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Microbial impacts on endocrine disrupting contaminants: Las Vegas Wash and Lake Mead, Nevada

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MICROBIAL IMPACTS ON ENDOCRINE DISRUPTING CONTAMINANTS: LAS VEGAS WASH AND LAKE MEAD, NEVADA

by

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ABSTRACT

Microbial Impacts on Endocrine Disrupting Contaminants: Las Vegas Wash and Lake Mead, NV

by

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International concern over endocrine disrupting chemicals (EDCs) has become heightened in recent years as more studies reveal their persistence in the environment and their detrimental effects on wildlife. However, little is known about the role of microorganisms in the fate and transport of these compounds in surface waters. Las Vegas Wash, a stream flowing into Lake Mead and fed primarily by treated wastewater effluent, provided a unique experimental system in which to study the role microorganisms play in the dispersal of these compounds in aquatic systems. Samples were collected from the Las Vegas Wash downstream of the Las Vegas Valley's three wastewater treatment plants, in Las Vegas Bay near the confluence of the Las Vegas Wash and Lake Mead, at the Drinking Water Intake site, and at the Colorado River where it enters Lake Mead. The biodegradation potential of 27 pharmaceuticals and EDCs was examined utilizing native microorganisms in microcosms from the four water samples over a 120 day period. Chemical analysis at the end of the incubation revealed that the

Las Vegas Wash site experienced the greatest removal, with 72% total mass reduction of the parent compounds. With the exception of the bacteriostatic control, the Colorado River site experienced the lowest degradation, with only 37% removal. Similarly, Biolog Ecoplate assays demonstrated that the Las Vegas Wash was able to use a considerably greater number of carbon substrates than any other site. Finally, microbial community composition analysis based on 16S rRNA gene censuses using Unifrac and LIBSHUFF statistical methods revealed the Las Vegas Wash community to be significantly distinct from other sampled locations, although all sites were similar with regard to overall diversity and richness. The results indicated that the Las Vegas Wash microbial community contained a broader metabolic potential for EDC biodegradation. This was further supported by phylogenetic analysis identifying a high number of phylotypes related to known isolates able to catabolize similar compounds.

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CHAPTER 1

INTRODUCTION

Emerging contaminants are a topic of increasing concern as studies reveal their persistence in the environment and the adverse effects they can have on wildlife. Among these are endocrine disrupting chemicals (EDCs), which are defined as "exogenous chemical substances or mixtures that alter the structure or function(s) of the endocrine system and cause adverse effects at the level of the organism, its progeny, populations, or subpopulations" (USEPA, 1997). Many commonly used synthetic chemicals, pharmaceuticals and personal care products are known or alleged EDCs, including the surfactants nonylphenol and octylphenol, the plastic additive bisphenol A (BPA), the pesticide atrazine, and the antimicrobial triclosan. However, while some of these compounds have been confirmed to be estrogenic, they are several orders of magnitude lower in estrogenicity than the natural and synthetic steroid hormones 17β-estradiol (E2), estrone (E1), and 17α-ethinylestradiol (EE2), as shown in *in vitro* studies by Routledge & Sumpter (1996). Chang et al. (2011) has suggested that natural estrogens such as E2 and estrone E1 are the primary compounds responsible for endocrine disruption in wildlife. In fact, some studies have attributed estrogens as the sole source for estrogenic effects in wastewater effluent (Desbrow et al. 1998). Steroid hormones, including estrogens, are excreted through human waste and although 90% or more may be removed through activated sludge treatment (Joss et al. 2004), it appears that many EDCs still make their way into streams and waterways through wastewater effluent. This can cause potential problems for wildlife downstream (Routledge et al. 1998).

The mechanisms driving the natural attenuation of wastewater-derived organic contaminants in the environment are poorly understood. These compounds are regularly detected in surface and groundwaters around the world, including, but not limited to the U.S., Italy, UK, Sweden, Germany, China, and Israel (Bendz et al. 2005, Bester et al. 2008, Kasprzyk-Hordern et al. 2009, Kolpin et al. 2002, Kuster et al. 2008, Petrovic et al. 2004). According to studies by the U.S. Geological Survey (USGS), prescription and over-the-counter pharmaceuticals, insect repellants, detergents, plasticizers, fire retardants, antibiotics, and hormones are the most frequently found EDCs in waterways nationwide (Barnes et al. 2008, Focazio et al. 2008). Their ubiquity suggests that as a class of compounds, they are somewhat refractory. However, the fate and transport of any individual compound is controlled by the relationship of biophysiochemical processes such as advection, photolysis, adsorption, desorption, and microbial degradation. As global water resources become more strained by population growth, drought, and climate change, beneficial wastewater reuse practices will increase, making dwindling water resources more susceptible to contamination from these compounds (Benotti et al. 2010). A better understanding of the fate and transport of wastewater-derived organic contaminants will help identify which compounds may pose a particular threat to ecosystem or human health.

Specific subclasses of organic wastewater contaminants, pharmaceuticals and EDCs have been the focus of many recent studies on occurrence, and to a lesser extent, fate and transport mechanisms (Loffler et al. 2005, Winkler et al. 2001, Kreuzig et al. 2003, Casey et al. 2004). While the presence of these compounds in the environment is not a new phenomenon, recent advances in analytical instrumentation have allowed for

robust quantification to the low ng/L concentrations, thus revealing their ubiquity in wastewater streams (Vanderford et al. 2003, Kolpin et al. 2002). Their presence in the environment has been implicated as a threat to the health of aquatic ecosystems, particularly in wastewater-dominated environments, as has been documented in the United Kingdom (Routledge et al. 1998) and the United States (Bevans et al. 1996, Snyder et al. 2010). Pharmaceuticals and EDCs have also been detected in drinking water (Benotti et al. 2009), raising the possibility that these compounds may pose a risk to human health as well.

Microorganisms are ubiquitous and largely responsible for the alteration and fate of many organic chemicals in the environment (Schwarzenbach 2003). Most of the information pertaining to the biodegradability of EDCs stems from investigations of their behavior at wastewater treatment plants (WWTP) or during soil aquifer treatment (SAT), in which their removal is most often attributed to microbial activity (Chang et al. 2011). Many studies have specifically focused on removal of these compounds by means of biodegradation under WWTP conditions with the majority being conducted in laboratory or pilot scale sludge reactors under varying conditions of incubation length and temperature. In a comprehensive study by Carballa et al. (2006), biodegradation of a suite of pharmaceuticals including sulfamethoxazole, carbamazepine, diazepam, diclofenac, EE2, ibuprofen, naproxen, and iopromide was examined after treatment in a pilot scale anaerobic digester with a sludge retention time (SRT) ranging from 6 to 20 days under both mesophilic and thermophilic conditions. While sulfamethoxazole was shown to be almost completely removed under both conditions, carbamazepine showed no degradation regardless of temperature. Diazepam, diclofenac, EE2, ibuprofen, and

naproxen all showed moderate to high removal under both settings. Trimethoprim biodegradation was examined by Junker et al. (2006) under lab scale WWTP conditions for three weeks, but no more than 1% removal was seen. Conversely, Batt and others (2006) observed approximately 70% removal of the same compound under WWTP conditions with nitrifying activated sludge and a longer SRT of 49 days. Fluoxetine biodegradation was studied by Kwon and Armbrust over a period of 30 days (2006), and 270 days by Redshaw et al. (2008), with activated sludge and biosolid-amended soil inoculum, respectively. However no removal was detected in either study.

Extrapolating the fate of pharmaceuticals and EDCs during WWTP conditions to that in surface water is difficult. It is assumed that compounds that persist through SAT systems (e.g. sulfamethoxazole and carbamazepine, among others (Drewes et al. 2003, Drewes et al. 2002)), are resistant to biotransformation or biodegradation and should have slower rates of biodegradation in surface waters. Conversely, compounds that are rapidly removed in SAT systems are often susceptible to biodegradation and should exhibit faster rates of biodegradation in surface waters. One notable exception is caffeine. Although caffeine is efficiently eliminated during wastewater treatment and rapidly attenuated during SAT, Buerge et al. (2003) demonstrated that it exhibited conservative behavior in surface waters and was an effective tracer of wastewater discharge. Thus, there is the need to measure microbial degradation rates of pharmaceuticals and EDCs in surface waters as their behavior in specific systems may not accurately predict outcomes in others.

In contrast to their behavior in WWTPs, relatively little is known about the microbial degradation or transformation of pharmaceuticals and EDCs in more natural

ecosystems. Benotti et al. (2009) calculated first order degradation rates for low (ng/L) concentrations of pharmaceuticals in a wastewater-impacted estuary based on laboratory experiments. Half-lives of the targeted compounds varied temporally and spatially throughout the estuary ranging from 0.68 to greater than 100 days. Similarly, Yamamoto et al. (2009) calculated half-lives of eight pharmaceuticals in water collected from two rivers on two different dates. In this work, half-lives attributed to microbial degradation ranged from 2.1 to 230 days and also varied spatially and temporally. This variability in pharmaceutical half-lives, as well as the stark differences in degradation rates between time points in the Yamamoto study suggests that the microbial degradation of pharmaceuticals and EDCs is a dynamic phenomenon which varies by time and environment. This variability may reflect the spatial and temporal differences in microbial populations in surface waters.

A number of studies have investigated bacteria capable of estrogen degradation, particularly those isolated from sewage sludge. Fujii et al. (2002) isolated a new *Novosphingobium* sp. from activated sludge capable of degrading E2, and Yu and others (2007) were able to isolate 14 E2-degrading bacteria from activated sludge, three of which were also able to degrade E1 which they suggested was due to nonspecific monooxygenase enzymes. A study by Czajka and Londry (2006), investigating the anaerobic biodegradation of the estrogens E1, E2, and EE2 from river water samples, demonstrated biotransformation of E1 and E2 but with little actual mineralization of the steroid compounds. Lee and Liu (2002) identified numerous metabolites and degradation pathways for E2 using a mixed culture of sewage bacteria and activated sludge supernatant. In their experiments, transformation of E2 to E1 via oxidation at ring D of

E2 was observed. This was followed by the transformation of E1 to a previously unnamed metabolite which they labeled X1, hypothesized to be a highly labile lactone. Actual elimination of E1 was observed after 14 days of incubation. Jurgens and others (2002) demonstrated the biodegradation of E2 and EE2 by microorganisms in bulk river water samples with half-lives as low as several hours for E2 and up to 17 days for EE2. Using radiolabeled E2, their research revealed steroid ring cleavage of E2 at the A ring with a transient formation to E1 with subsequent complete mineralization. This is similar to results found by Coombe et al. (1966) whose experiments displayed the transformation pathways of E1 by *Nocardia* sp. (E110), a microorganism isolated from soil. In their experiment E1 degradation occurred via cleavage of the A ring followed by the B ring. Their research also revealed the transformation of estrone to a pyridine carboxylic acid which they suggested was formed from either hydroxylation, oxidative fission via dioxygenase, or by nonenzymatic means, by a reaction with NH_3. Yi and others (2006), were able to show removal of EE2 using the ammonium monooxygenase enzyme which they obtained from a mixed culture of nitrifying microorganisms.

