Kruskal Wallis test result table comparing D.org optical density means by carbon source. D.org has significant differences among the carbon source types.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
2-5	-1.333	13.539	-.098	.922	1.000
2-4	-3.488	9.225	-.378	.705	1.000
2-3	-14.933	8.563	-1.744	.081	1.000
2-1	20.375	10.703	1.904	.057	.854
2-6	-37.958	10.703	-3.546	.000	.006
5-4	2.155	13.295	.162	.871	1.000
5-3	13.600	12.844	1.059	.290	1.000
5-1	19.042	14.360	1.326	.185	1.000
5-6	-36.625	14.360	-2.550	.011	.161
4-3	11.445	8.171	1.401	.161	1.000
4-1	16.887	10.393	1.625	.104	1.000
4-6	-34.470	10.393	-3.317	.001	.014
3-1	5.442	9.810	.555	.579	1.000
3-6	-23.025	9.810	-2.347	.019	.284
1-6	-17.583	11.725	-1.500	.134	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.
Significance values have been adjusted by the Bonferroni

Figure B5. Ecoplate D.org Pairwise Comparison (Top) Visual representation of significant differences among optical densitycategorized by carbon source type within D.org. Connecting lines represent a significant difference and samples that are not connected by lines are not significantly different. (Bottom) Pairwise comparison table, D.org had significantly greater use of polymers than amino acids (H=0.006) and carboxylic acids (H=0.014).



Total N	93
Test Statistic	16.300
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.006

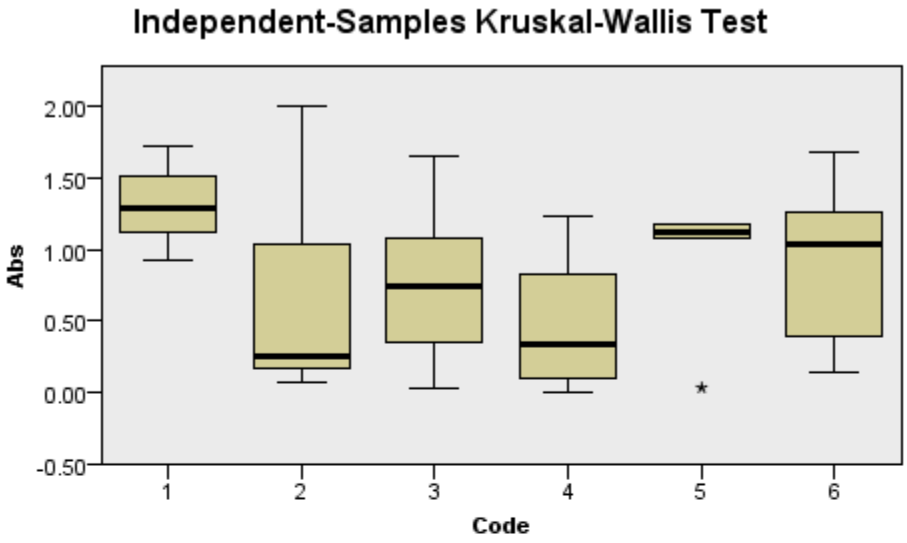
1. The test statistic is adjusted for ties.

Figure B6. Ecoplate D.mix Kruskal Wallis Results (Top) Carbon category (amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6) (grouping factor) versus optical density box plot. (Bottom) Kruskal Wallis test result table comparing D.mix optical density means by carbon source. D.mix has significant differences among the carbon source types.

Sample 1 Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
2-4	-3.278	8.670	-.378	.705	1.000
2-5	-9.111	12.723	-.716	.474	1.000
2-3	-21.211	8.047	-2.636	.008	.126
2-1	24.278	12.723	1.908	.056	.846
2-6	-31.319	10.059	-3.114	.002	.028
4-5	-5.833	12.494	-.467	.641	1.000
4-3	17.933	7.679	2.335	.020	.293
4-1	21.000	12.494	1.681	.093	1.000
4-6	-28.042	9.767	-2.871	.004	.061
5-3	12.100	12.070	1.002	.316	1.000
5-1	15.167	15.583	.973	.330	1.000
5-6	-22.208	13.495	-1.646	.100	1.000
3-1	3.067	12.070	.254	.799	1.000
3-6	-10.108	9.219	-1.096	.273	1.000
1-6	-7.042	13.495	-.522	.602	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.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