Although numerous studies have examined the biodegradation of EDCs and pharmaceuticals in WWTPs and SATs (Junker et al. 2006, Batt et al. 2006, Drewes et al. 2002 and 2003), few studies have been performed which investigate the microbial communities or specific microbes involved in the biodegradation of the other less estrogenic compounds. Cai et al. (2003) discovered an *Arthrobacter* sp. isolated from industrial wastewater capable of growing on atrazine as its sole nitrogen source. Zhang et al. (2009) isolated two atrazine-degrading strains, including another *Arthrobacter* sp. and a *Microbacterium* sp., which demonstrated 66 and 78% degradation, respectively,

after 14 days in liquid culture. Yamanaka et al. (2007) discovered three strains of BPA-degrading bacteria (identified as *Bacillus pumilis*) from the traditional fermented food kimchi, capable of complete removal of BPA in liquid culture within 7 days. Similarly, two *Pseudomonas* strains isolated from river water, including *Pseudomonas putida* and another *Pseudomonas* sp., were found to be capable of BPA degradation by Kang & Kondo (2002). Gummadi and others (2009) were able to isolate a *Pseudomonas* strain from coffee plantation soil which could grow on caffeine as both a sole carbon and nitrogen source. The compound N,N-diethyl-m-toluamide, better known as DEET, a commonly used insect repellant, has been detected throughout numerous U.S. streams (Kolpin et al. 2002). Rivera-Cancel et al. (2007) observed *P. putida* capable of growing aerobically on DEET as a sole carbon source and subsequently forming the metabolites 3-methylbenzoate and diethylamine. The antidepressant fluoxetine was investigated by Redshaw et al. (2008) and shown to be resistant to any form of biological degradation even after incubation in liquid cultures for 60 days and in sewage sludge-amended soils for greater than 200 days. No known bacterial species capable of degrading fluoxetine have been isolated to date.

There is a relative absence of studies examining microbial degradation of pharmaceuticals and EDCs in surface water systems while concurrently examining the microbial community composition which may be responsible for the degradation. Las Vegas Wash and Lake Mead, ecosystems impacted by drought and anthropogenic point sources, provide unique environments to study the fate and degradation of EDCs by microorganisms while additionally assisting in understanding the factors involved in the persistence of these compounds in the environment. Due to the fact that much of the Las

Vegas Valley's treated wastewater flows through the Las Vegas Wash and into the Boulder Basin of Lake Mead, it is not surprising that various pharmaceuticals and EDCs including atrazine, DEET, diazepam, fluoxetine, progesterone, TCEP, and meprobamate have been found in in the Wash and Las Vegas Bay (Vanderford et al. 2003 and Trenholm et al. 2006). Additionally, other studies have found steroid estrogens including E2 at concentrations as high as 2.7 ng/L (Snyder et al. 1999). For perspective, concentrations of estrogens as low as 5 ng/L have been shown to cause total collapse of fish populations due to adverse effects on reproductive health (Kidd et al. 2007). Correspondingly, carp collected from Las Vegas Wash and Las Vegas Bay have been found to contain high concentrations of synthetic organic chemicals and significantly higher levels of endocrine disrupting biomarkers such as vitellogenin, an egg yolk precursor (Bevans et al. 1996).

Lake Mead is the largest reservoir by volume in the United States and a principal water source for more than 25 million people in Nevada, Arizona, and California (Holdren & Turner 2010). Inflows to Lake Mead include the Colorado River, the Virgin River, the Muddy River, and the aforementioned Las Vegas Wash (LaBounty & Burns 2005). Approximately 900 million gallons per day (MGD) of water is withdrawn from Lake Mead for Las Vegas residents, and approximately half is returned as treated wastewater via the Las Vegas Wash (LaBounty & Horn 1997, Drury et al. 2006). The remaining water is lost to groundwater via irrigation and evapotranspiration. Though the flow of the Las Vegas Wash to Lake Mead is relatively small (approximately 2.2%, Holdren & Turner 2010), it is almost entirely (90 percent) treated wastewater and represents a major point source discharge of wastewater-derived contaminants to Lake

Mead (Shanahan & Zhou 2011). Thus, the Las Vegas Wash and Las Vegas Bay provide ideal sites to study microbial contaminant degradation in wastewater-impacted surface waters. Moreover, it provides some insight into how microbial communities differ between a wastewater-dominated system (e.g. the Las Vegas Wash) and a contrasting environment with little wastewater influence (e.g. the Colorado River entering Lake Mead), as well as how differences in microbial communities relate to changes in relative rates of microbial degradation.

This study examined the primary microbial degradation rates of a diverse suite of pharmaceuticals and EDCs at four representative locations around Lake Mead, comparing the observed degradation patterns to characterizations of microbial composition, metabolism, and water quality characteristics. The objectives were to:

1. Investigate rates of EDC and pharmaceutical biodegradation along a gradient of highly wastewater-impacted to less impacted sites (Las Vegas Wash > Las Vegas Bay > Drinking Water Intake> Colorado River).
2. Identify differences in bacterial abundance, diversity, community composition, and overall community metabolic activity between the four sites.

This research will help elucidate characteristics of the microbial communities responsible for pharmaceutical and EDC biodegradation by evaluating the abundance, diversity, and metabolic capabilities at the individual sites. Factors controlling the metabolisms of these compounds (i.e., microbial community structure and naturally occurring nutrient availability) will also be determined. This information will assist in future risk assessments detailing the environmental fate and transport pathways of contaminants and their elimination via microbially-mediated processes.

CHAPTER 2

MATERIALS AND METHODS

Sampling Area

Water samples were collected from four sites throughout Lake Mead and the Las Vegas Wash (Figure 2.1) on October 21 and 22, 2008 in coordination with routine water quality sampling by the Southern Nevada Water Authority (SNWA). Each sample was collected using a peristaltic pump (Masterflex® E/S™ Portable Sampler, Cole-Parmer) and sterile platinum-cured silicone tubing (Masterflex® 96420-24, Saint-Gobain Performance Plastics Corporation) from shore (in the case of the Las Vegas Wash site), from a boat (in the case of the Las Vegas Bay and Colorado River Sites), or from a tap (in the case of the drinking water intake site. The tap is connected to a water intake pipe which transfers raw water from Lake Mead to the Alferd Merrit Smith Drinking Water Treatment Facility (Boulder City, NV). All samples were taken from within a meter of the surface with the exception of the drinking water intake which drew water from approximately 32 meters below the surface at the time of sampling. Coordinates for the sites are as follows: Las Vegas Wash (36.092°N,-114.969°W), Las Vegas Bay (36.106°N,-114.780°W), Drinking Water Intake (36.064°N,-114.801°W), and Colorado River (36.100°N,-114.116°W).

General chemical and physical parameters were measured at each site by SNWA and included temperature, dissolved oxygen concentration, pH, and specific conductance, using a multi-parameter sonde (Hydrolab Corporation Model Surveyor®). Samples were also collected at each site to provide a comprehensive understanding of water chemistry parameters including major ions, metals, and nutrients. Bottles for samples not requiring

preservatives were triple-rinsed with sample water before collections. Sample bottles for metals, major ions and nutrients were prepared and analyzed by Weck Laboratories (Monrovia, CA) or SNWA. Sterile bottles for bacterial heterotrophic plate counts (HPCs) were prepared and analyzed by SNWA Laboratory Support Services personnel according to in-house SOPs. On site, samples for the microcosm experiments were pumped into 5 liter sterile glass carboys (Pyrex # CG-8106) containing a sterile teflon stir bar and having a hose-barbed tap and fitted with sterile tubing and "t" valves. Prior to collection, the pump line was flushed with 5 liters of sample water and a 100 µm prefilter was fitted upstream of collection bottles to screen out larger debris and zooplankton. All samples were maintained in a cooler on ice until distributed to the corresponding laboratory for analysis.

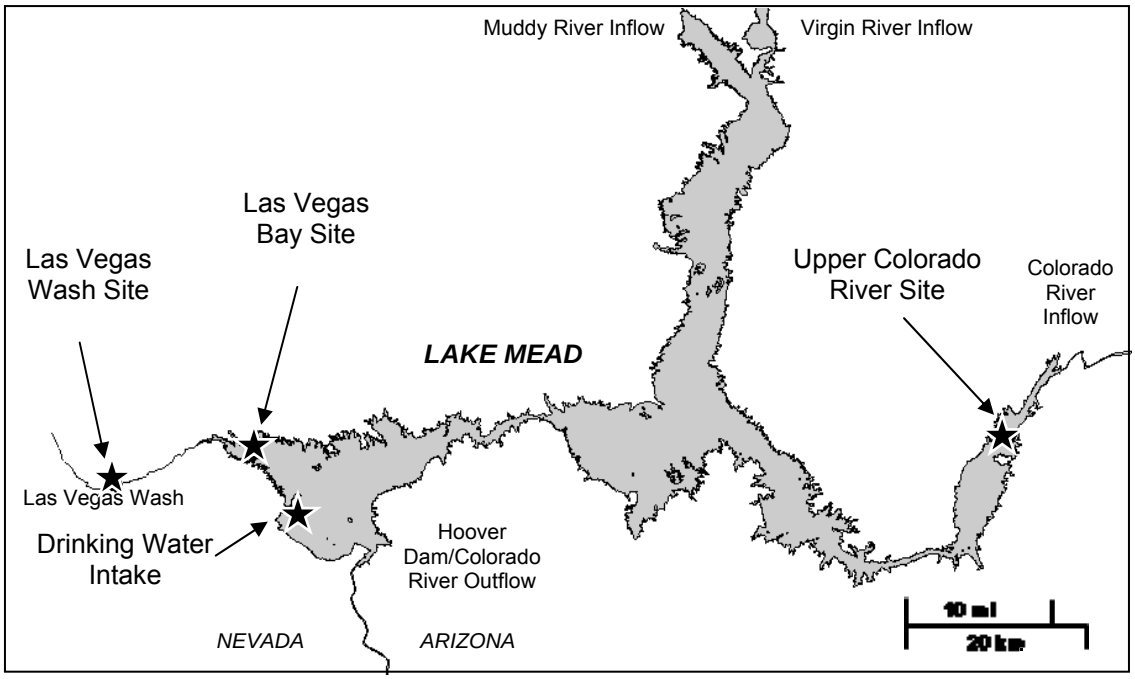


Figure 2.1. Map of study site and sampling locations.

Microcosm setup

In the laboratory, carboys were capped with a sterile rubber stopper fitted with a 0.2 μm filter to allow gas transfer and maintained in the dark (to prevent photodegradation) with gentle stirring at room temperature (Figure 2.2). A total of six incubations were maintained, one for each of the four samples described above, and two controls: 1) Las Vegas Bay water with 1 g/L sodium azide as a bacteriostatic control and 2) Las Vegas Bay water without pharmaceuticals and EDCs added to monitor microbial community responses to bottle effects. Samples were collected from the four experimental incubations prior to EDC and pharmaceutical addition to determine native concentrations of EDCs and pharmaceuticals (Tables A.2 – A.5). Each microcosm (except for the non-amended control) was amended with twenty-seven pharmaceuticals and EDCs (Table 2.1) to an initial concentration of 100-500 ng/L (see Appendix Tables A.6 – A.10 for actual concentrations). Stock pharmaceuticals and EDCs were dissolved in sterile deionized (DI) water, rather than organic solvents, to prevent the unintentional introduction of organic matter, which might otherwise serve as a supplemental carbon or energy substrate in the incubations. The low aqueous solubility of some of these compounds (e.g. the steroid hormones) precluded the entire stock from dissolving and explains some of the lower than desired initial concentrations. Samples were collected using a "t" valve assembly for chemical analyses (including pharmaceuticals/EDCs and steroid hormones) and biological analyses (including terminal restriction fragment length polymorphism (T-RFLP), 16S rRNA gene libraries, flow cytometry, and community metabolism analysis using the Biolog Ecoplate assay). At each time point, the oxygen content of the sample was checked using an Oxygen CHEMets® Kit (K-7512,

Chemetrics, Calverton, VA) to ensure that incubations were aerobic. All sites remained stable with values between 6 and 7 mg/L with the exception of the bacteriostatic control which had slightly higher DO levels between 8 and 9 mg/L. Time points for chemistry analysis included 0, 1, 2, 4, 7, 14, 29, 56, and 120 days.

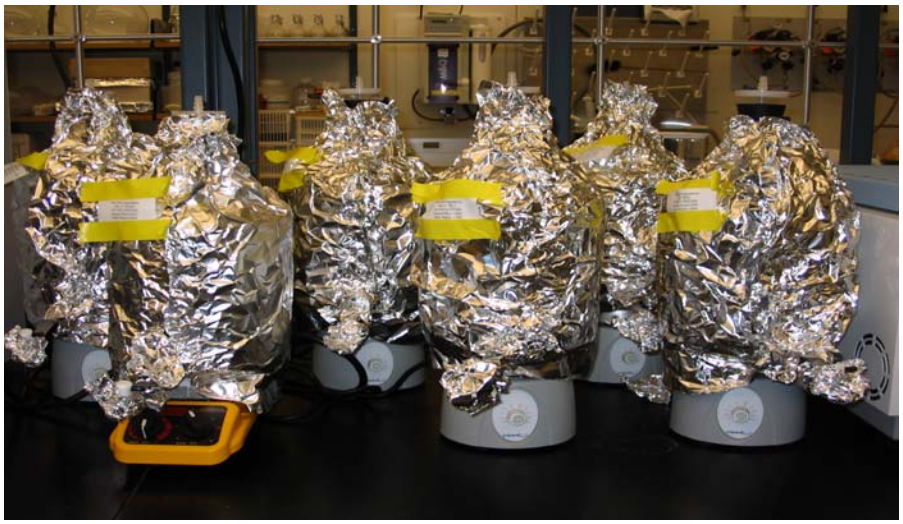
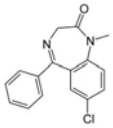
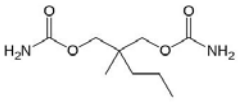
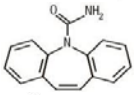
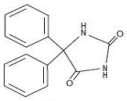
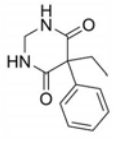
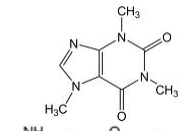
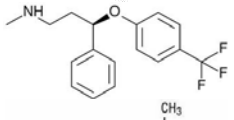
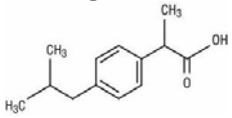
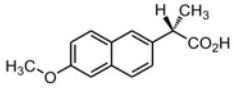
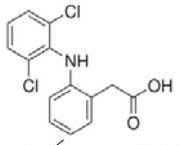
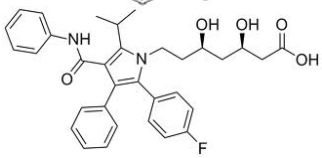
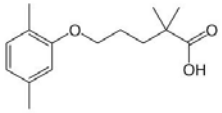
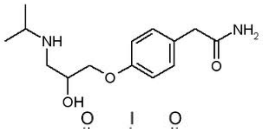
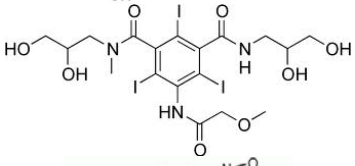
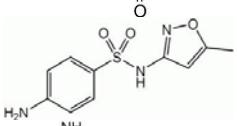
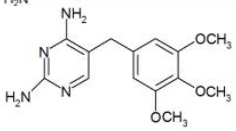
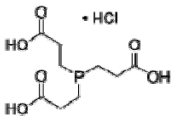
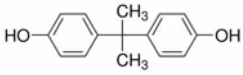
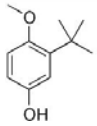
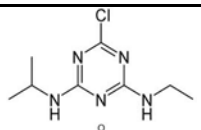
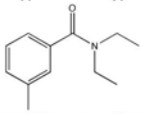
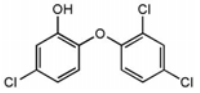
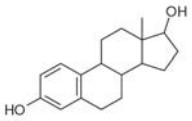
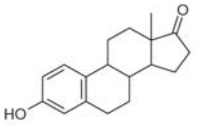
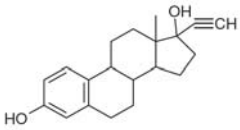
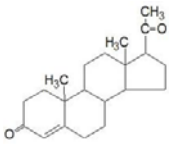
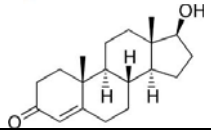


Fig 2.2. Microcosm setup showing the 4 representative sites with the 2 controls.

Table 2.1. Chemical names and structures of the spiked compounds.

Compound	Class	Structure
Diazepam	Antianxiety	
Meprobamate	Antianxiety	
Carbamazepine	Anticonvulsant	
Phenytoin	Anticonvulsant	
Primidone	Anticonvulsant	

Compound	Class	Structure
Caffeine	Stimulant	
Fluoxetine	Antidepressant	
Ibuprofen	Nonsteroidal anti-inflammatory	
Naproxen	Nonsteroidal anti-inflammatory	
Diclofenac	Nonsteroidal anti-inflammatory	
Atorvastatin	Antilipidemic	
Gemfibrozil	Antilipidemic	
Atenolol	Beta blocker	
Iopromide	Radiographic contrast medium	
Sulfamethoxazole	Antibiotic	
Trimethoprim	Antibiotic	
TCEP	Flame retardant	
Bisphenol A	Plastic component	
BHA	Food preservative	

Compound	Class	Structure
Atrazine	Herbicide	
DEET	Insect repellant	
Triclosan	Antimicrobial	
Estradiol	Steroid hormone	
Estrone	Steroid hormone	
Ethynylestradiol	Steroid hormone	
Progesterone	Steroid hormone	
Testosterone	Steroid hormone	

Analytical Methods

At each time point, 500 mL subsamples were collected into two 250 mL amber glass bottles, pre-amended with 0.25 g sodium azide. One of each of the samples was analyzed for 1) pharmaceuticals and EDCs, and 2) steroid hormones. Both analyses employ cleanup and sample concentration by solid phase extraction (SPE) and analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The pharmaceutical and EDC procedure is based on a method developed by Vanderford and Snyder (2006), and the steroid hormone procedure is based on a method developed by Trenhom et al. (2006) with the only differences being slightly modified analyte lists. As

part of quality control measures for the SPE system, laboratory grade DI blanks and DI spiked samples were extracted alongside experimental samples. Water samples were spiked with isotopically-labeled standards for isotope dilution quantitation of each of the target compounds analyzed and extracted using 5 cc glass, 200 mg, Oasis hydrophilic-lipophilic balance (HLB) cartridges (Waters Corp., Milliford, MA), using the Autotrace automated SPE system (Zymark Cop., Hopkington, MA). Cartridges were preconditioned with 5 ml each of dichloromethane, tert-butyl methyl ether, methanol and reagent water. The water samples were then filtered through the cartridges at 15 ml/min, after which the cartridges were rinsed with 5 ml reagent water and dried with nitrogen gas for 60 minutes. Samples were eluted with methanol, evaporated to 250 µl, and stored at -20°C until analysis. LC/MS/MS analysis was performed using an Agilent G1312A (Palo Alto, CA) with a binary mobile phase of 0.1% formic acid in water and 100% methanol at a flow rate of 0.7 ml/min and an injection volume of 10 µl. Mass Spectrometry was done with an Applied Biosystems API 4000 triple quadrupole mass spectrometer (Foster City, CA), using multiple reaction monitoring (MRM) with electrospray ionization (ESI) in positive and negative modes. Method reporting limits (MRL) were three times the method detection limit (MDL).

Microbial Characterization and Molecular Analysis

Sample water for flow cytometric analysis was collected into sterile 15 ml conical polypropylene centrifuge tubes containing the preservative gluteraldehyde at a final concentration of 2.5%. Total cell counts were performed using a MicroPRO™ flow

cytometer ("Total Biomass" assay, Advanced Analytical Technology Inc.) according to manufacturer designed methodologies.

Cell biomass for DNA analysis was collected at each analytical time point by filtration onto 0.2 micron membrane filters (25 mm, Supor Polysulfone, Pall) from 100 mL subsamples and stored at -80 °C. DNA was extracted from archived filters using MoBio Ultraclean® Soil DNA kits (MoBio, Solano Beach, CA), and bacterial community analysis was conducted using terminal restriction fragment length polymorphism (T-RFLP, Liu et al., 1997) at Nevada Genomics Center in Reno, NV. Amplicons were generated using the polymerase chain reaction (PCR), using a FAM-labeled forward primer (9bF, GRGTTTGATCCTGGCTCAG) and universal reverse (1512uR, ACGGHTACCTTGTTACGACTT) (Eder and Ludwig, 1999), on a PXE 0.2 thermal cycler (Thermo Electron Corp., Milford, MA). Fifty microliter PCR reactions contained 5 U LA Taq™ (Takara Bio Inc, Japan), 200 nmol/L of each primer, 8 µL of 2.5 mM dNTP mixture, 5 µL of 10X LA PCR Buffer, 5 µL of 25 mM $MgCl_2$, and 2 µL of DNA template. Thermal cycler conditions included an initial denaturation step (5 min at 95°C); 35 cycles of denaturation (30 s at 95°C), annealing (60 s at 50°C), and extension (90 s at 72°C); and a final elongation step of 20 min at 72°C. PCR products were visualized in 1% agarose gels stained with 1 mg/mL ethidium bromide, run on a horizontal electrophoresis system (Owl Separation Systems, Portsmouth, NH) in 1X TAE buffer at 100 V for 45 min. The crude product was submitted for purification and T-RFLP analysis to the Nevada Genomics Center. T-RFLP digests were performed using the restriction endonucleases *Hha*I and *Hae*III (New England Biolabs, Ipswich, MA).

Patterns were analyzed using PeakScanner™ software (Applied Biosystems, Inc., Carlsbad, CA).

For bacterial 16S rRNA gene library construction, PCR was performed in 3 replicate 25 μL reaction mixtures for each sampling site to reduce PCR bias. Bacterial primers 9bF and 1512uR were used and PCR products were purified (UltraClean™ GelSpin™ DNA Purification Kit, MoBio Laboratories, Inc.) and subjected to molecular cloning methodology using TOPO®-TA kits (Invitrogen). Based on forward reads, clones representative of unique OTUs were bidirectionally sequenced (Functional BioSciences, Madison, WI) and contigs generated using Sequencher™ 4.8 (Gene Codes) and aligned, matched with nearest neighbors and checked for chimeras using Greengenes (DeSantis et al., 2006). Alignments were refined and phylogenetic relationships determined using MEGA (Tamura et al., 2007). Evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 2003). The tree with the highest log likelihood (-26421.8378) was used. The initial tree for the heuristic search was obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The analysis involved 218 nucleotide sequences. All positions containing gaps and missing data were eliminated.