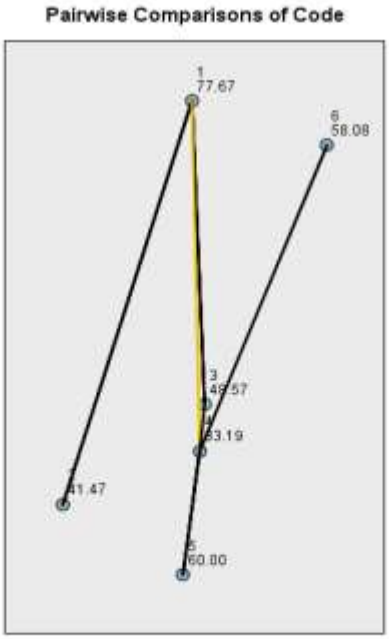
Figure B7. Ecoplate D.mix Pairwise Comparison (Top) Visual representation of significant differences among optical density categorized by carbon source type within D.mix. Connecting lines represent a significant difference and samples that are not connected by lines are not significantly different. (Bottom) Pairwise comparison table, D.mix had significantly greater use of polymers than amino acids (H=0.028).



Total N	94
Test Statistic	17.155
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.004

1. The test statistic is adjusted for ties.

Figure B8. Ecoplate D.con Kruskal Wallis Results (Top) Carbon category (amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6) (grouping factor) versus optical density box plot. (Bottom) Kruskal Wallis test result table comparing D.con optical density means by carbon source. D.com has significant differences among the carbon source types.

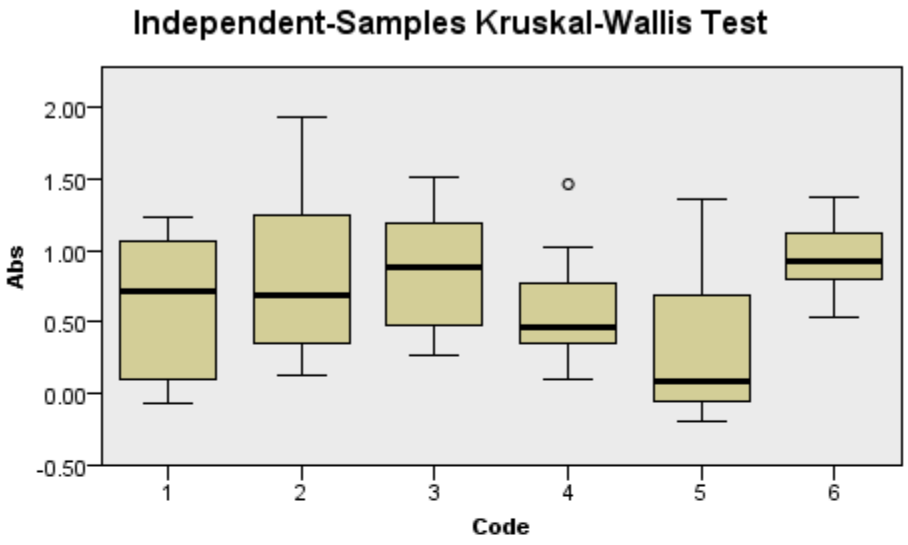


Each node shows the sample average rank of Code.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
4-2	8.283	8.637	.959	.338	1.000
4-3	15.376	7.762	1.981	.048	.714
4-6	-24.893	9.872	-2.522	.012	.175
4-5	-26.810	12.628	-2.123	.034	.506
4-1	44.476	12.628	3.522	.000	.006
2-3	-7.093	7.998	-.887	.375	1.000
2-6	-16.610	10.059	-1.651	.099	1.000
2-5	-18.526	12.775	-1.450	.147	1.000
2-1	36.193	12.775	2.833	.005	.069
3-6	-9.517	9.318	-1.021	.307	1.000
3-5	-11.433	12.200	-.937	.349	1.000
3-1	29.100	12.200	2.385	.017	.256
6-5	1.917	13.640	.141	.888	1.000
6-1	19.583	13.640	1.436	.151	1.000
5-1	17.667	15.750	1.122	.262	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.
Significance values have been adjusted by the Bonferroni

Figure B9. Ecoplate D.con Pairwise Comparison (Top) Visual representation of significant differences among optical density categorized by carbon source type within D.con. Connecting lines represent a significant difference and samples that are not connected by lines are not significantly different. (Bottom) Pairwise comparison table, D.con had significantly greater use of amines than carboxylic acids.