Phylogenetic and Diversity Analysis

Sequences from 16S rRNA gene libraries were clustered using the MOTHUR software package (Schloss et al. 2009) at operational taxonomic unit (OTU) cutoffs of

97%, 90%, and 80% using the average neighbor algorithm. Diversity and richness indices including the Shannon diversity index (H'), the Simpson index, and Chao1 richness estimator were also calculated in MOTHUR. Evenness was calculated as H/Hmax, where H is the Shannon diversity estimate and Hmax = log2(S) with S being the total number of corresponding OTUs. Community overlap and differences were also determined using rarefaction analysis. LIBSHUFF was used in order to determine whether any statistically significant differences existed between sites (Schloss et al., 2004).

Community metabolic potential was assessed using Biolog EcoPlates (Biolog Inc., Hayward, CA) according to the manufacturer's standard protocol. Briefly, each 96 well microtiter EcoPlate contains three replicates of 31 different carbon substrates and an additional three wells containing only water as a control. As the various substrates are metabolized, a tetrazolium dye in each well produces a color change when reduced, which can then be measured using a microplate reader to provide optical density (OD_{595}) values and subsequently quantified. Approximately 10 mL of sample water from each of the four spatial sites were added to an EcoPlate at the beginning of the experiment and incubated at room temperature in the dark. Absorbance readings at 595 nm were taken at intervals up to 12 days. Averages were calculated from the 3 replicates and absorbance values above a threshold of 0.3 were considered positive for usage of the carbon source for that well.

CHAPTER 3

RESULTS AND DISCUSSION

Water Chemistry

Table 3.1 shows the physical and chemical parameters at each site. Las Vegas Wash had the highest temperature (23.4^{o}C vs. 21.6 ^{o}C, 20.8 ^{o}C and 17.8 ^{o}C for the Bay, River and mid water column drinking water intake). pH was slightly alkaline at all sites and highest at the river and LVB sites, at 8.38, and 8.27, respectively. Dissolved oxygen (DO), 6.57 mg/L (LVW), 7.81 mg/L (LVB), and 5.87 mg/L (DWI), was under-saturated at all of the sites, except for the Colorado River, 8.57 mg/L. The lower temperature and DO concentrations at the drinking water intake are consistent with this site's location at a greater depth within the reservoir.

Overall, major ion concentrations were similar at three of the sites, LVB, DWI, and CR, and markedly higher in LVW. Specifically, the relatively conservative cations, magnesium, sodium, and potassium, as well as the major anions, sulfate and nitrate were all much higher in the Las Vegas Wash. The higher ion (salt) concentrations in the Wash are reflected in a much higher conductivity value (2,433 uS/cm, vs. 908 – 1,068 at the other sites) and are similar to previously reported data which were attributed to high evaporation rates at this site (Zhou et al. 2005). The higher conductance values in Las Vegas Wash are consistent with data from LaBounty & Burns (2005), which showed a decreasing gradient in TOC and conductivity values from the inner basin where the Wash enters to sites within Lake Mead and on toward the Colorado River.

A combined graphic expression of the water chemistry components in a Piper diagram (Figure 3.1) reveals that all sites group together, again with the exception of the

Las Vegas Wash, which is a slight outlier with respect to both cations and anions. Conversely, water from the drinking water intake and Colorado River sites plot on top of one another.

Table 3.1. Water chemistry parameters

	Las Vegas Wash	Las Vegas Bay	DW Intake	Colorado River
Temperature (°C)	23.4	21.6	17.8	20.8
pH	7.71	8.27	7.93	8.38
DO (mg/L)	6.57	7.81	5.87	8.57
Conductivity (µS/cm)	2433	1068	1030	908
TOC (mg/L)	6.0	3.2	2.7	2.9
Tot. Alkalinity (mg/L)	129	132	143	134
Nitrate (mg/L N)	14	0.88	0.5	0.2
Nitrite (mg/L N)	<0.05	<0.05	<0.05	<0.05
T-Phosphate (mg/L P)	0.12	<0.005	<0.005	0.0055
Ammonia (mg/L N)	0.205	0.024	<0.02	<0.02
Calcium (mg/L)	130	75	81	69
Chloride (mg/L)	340	100	88	80
Iron (mg/L)	<0.1	<0.1	<0.1	<0.1
Magnesium (mg/L)	63	28	27	24
Potassium (mg/L)	32	6.9	5.9	5
Sodium (mg/L)	280	99	96	81
Tot. Hardness (mg/L $CaCO_3$)	590	300	320	270
Alkalinity, CO_3^{-2}	0	0.592	0	2.39
Alkalinity, HCO_3^-	129	131	143	131
Silica (mg/L)	19	6.7	7.3	6.2
Sulfate (mg/L)	570	270	250	220
TDS (mg/L)	1541	649.4	618.4	553.6
Turbidity (NTU)	2.68	0.51	0.33	2.66

DO – Dissolved oxygen; TOC – total organic carbon; TDS – total dissolved solids

Carbon, nitrogen, and phosphorus are nutrients which are vital for microbial growth and in some cases can have significant impacts on biodegradation rates (Schwarzenbach et al., 2003). TOC, concentrations of wastewater-derived constituents such as nutrients (nitrate, phosphate, ammonium), and TDS were all considerably higher at the Las Vegas Wash site, but decrease spatially towards the Bay. TOC levels in the

Wash were approximately double those at the other three sites (6.0 mg/L compared with 2.7 - 3.2 mg/L), and nitrate was considerably higher in Las Vegas Wash (14.0 mg/L) than in the Bay, DW Intake or Colorado River (0.88, 0.5, and 0.2 mg/L, respectively). Phosphate (as mg/L P) was only detected in the Las Vegas Wash and Colorado River (0.12 and 0.055), but was below detection limit at the other two sites. This supports previously collected data which demonstrated that Lake Mead is extremely phosphorus limited (LaBounty and Horn 1997, Reginato and Peichota 2004, LaBounty 2005).

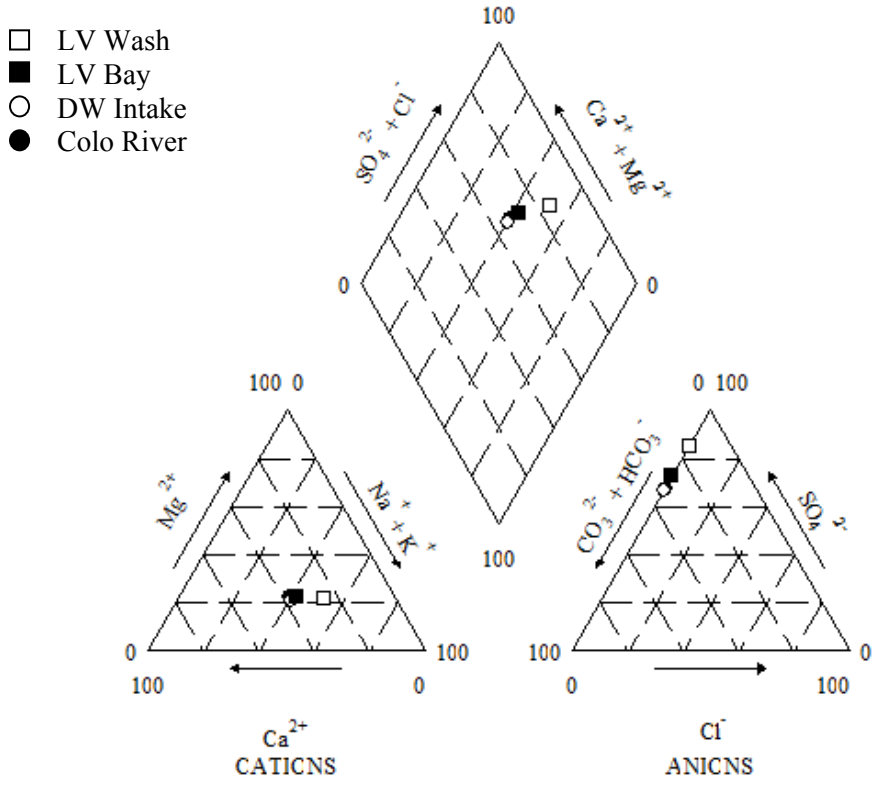


Fig. 3.1. Piper diagram of the water chemistry from the four sampling sites. Diagram was made using GW Chart (Version 1.23.5.0), a free software program available from the USGS.#

Although the carbon, nitrogen, and phosphorus concentrations may be limiting factors for microbial activity and ultimately, biodegradation, it is less clear what concentrations of TOC may hinder (by providing competitive food sources) or assist (through cometabolism) microbial attenuation of xenobiotic compounds. According to Cotner et al. 2010, aquatic bacteria (specifically those from freshwater lakes) are flexible in their nutrient requirements. In their experiment, freshwater bacterial isolates grown under conditions of phosphorus limitation (875:179:1 C:N:P) produced bacterial biomass stoichiometries of 259:69:1, indicating that bacterial populations can subsist with nutrient ratios that diverge considerably from the commonly accepted Redfield ratio of C:N:P of 106:16:1 (Redfield, 1934). Chenier and others (2003) showed that the addition of carbon, nitrogen and phosphorus increased the mineralization of hexadecane by river water biofilms. When no additional nutrients were added, mineralization was minimal, however, with the addition of 67 μM carbon, 80 μM nitrogen, and 5 μM phosphorus, up to 70% mineralization of hexadecane was observed after 42 days of incubation. Additionally, an experiment by Leys et al. (2005) noted that a molar C:N:P ratio of 100:10:1 resulted in efficient polycyclic aromatic hydrocarbon (PAH) biodegradation although this rate was not affected when the C:N:P ratio was imbalanced by a lower molar N ratio (100:2:1), indicating that lower N conditions did not affect the biodegradation by the bacteria involved. However, Rojas-Avelizapo et al. (2000) found that modifying the C:N:P ratio from 2700:140:1 to 100:10:1 caused an increase in the heterotrophic bacterial activity in soil but did not improve PCB degradation, although other researchers have found that adjusting this ratio can stimulate petroleum hydrocarbon degradation in contaminated soils and surface waters (Dibble and Bartha

1979, and Rogers et al. 1993). Since these nutrients were not monitored throughout the time points of the experiment, the rate at which they were utilized is unknown, but the Las Vegas Bay and DW Intake microcosms may have been at a disadvantage due to the low initial phosphorus concentrations at these sites (<0.005 mg/L).

Transformation of Pharmaceuticals and EDCs

Primary degradation (e.g. loss of parent compound) at each site varied considerably; however, the Las Vegas Wash microcosm showed the highest degradation capabilities of the four sites overall, with an aggregate 28% remaining of the compounds over the course of 120 days (Table 3.2). This was followed by Las Vegas Bay with 49% remaining, the Drinking Water Intake with 60%, and the Colorado River site with 63%. The Las Vegas Bay Control microcosm exhibited markedly lower removal than the other 4 sites, with 80% of the parent compounds still remaining at the conclusion of the experiment.

Table 3.2. Total contaminant mass (%) remaining at each time point over the course of the experiment.

	Time Point (days)								
	0	**1**	**2**	**4**	**7**	**14**	**29**	**56**	**120**
LV Wash	100	103	ND*	92	86	70	47	39	**28**
LV Bay	100	99	102	102	102	95	86	68	**49**
DW Intake	100	102	97	96	95	85	79	66	**60**
Colo River	100	99	92	97	92	86	83	71	**63**
LV Bay Control	100	98	96	101	102	90	88	81	**80**

ND* = No data for this time point due to failure of SPE pump. Percent contaminant remaining calculated as C_t/C_0, where C_t = sum of the total mass of the 27 compounds at each individual time point, and C_0 = sum of the total mass of the compounds at the beginning of the experiment.

Analysis of the steroid estrogens, the compounds with the highest EDC potential (Routledge & Sumpter 1996), revealed that estrone, estradiol, and progesterone were reduced to below detection limits after 120 days in all samples with the exception of the control (which still contained 10% and 128% of estrone and estradiol, respectively) (Table 3.3). Although estradiol was almost completely removed by the end of the 120 day incubation in the control microcosm, it appears that it was converted to estrone, given the stoichiometric increase of this compound in the control (Fig. 3.2). This could likely be due to abiotic transformation of estradiol to estrone, as this has previously been reported in the literature by Sheng et al. (2009), who showed complete abiotic oxidation of estradiol to estrone due to manganese in autoclaved soil samples. Ethinylestradiol, which was reduced in concentration but persisted in all samples throughout the duration of the experiment, saw greatest reduction in the Las Vegas Wash microcosm.

As was noted earlier, caffeine has been previously utilized as a wastewater tracer due to its reported refractory characteristics in surface water (Buerge et al., 2003). However, the Las Vegas Wash and Las Vegas Bay microcosms exhibited almost complete removal with 14% and 0% remaining, respectively. A similar pattern was obtained for the antianxiety drug, meprobamate. Consistent with the previously reported recalcitrance to degradation in surface waters noted by Snyder et al. (2004), after 120 days no degradation was observed for three of the four sites tested. In marked contrast, however, this compound was effectively removed from the Las Vegas Wash microcosm, with only 6%, remaining after 120 days (Table 3.3).

Although degradation of some compounds was observed in the Las Vegas Bay bacteriostatic control microcosm, this loss could be due to abiotic processes such as

chemical oxidation, volatilization, or hydrolysis. In the current experiment, the bacteriostatic agent used in the control was 1% sodium azide. This compound has been used extensively as an industrial and experimental preservative as well as bacteriostatic agent since at least 1891 (Loew). Keilin and Hartree (1934) demonstrated that sodium azide interferes with cellular metabolism by inhibiting cytochrome oxidase, the terminal electron transport complex in aerobic organisms. Lichstein and Soule (1943) reported that lower concentrations of sodium azide (0.005 to 0.02%) were sublethal, and decreased aerobic respiration of bacteria. However, higher concentrations (1%) have been shown to be bacteriostatic to both aerobes and anaerobes in liquid media, and bactericidal to many organisms including *Bacillus subtilis*, *E. coli*, and *Clostridium perfingens* (Forget and Fredette, 1962). Due to its affordability, ease of use, and relatively safe disposal (unlike other bactericides, such as HgCl), sodium azide is often used to provide an abiotic control in biodegradation tests (Patterson et al., 2010, Bergheim et al., 2011, Murialdo et al., 2003). In addition to these benefits, it was also chosen in this experiment instead of autoclaving, over concern that the heat and pressure could potentially alter the natural organic matter and sorption potential of the treated sample.

Removal via sorption was not specifically examined in this study, however, a similar study conducted by Benotti and Brownawell (2009), examined loss of pharmaceuticals due to sorption on suspended sediment particles using adsorption experiments. After testing 19 pharmaceuticals, many of which overlap with those used in this study, they determined an average fraction of 0.92 of the original compounds which remained dissolved in solution, indicating sorption was insignificant. Therefore, it is

unlikely that sorption played a major role in the loss of any compounds seen in the current study.

Table 3.3. Total % remaining of parent compound after incubation for 120 days.

		LV Wash	LV Bay	DW Intake	Colorado River	LV Bay Control
Diazepam	Anti-anxiety	82	65	47	54	76
Meprobamate		6	100	103	106	106
Carbamazepine	Anti-convulsant	76	85	105	88	69
Phenytoin		14	122	74	104	84
Primidone		95	108	108	96	97
Caffeine	Stimulant	14	0	85	104	109
Fluoxetine	Anti-depressant	53	20	3	22	64
Ibuprofen	NSAID	0	0	1	9	103
Naproxen		0	0	26	0	93
Diclofenac		30	26	84	77	53
Atorvastatin	Anti-lipidemic	8	48	17	45	73
Gemfibrozil		4	96	100	100	100
Atenolol	Beta blocker	0	3	44	9	81
Iopromide	X-ray contrast	0	89	190	71	148
Sulfamethoxazole	Antibiotic	5	91	113	116	82
Trimethoprim		4	81	83	50	82
TCEP	Flame retardant	87	82	53	83	67
Bisphenol A	Plastic component	7	0	9	76	90
BHA	Food preservative	0	2	0	2	0
Atrazine	Herbicide	83	76	68	72	62
DEET	Insect repellant	11	93	86	115	107
Triclosan	Antimicrobial	0	34	44	34	71
Estradiol	Steroid hormone	0	0	0	0	10
Estrone		0	0	0	0	128
Ethynylestradiol		32	61	61	48	62
Progesterone		0	0	0	0	0
Testosterone		0	0	0	0	0

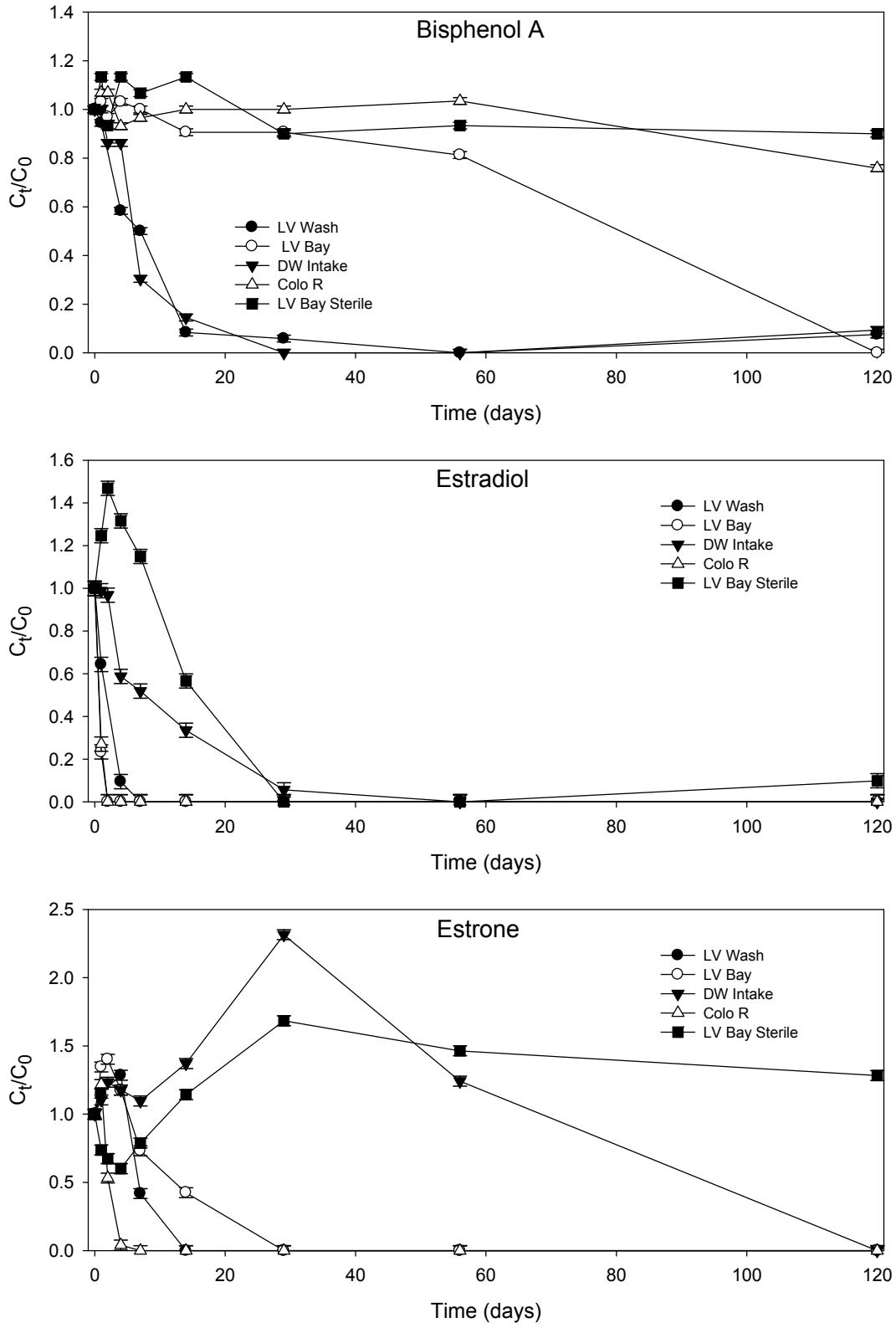


Figure 3.2. Degradation curves for bisphenol A, estradiol, and estrone. Error bars represent analytical error. For additional compound figures, see Appendix.