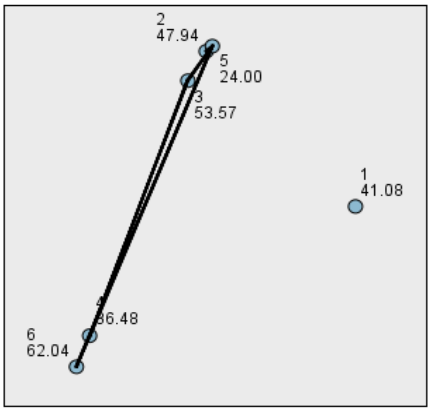


Total N	93
Test Statistic	13.363
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.020

1. The test statistic is adjusted for ties.

Figure B10. Ecoplate C.org Kruskal Wallis Results (Top) Carbon category (amine (1), amino acid (2), carbohydrate (3), carboxylic acids (4), phenolic compounds (5), and polymers (6) (grouping factor) versus optical density box plot. (Bottom) Kruskal Wallis test result table comparing C.org optical density means by carbon source. C.org has significant differences among the carbon source types.

Pairwise Comparisons of Code



Each node shows the sample average rank of Code.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
5-4	12.476	12.494	.999	.318	1.000
5-1	17.083	15.583	1.096	.273	1.000
5-2	23.944	12.723	1.882	.060	.898
5-3	29.567	12.070	2.450	.014	.215
5-6	-38.042	13.495	-2.819	.005	.072
4-1	4.607	12.494	.369	.712	1.000
4-2	11.468	8.669	1.323	.186	1.000
4-3	17.090	7.679	2.226	.026	.391
4-6	-25.565	9.767	-2.618	.009	.133
1-2	-6.861	12.723	-.539	.590	1.000
1-3	-12.483	12.070	-1.034	.301	1.000
1-6	-20.958	13.495	-1.553	.120	1.000
2-3	-5.622	8.047	-.699	.485	1.000
2-6	-14.097	10.059	-1.402	.161	1.000
3-6	-8.475	9.219	-.919	.358	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.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure B11. Ecoplate C.org Pairwise Comparison (Top) Visual representation of significant differences among optical density categorized by carbon source type within D.org. Connecting lines represent a significant difference and samples that are not connected by lines are not significantly different. (Bottom) Pairwise comparison table of C.org data set detects no significant differences after adjustments.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Abs is the same across categories of Code.	Independent-Samples Kruskal-Wallis Test	.667	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Figure B12 Kruskal-Wallis test C.tap No significant differences due to absorbance of carbon source type was detected in this test.

Biphenyl Tolerance Test Assumption Tests

Table B2. Biphenyl Tolerance Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Absorbance_ln	Based on Mean	1.882	4	532	.112
	Based on Median	1.865	4	532	.115
	Based on Median and with adjusted df	1.865	4	498.746	.115
	Based on trimmed mean	1.886	4	532	.111

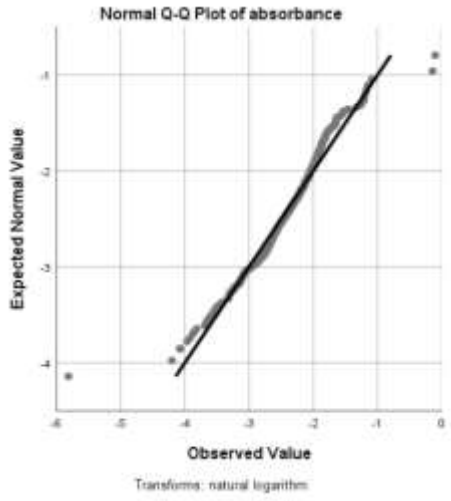


Figure B13. Q-Q Test for Normal Distribution of Growth Curve Optical Density After transforming the data with a natural log transformation the data met normality assumptions

Among Samples Biphenyl Tolerance Test ANOVA and LSD Multiple Comparison Test

Table B3. Biphenyl Tolerance Test ANOVA: Among Samples

Absorbance_ln

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.685	4	10.421	46.214	.000
Within Groups	119.965	532	.225		
Total	161.650	536			

Table B4. Biphenyl Tolerance Test Multiple Comparisons Among Samples

Dependent Variable: ABSORBANCE_LN

LSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.26466*	.06493	.000	.1371	.3922
	3.00	.27677*	.06462	.000	.1498	.4037
	4.00	.01292	.06477	.842	-.1143	.1402
	5.00	.76764*	.06462	.000	.6407	.8946
2.00	1.00	-.26466*	.06493	.000	-.3922	-.1371
	3.00	.01211	.06493	.852	-.1154	.1397
	4.00	-.25174*	.06508	.000	-.3796	-.1239
	5.00	.50298*	.06493	.000	.3754	.6305
3.00	1.00	-.27677*	.06462	.000	-.4037	-.1498
	2.00	-.01211	.06493	.852	-.1397	.1154
	4.00	-.26385*	.06477	.000	-.3911	-.1366
	5.00	.49087*	.06462	.000	.3639	.6178
4.00	1.00	-.01292	.06477	.842	-.1402	.1143
	2.00	.25174*	.06508	.000	.1239	.3796
	3.00	.26385*	.06477	.000	.1366	.3911
	5.00	.75473*	.06477	.000	.6275	.8820
5.00	1.00	-.76764*	.06462	.000	-.8946	-.6407
	2.00	-.50298*	.06493	.000	-.6305	-.3754
	3.00	-.49087*	.06462	.000	-.6178	-.3639
	4.00	-.75473*	.06477	.000	-.8820	-.6275

*. The mean difference is significant at the 0.05 level.

Among Treatment Groups Biphenyl Tolerance Test ANOVA and LSD Multiple Comparisons Test

Table B5. Biphenyl Tolerance Tests ANOVA: Control Treament

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.884	4	4.721	19.180	.000
Within Groups	42.828	174	.246		
Total	61.712	178			

Table B6. Biphenyl Tolerance Tests Multiple Comparisons: Control Treatment

Dependent Variable: ln_abs

LSD

					95% Confidence Interval	
(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1.00	2.00	.31513*	.11777	.008	.0827	.5476
	3.00	.35467*	.11694	.003	.1239	.5855
	4.00	.01095	.11694	.925	-.2198	.2418
	5.00	.89060*	.11694	.000	.6598	1.1214
2.00	1.00	-.31513*	.11777	.008	-.5476	-.0827
	3.00	.03954	.11777	.737	-.1929	.2720
	4.00	-.30418*	.11777	.011	-.5366	-.0717
	5.00	.57546*	.11777	.000	.3430	.8079
3.00	1.00	-.35467*	.11694	.003	-.5855	-.1239
	2.00	-.03954	.11777	.737	-.2720	.1929
	4.00	-.34372*	.11694	.004	-.5745	-.1129
	5.00	.53593*	.11694	.000	.3051	.7667
4.00	1.00	-.01095	.11694	.925	-.2418	.2198
	2.00	.30418*	.11777	.011	.0717	.5366
	3.00	.34372*	.11694	.004	.1129	.5745
	5.00	.87964*	.11694	.000	.6488	1.1104
5.00	1.00	-.89060*	.11694	.000	-1.1214	-.6598
	2.00	-.57546*	.11777	.000	-.8079	-.3430
	3.00	-.53593*	.11694	.000	-.7667	-.3051
	4.00	-.87964*	.11694	.000	-1.1104	-.6488

*. The mean difference is significant at the 0.05 level.

Table B7. Biphenyl Tolerance Test ANOVA: Low Treatment

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.862	4	2.966	12.906	.000
Within Groups	39.982	174	.230		
Total	51.845	178			

Table B8. Biphenyl Tolerance Test Multiple Comparisons: Low Treatment

Dependent Variable: ln_abs

LSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.32015*	.11379	.005	.0956	.5447
	3.00	.29038*	.11299	.011	.0674	.5134
	4.00	-.00366	.11299	.974	-.2267	.2193
	5.00	.69354*	.11299	.000	.4705	.9165
2.00	1.00	-.32015*	.11379	.005	-.5447	-.0956
	3.00	-.02976	.11379	.794	-.2543	.1948
	4.00	-.32381*	.11379	.005	-.5484	-.0992
	5.00	.37339*	.11379	.001	.1488	.5980
3.00	1.00	-.29038*	.11299	.011	-.5134	-.0674
	2.00	.02976	.11379	.794	-.1948	.2543
	4.00	-.29405*	.11299	.010	-.5170	-.0710
	5.00	.40315*	.11299	.000	.1802	.6262
4.00	1.00	.00366	.11299	.974	-.2193	.2267
	2.00	.32381*	.11379	.005	.0992	.5484
	3.00	.29405*	.11299	.010	.0710	.5170
	5.00	.69720*	.11299	.000	.4742	.9202
5.00	1.00	-.69354*	.11299	.000	-.9165	-.4705
	2.00	-.37339*	.11379	.001	-.5980	-.1488
	3.00	-.40315*	.11299	.000	-.6262	-.1802
	4.00	-.69720*	.11299	.000	-.9202	-.4742

*. The mean difference is significant at the 0.05 level.