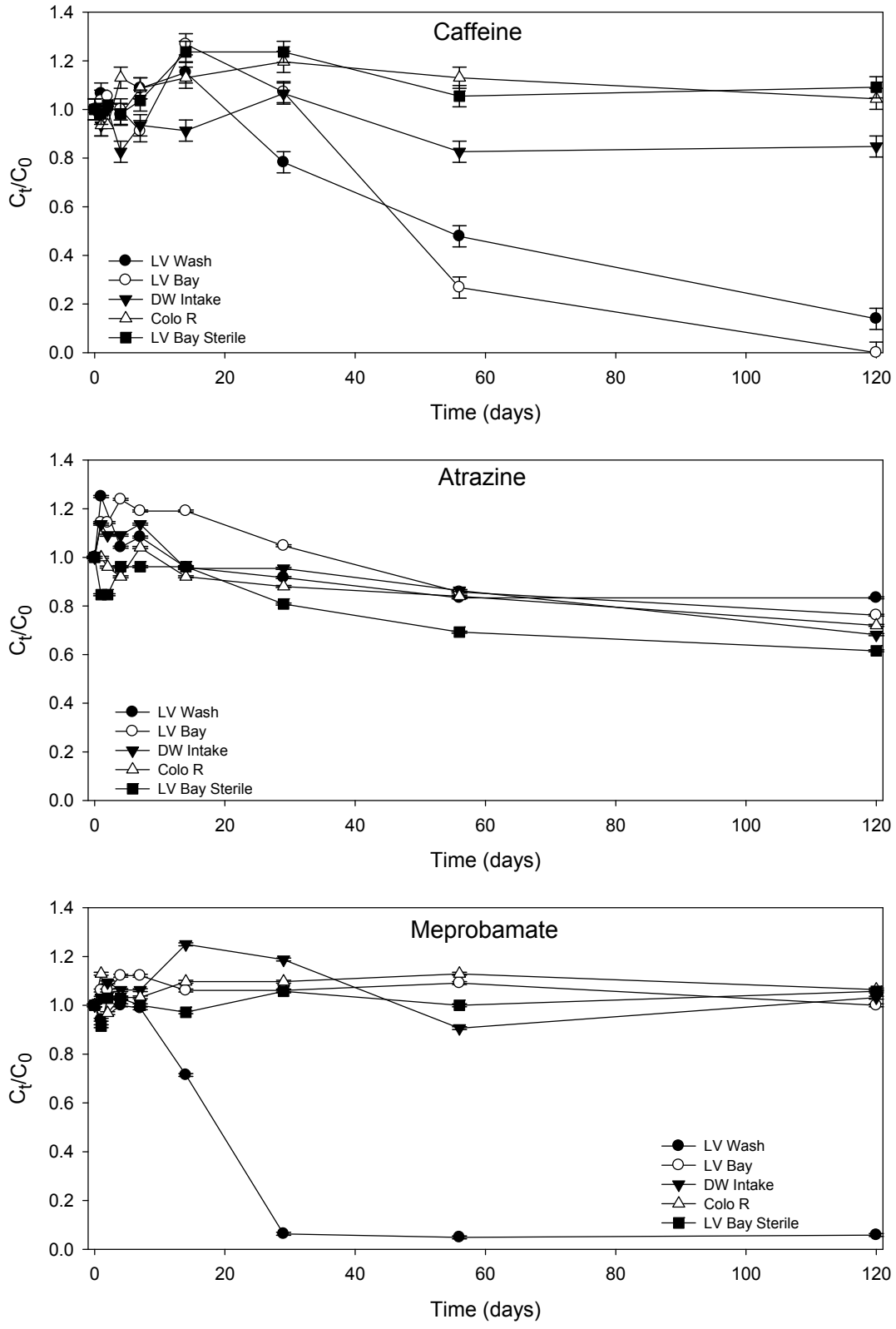


Figure 3.3. Degradation curves for caffeine, atrazine, and meprobamate. Error bars represent analytical error. For additional compound figures, see Appendix.

Characterization of the Microbial Community

Community diversity and richness

Table 3.4 shows the results of the analysis of the 16S rRNA gene library coverage for planktonic microbial communities collected from each of the four sites at the beginning of the incubation. OTUs are given for unique sequences and those roughly corresponding to the species, genus, and phylum levels (97%, 90% and 80%, respectively). There were a total of 268 non-chimeric sequences among the four sites (Table 3.4). Sites ranged from 32 – 40 observed OTUs and 63 – 90 predicted OTUs at the species level. Values for the Chao 1 richness estimator revealed that the Las Vegas Bay and Colorado River were higher than either the Drinking Water Intake or Las Vegas Wash at the species level, with values of 89.8 and 90.2, respectively as opposed to 63.0 and 65.1. Since the Chao 1 estimate values are much higher at the species level than the total number of OTUs, it is assumed that total coverage of bacterial richness was not observed. Rarefaction curves performed at the species level (Fig. 3.4) show similar richness between all four sites, with no observable significant difference between them. Rarefaction curves also indicate sampling was not exhaustive.

Diversity at the species level according to the Shannon index indicated similar levels among the four sites. The Drinking Water was slightly greater (3.43) than the Las Vegas Wash (3.38), the Las Vegas Bay (3.21) and the Colorado River (3.16) (Table 3.4). The Simpson index, with values inversely related to diversity, indicated that the Colorado River bacterial community was slightly less diverse than the other three sites at the species level, which were all close in value (0.0308 -0.0363). At higher phylogenetic levels, the Drinking Water Intake site exhibited the greatest diversity at the genus and the

phylum levels for the Shannon index. Evenness did not vary appreciably between sites at any of the distance levels analyzed (0.55-0.69).

Table 3.4. Diversity and richness estimates from 16S rRNA gene sequences.

	LV Wash n=64	**LV Bay** n=57	**DW Intake** n=75	**Colorado R** n=72
[a]OTUs				
unique	47	48	71	71
97%	37	32	40	35
90%	24	24	35	27
80%	14	12	18	13
[a]Shannon index (H')				
unique	3.70	3.81	4.24	4.26
97%	3.38	3.21	3.43	3.16
90%	2.88	2.85	3.25	2.93
80%	2.10	1.97	2.53	2.07
[a]Chao 1				
unique	110.0	184.7	513.2	1278.5
97%	65.1	89.8	63.0	90.2
90%	33.0	35.0	50.3	38.1
80%	24.5	17.0	19.0	15.0
[b]Evenness				
unique	0.67	0.68	0.69	0.69
97%	0.65	0.64	0.64	0.62
90%	0.63	0.62	0.63	0.62
80%	0.55	0.55	0.61	0.56
[a]Simpson index				
unique	0.0164	0.0069	0.0014	0.0004
97%	0.0308	0.0363	0.0317	0.0509
90%	0.0610	0.0658	0.0418	0.0610
80%	0.1483	0.1704	0.0941	0.1616

[a]Diversity and richness measurements were determined in MOTHUR (Schloss et al. 2009)
[b]Evenness was calculated as $E=H/H_{max}$, where H_{max} =log2(S), and S = the total number of phylotypes

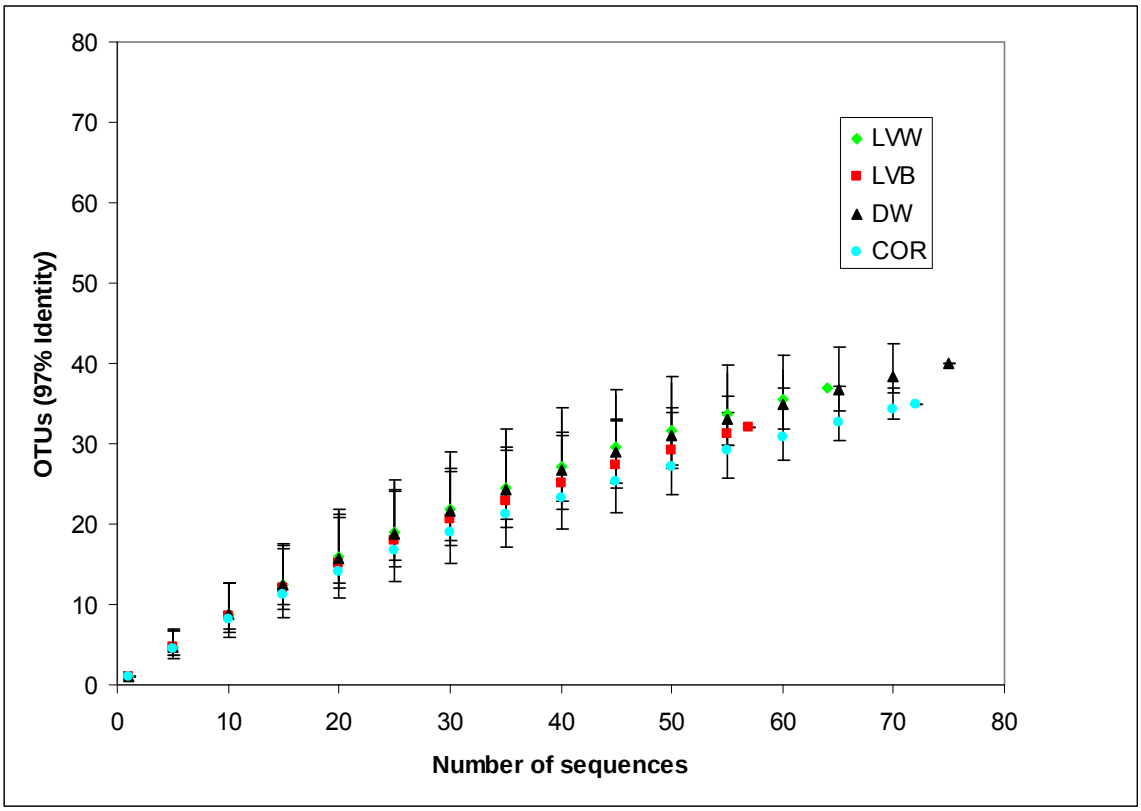


Fig 3.4. Rarefaction analysis of 16S rRNA clone libraries at distance level of 97%. LVW = Las Vegas Wash, LVB = Las Vegas Bay; DWI = Drinking water intake; COR = Colorado River.

Community composition

Bacterial groups from the four sites at the start of the incubation were assigned at the phylum level using the RPD classifier tool (Wang et al. 2007) (Fig 3.5). In all, samples showed significant coverage among the recognized bacterial phyla, even though the rRNA gene libraries were relatively small (64, 57, 75, and 72, for the Las Vegas Wash, Las Vegas Bay, Drinking Water Intake, and Colorado River, respectively (see Table 3.4)). Proteobacteria dominated the community from the Las Vegas Wash (68%, Fig. 3.5), whereas Las Vegas Bay showed an equal proportion of Bacteroidetes (33%) and Proteobacteria (33%). Cyanobacteria were relatively uniformly distributed across the

dataset, although at a somewhat lower proportion in the Drinking Water Intake, as expected due to the lower depth of this site. The Drinking Water site was the only site to contain Nitrospira and Gemmatimonadetes. All sites contained Actinobacteria and a small proportion of Verrucomicrobia, with the exception of the Las Vegas Wash, which had none.

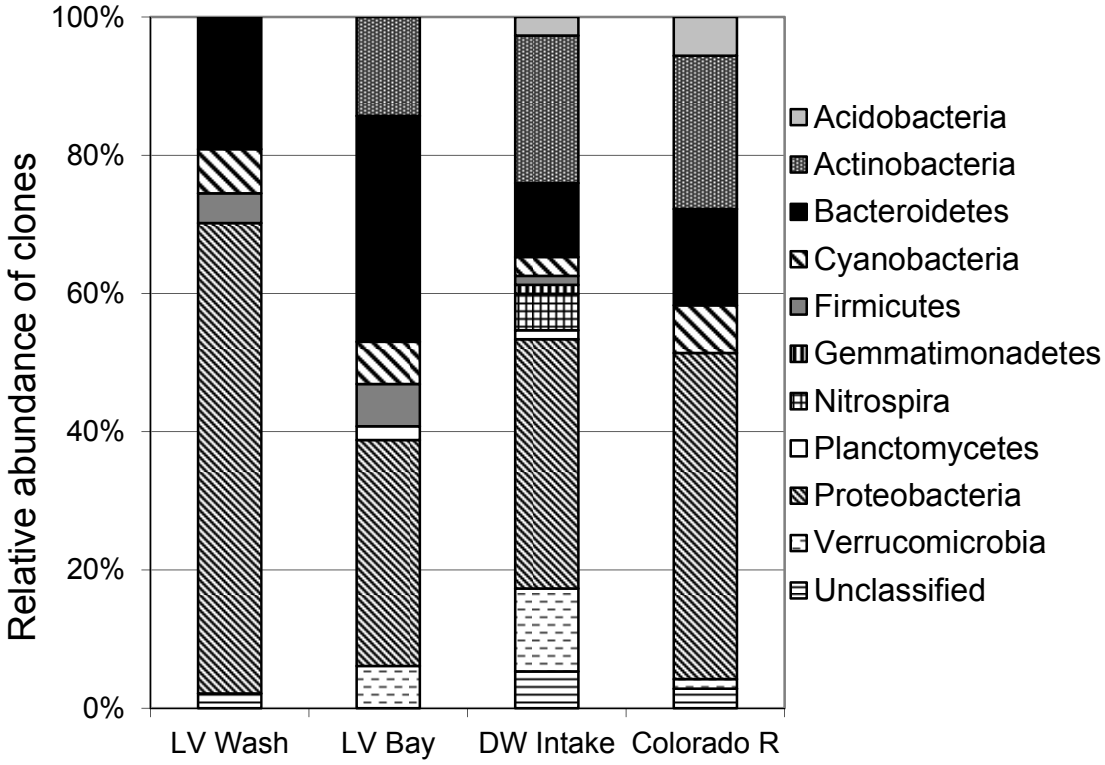


Figure 3.5. Phylum-level distributions assigned to sequences using the Ribosomal Database Project classifier tool (http://rdp.cme.msu.edu/classifier).