Table B9. Biphenyl Tolerance Test ANOVA: High Treatment

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.095	4	3.024	16.396	.000
Within Groups	32.090	174	.184		
Total	44.185	178			

Table B10. Biphenyl Tolerance Test Multiple Comparisons: High Treatment

Dependent Variable: ln_abs

LSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.16087	.10122	.114	-.0389	.3606
	3.00	.18526	.10122	.069	-.0145	.3850
	4.00	.03279	.10194	.748	-.1684	.2340
	5.00	.71879*	.10122	.000	.5190	.9186
2.00	1.00	-.16087	.10122	.114	-.3606	.0389
	3.00	.02439	.10122	.810	-.1754	.2242
	4.00	-.12808	.10194	.211	-.3293	.0731
	5.00	.55793*	.10122	.000	.3581	.7577
3.00	1.00	-.18526	.10122	.069	-.3850	.0145
	2.00	-.02439	.10122	.810	-.2242	.1754
	4.00	-.15247	.10194	.137	-.3537	.0487
	5.00	.53354*	.10122	.000	.3338	.7333
4.00	1.00	-.03279	.10194	.748	-.2340	.1684
	2.00	.12808	.10194	.211	-.0731	.3293
	3.00	.15247	.10194	.137	-.0487	.3537
	5.00	.68601*	.10194	.000	.4848	.8872
5.00	1.00	-.71879*	.10122	.000	-.9186	-.5190
	2.00	-.55793*	.10122	.000	-.7577	-.3581
	3.00	-.53354*	.10122	.000	-.7333	-.3338
	4.00	-.68601*	.10194	.000	-.8872	-.4848

*. The mean difference is significant at the 0.05 level.

Treatment Effects Within Samples Biphenyl Tolerance Test ANOVA and Multiple Comparison

Treatment groups are represented as: Control=1, High=2, Low=3

Table B11. Biphenyl Tolerance Test ANOVA: D.org Treatment Effects

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.461	2	.230	1.090	.340
Within Groups	22.192	105	.211		
Total	22.653	107			

Table B12. Biphenyl Tolerance Test ANOVA: D.mix Treatment Effects

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.695	2	.347	1.835	.165
Within Groups	19.495	103	.189		
Total	20.190	105			

Table B13. Biphenyl Tolerance Test ANOVA: D.con Treatment Effects

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.065	2	.532	3.101	.049
Within Groups	18.021	105	.172		
Total	19.086	107			

Table B14. Biphenyl Tolerance Test Multiple Comparison Test: D.con Treatment Effects

Dependent Variable: ln_abs

LSD

(I) treat_numb	(J) treat_numb	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound

1.00	2.00	-.20381*	.09765	.039	-.3974	-.0102
	3.00	-.21681*	.09765	.029	-.4104	-.0232
2.00	1.00	.20381*	.09765	.039	.0102	.3974
	3.00	-.01300	.09765	.894	-.2066	.1806
3.00	1.00	.21681*	.09765	.029	.0232	.4104
	2.00	.01300	.09765	.894	-.1806	.2066

*. The mean difference is significant at the 0.05 level.

Table B15. Biphenyl Tolerance Test ANOVA: C.org Treatment Effects

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.622	2	.311	1.420	.246
Within Groups	22.759	104	.219		
Total	23.381	106			

Table B16. Biphenyl Tolerance Test ANOVA: C.tap Treatment Effects

ln_abs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.223	2	1.112	3.599	.031
Within Groups	32.432	105	.309		
Total	34.656	107			

Table B17. Biphenyl Tolerance Test Multiple Comparison Test: C.tap Treatment Effects

Dependent Variable: ln_abs

LSD

(I) treat_numb	(J) treat_numb	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	2.00	-.20620	.13100	.118	-.4659	.0535
	3.00	-.34959*	.13100	.009	-.6093	-.0898
2.00	1.00	.20620	.13100	.118	-.0535	.4659
	3.00	-.14338	.13100	.276	-.4031	.1164
3.00	1.00	.34959*	.13100	.009	.0898	.6093

2.00	.14338	.13100	.276	-.1164	.4031

*. The mean difference is significant at the 0.05 level.

BIOGRAPHICAL SKETCH

Alisha Janiga was born in Indio, California, and moved to the Rio Grande Valley, Texas as a child. She grew up in the South Padre Island, Texas area and graduated high school from the Mathematics and Science Academy among the top students in her class. Following this, she continued her studies at the University of Texas at Austin. Here she researched neurobiology and addiction sciences in preparation to pursue pathology. After an academic break, she was inspired to studied environmental toxicology and human impacts on novel ecosystems. In 2016, Alisha was admitted to the master's program at the University of Texas Rio Grande Valley. During this time, she also served as a teaching assistant for general biology laboratories as well as a tutor for her niece and nephew in her free time. Completing this master's program has led her in career directions she would have never imagined, and she plans to pursue a doctoral degree in the near future.

Alisha.janiga@gmail.com

Earned Masters of Science in Agriculture, Environmental, and Sustainable Sciences