Bacterial community compositions of the four microcosm environments were compared using the Unifrac distance matrix (Lozupone et al. 2006). A UPGMA tree illustrated the uniqueness of the Las Vegas Wash bacterial community relative to the other sites (Fig. 3.6) with the Las Vegas Wash branch clearly distinct from the other site

branches. Unifrac Significance Analysis further showed that the Las Vegas Wash community was significantly different than the other three sites ($p < 0.01$). Analysis using LIBSHUFF statistical comparison in Mothur (Schloss et al. 2009), also showed significant differences ($p<0.0001$) between the Wash bacterial community and each of the other three sites (data not shown). Comparison of bacterial community structure from the four sites was further examined using T-RFLP profiling (Fig. 3.7). Conspicuous shared ribotypes exist between Las Vegas Bay and the Colorado River (130 bp, blue arrows, Fig. 3.7), and between Las Vegas Bay, the Drinking Water Intake, and the Colorado River (291 bp, red arrows, Fig. 3.7).

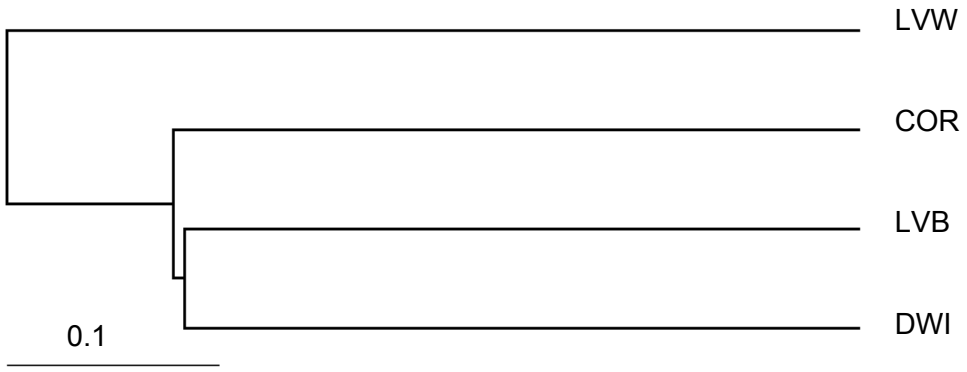


Figure 3.6. Comparison of the bacterial community profiles. Dendogram was created from the 16S rRNA gene library OTUs and the UPGMA method calculated in UNIFRAC (Lozupone et al. 2006). LVW = Las Vegas Wash, LVB = Las Vegas Bay; DW = Drinking water intake; COR = Colorado River.

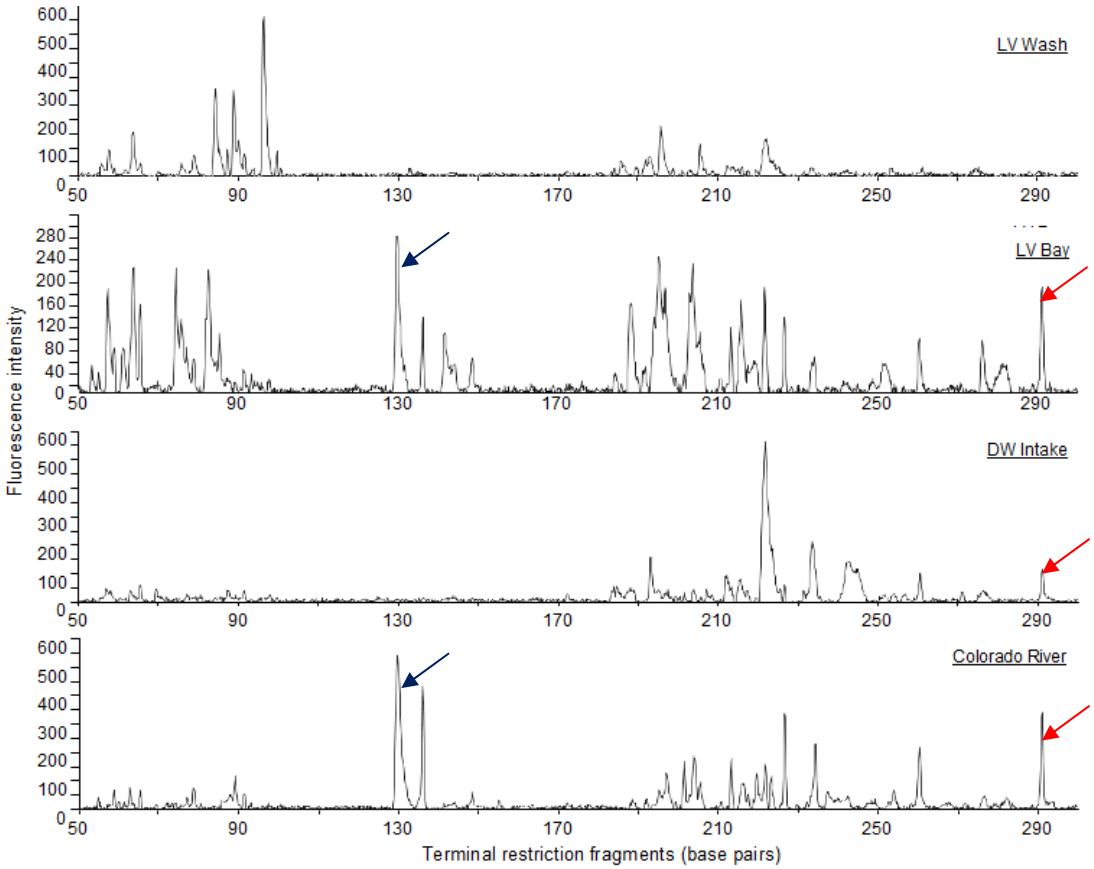


Figure 3.7. Qualitative overview showing patterns of bacterial diversity across the sample set (T-RFLP profiles). Several prominent shared peaks are denoted at 130 bp (blue arrow) and 291 bp (red arrow).

Phylogenetic trees summarized the phylogenetic relationships between library clones in the four datasets and their cultivated relatives (Fig. 3.8 and 3.9). Many of the clone sequences were affiliated with isolates previously shown to be capable of pollutant biodegradation or isolated from contaminant containing sites. This was evident particularly from the Las Vegas Wash community. Within the phylum Proteobacteria, the class Epsilonproteobacteria was exclusively represented by Las Vegas Wash clones, two of which were 98% identical to *Sulfurospirillum* sp. str. JPD-1 (Acc. AY189928.1), a strain capable of biotransformation of tetrachloroethene. Additionally, five other Las Vegas Wash clones were 99% related to the bacterium *Sulfuricurvum kujiense* str. YK-3

(Acc. AB080644.1), a sulfur-oxidizing chemolithoautotroph able to grow on crude oil. Three Las Vegas Wash clones contained phylotypes with 97% identity to *Denitromonas aromaticus* str. AS-7 (AB049763.1), an aromatic compound-degrading bacterium previously isolated from activated sludge belonging to the Betaproteobacteria class. *Methylophilus* sp. U33 (EU375653.1), a betaproteobacterial strain capable of degrading organic pollutants, was the closest isolate (95% identity) related to three Drinking Water Intake clones, eight Colorado River, and two Las Vegas Bay clones. The trichloroethylene degrader, Bacterium C115 (AB167243.1), with 98% identity to the Colorado River clone COR 04G, in the Betaproteobacteria class, was isolated from a chemostat enrichment culture. Other clones within the Betaproteobacteria class included two Las Vegas Wash clones with 97% identity to *Azoarcus* sp. LU1 (AJ007007.1), a bacterium isolated from a compost biofilter which is able to degrade toluene, and *Burkholderia* sp. str. IMER-A1-13 (FJ434112.1), which can catabolize aromatic compounds under oligotrophic conditions. Two Drinking Water clones, 09B and 01G, were most closely related to *Steroidobacter denitrificans* str. FS (89% identity; EF605262.1). This isolate, in the Gammaproteobacteria class, has steroid hormone-degrading abilities. *Sphingomonas* sp str. D12 (AB105809.1), within the Alphaproteobacteria class and a known estrogen-degrading bacterium, is 95% related to two Colorado River clones. *Agrobacterium sanguineum* str. ATCC 25660 (AB062105.1), also within the class Alphaproteobacteria, has the ability to degrade biphenyl and dibenzofuran, and has 99% identity to a Colorado River clone. *Holophaga foetida* str. TMBS4-T (X77215.1), a strain able to degrade methoxylated aromatic compounds in the phylum Acidobacteria, is 91% related to the Colorado River clone

07D. Within the Firmicutes phylum, one OTU from the Las Vegas Wash site shared 97% identity to *Paenibacillus* sp. Sphe2 (AJ699168), a PAH-degrading bacterium isolated from a creosote-contaminated site (Kallimanas 2004). The high occurrence of clones related to known contaminant-degrading bacteria, particularly in the Las Vegas Wash and Colorado River, suggests the presence of organisms at these sites with the ability to degrade or at a minimum tolerate xenobiotic compounds and contaminants, particularly those of an aromatic nature. This is particularly noteworthy in lieu of the fact that the majority of the compounds used in this study are aromatic compounds themselves.

Microbial functional diversity

Results from the Biolog EcoPlates, which were used to estimate microbial functional diversity, demonstrated that the microbial community of the Las Vegas Wash was capable of using most (30 of 31) of the suite of carbon substrates provided (Table 3.5). In comparison, the Las Vegas Bay community was able to use little more than half (16) of the substrates, whereas the Drinking Water Intake and Colorado River were far lower, with an ability to utilize 5 and 3 of the substrates, respectively. The Las Vegas Wash's considerably greater metabolic diversity is consistent with other results showing the uniqueness of the Wash bacterial community relative to the other sites in addition to the relatively high number of Las Vegas Wash clones known for their degradation capabilities.

Fig. 3.8. Maximum-likelihood tree with Bacteriodetes, Nitrospira, Gemmatimonadetes, Verrucomicrobia, and Cyanobacteria compressed.

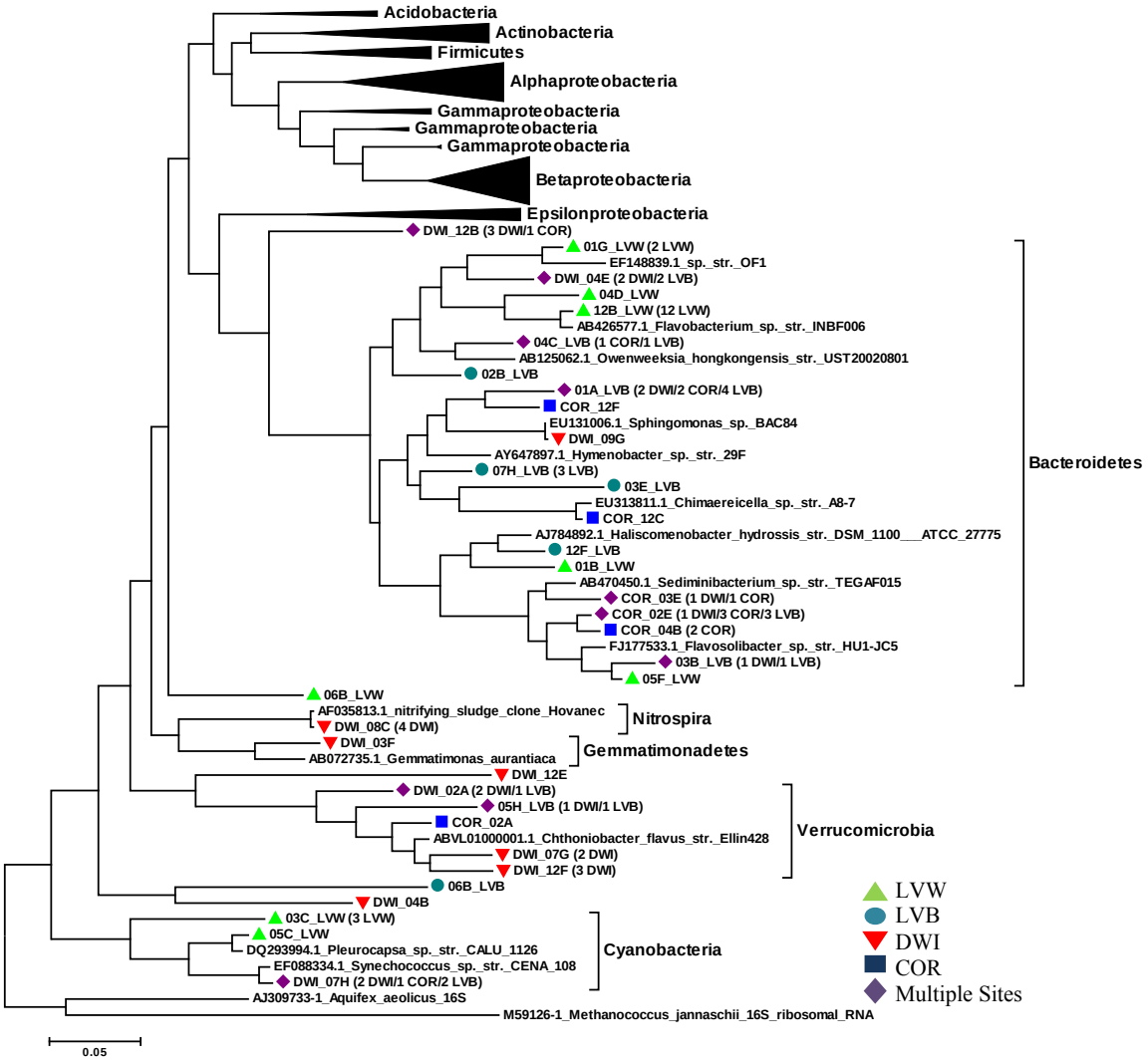


Fig. 3.9. Maximum-likelihood tree with Acidobacteria, Actinobacteria, Firmicutes, and Proteobacteria compressed.

Table 3.5. Community metabolic diversity using Biolog EcoPlates at T=0.

Substrate	LV Wash	LV Bay	DW Intake	Colo. R	Control
Water					
pyruvic acid methyl ester	X	X			
Tween 40	X	X	X		
Tween 80	X		X		
alpha-cyclodextrix	X				
glycogen	X	X		X	
D-cellulose	X	X			
alpha-D-lactose	X	X			

Substrate	LV Wash	LV Bay	DW Intake	Colo. R	Control
beta-methyl-D-glucoside	X				
D-xylose	X				
i-erythritol	X	X			
D-mannitol	X	X		X	
n-acetyl-D-glucosamine	X	X			
D-glucosaminic acid	X				
glucose-1-phosphate	X				
D,L-alpha-glycerol phosphate	X				
D-galactonic acid gamma-lactone	X	X			
D-galacturonic acid	X		X	X	
2-hydroxy benzoic acid					
4-Hydroxy Benxoic acid	X	X	X		
gamma-hydroxuybutyric acid	X		X		
itaconic acid	X				
alpha-ketobutyric acid	X	X			
D-malic acid	X				
L-arginine	X	X			
L-asparagine	X	X			
L-phenylalanine	X				
L-serine	X	X			
L-threonine	X	X			
glycyl-L-glutamic acid	X	X			
phenylethylamine	X				
putrescine	X				
Total substrates used	30	16	5	3	0

Bacterial counts

Bacterial abundance was measured at the initiation of the experiment using both flow cytometry and heterotrophic plate counts (HPCs). Cell counts from the initial time point of the experiment (Table 3.6) were greatest for the Las Vegas Wash site for both flow cytometry counts (viable and non-viable) and HPCs, numbering 3.4 X 10^5 and 1.2 X 10^5 mL^{-1}, respectively. The Drinking Water Intake had by far the lowest numbers of cells, with almost an order of magnitude lower cell counts than the Wash as measured by flow cytometry, and far lower HPCs, with only 2.0 X 10^2 mL^{-1}. The Las Vegas Bay (with 1.1 X 10^5 mL^{-1}), and consecutively, the Colorado River (with 2.5 X 10^5 mL^{-1}) each had slightly higher counts than the Drinking Water Intake with regard to flow cytometry

values. HPC numbers were again, far lower at these sites when compared to the Wash (8.7×10^2 and 2.0×10^2 mL^{-1}, respectively). These values are generally consistent with the literature as HPCs have been shown to be 1 – 4 orders of magnitude lower than those observed for flow cytometry counts of raw surface and drinking waters (Hoefel et al. 2003, Hammes et al. 2008). This is not unexpected as it is generally acknowledged that only a small percentage of environmental bacteria are culturable using current methodologies (Amann et al. 1995, Oliver 2005). Therefore, it is somewhat surprising to see such a close agreement between the HPC and flow cytometry counts for the Las Vegas Wash site. It is possible that heterotrophic bacteria are more abundant and occur in higher proportions in the Wash due to the higher nutrient concentrations at this location. Additionally, the higher metabolic diversity of the Las Vegas Wash as seen from the EcoPlates, could be attributable to the higher density of heterotrophic bacteria from this site and their ability to utilize a more diverse set of carbon substrates.

Table 3.6. Cell counts (mL^{-1}) at initiation of microcosm experiment using flow cytometry and heterotrophic plate counts (HPCs).

	LV Wash	**Colorado R**	**DW Intake**	**LV Bay**
[a]Flow cytometry	3.4E+05	2.5E+05	5.6E+04	1.1E+05
HPC	1.2E+05	2.4E+03	2.0E+02	8.7E+02

[a]Flow cytometry counts include both viable and non-viable cells

CHAPTER 4

CONCLUSIONS

This study examined the degradation potential of 27 pharmaceuticals and EDCs from four sites differentiated by their proximity to a wastewater effluent source. The goals of the study were to identify what roles bacterial community composition, diversity, and abundance had relative to pharmaceutical and EDC degradation. Of the four microcosms, the greatest overall contaminant removal occurred in the Las Vegas Wash, with 72% overall compound mass reduction after 120 days. The other microcosms exhibited removal to a lesser extent, with 51%, 40%, and 37% for the Las Vegas Bay, Drinking Water Intake, and the Colorado River microcosm, respectively (Table 3.2). The metabolic potential of microorganisms from the four sites measured with Biolog Ecoplates showed a similar pattern, with the Wash community able to use a more extensive range of carbon substrates than any other microcosm community (Table 3.5). Furthermore, community composition analysis using Unifrac (Fig 3.6) and Libshuff statistical methods revealed the Las Vegas Wash phylotypes to be significantly distinct from those of any other site, although there was little difference between the microcosms with regard to diversity and richness. Water chemistry parameters also distinguished the Las Vegas Wash site from the others due to the higher concentrations of nutrients including carbon, nitrogen, and phosphorus. Due to the higher nutrient availability in the Las Vegas Wash, it is difficult to differentiate whether the higher degradation potential exhibited by the Wash microcosm was due to the microbial community itself, or to a more nutrient-favorable environment better suited to heterotrophic activity and subsequent biodegradation. However, 16S rRNA gene libraries did indicate a greater

number of phylotypes related to known isolates with biodegradation abilities, which indicates that microorganisms from the Las Vegas Wash have a greater metabolic potential for EDC catabolism.

Future studies of this type could correct for this ambiguity by providing microcosms supplied with the same nutrient and water chemistry parameters, with different microbial community inocula standardized to the same initial cell concentration. Additionally, nutrients and other abiotic parameters such as pH and temperature could be amended to determine which conditions might favor enhanced degradation. Furthermore, due to the fact that many contaminants are hydrophobic in nature, experiments with microcosms looking at the sediment water interface and any associated microbial biofilms would likely be useful.

Finally, while primary degradation information can assist in estimating the fate and transport of these compounds, there is still very little known about the metabolites, pathways, and transformation products formed as a result of initial biodegradation. Transformation by bacteria can often cause an increase in toxicity and/or estrogenicity of some compounds (i.e. when mercury or triclosan becomes methylated (Schwarzenbach et al. 2006)). Toxicity Identification Evaluations (TIEs) and metabolite identification would be helpful in determining if the primary degradation reduced the toxicity or estrogenicity of the water samples.

APPENDIX

Table A.1. Water Quality Data

	Las Vegas Wash	Las Vegas Bay	DW Intake	Colorado River
Tot. Alkalinity (mg/L)	129	132	143	134
Ammonia (mg/L N)	0.205	0.024	<0.02	<0.02
Boron (mg/L)	0.57	0.12	0.1	<0.1
Bromide (mg/L)	0.99	0.09	0.0817	0.0693
Chloride (mg/L)	340	100	88	80
Conductivity (µs/cm)	2433	1068	1030	908
DO (mg/L)	6.57	7.81	5.87	8.57
Fluoride (mg/L)	0.9	0.35	0.33	0.31
Iron (mg/L)	<0.1	<0.1	<0.1	<0.1
Calcium (mg/L)	130	75	81	69
Magnesium (mg/L)	63	28	27	24
Potassium (mg/L)	32	6.9	5.9	5
Sodium (mg/L)	280	99	96	81
Tot. Hardness (mg/L $CaCO_3$)	590	300	320	270
Nitrate (mg/L N)	14	0.88	0.5	0.2
Nitrite (mg/L N)	<0.05	<0.05	<0.05	<0.05
pH	7.71	8.27	7.93	8.38
o-Phosphate (mg/L P)	0.098	<0.001	0.0014	<0.001
T-Phosphate (mg/L P)	0.12	<0.005	<0.005	0.0055
Silica (mg/L)	19	6.7	7.3	6.2
Sulfate (mg/L)	570	270	250	220
TDS (mg/L)	1541	649.4	618.4	553.6
Temperature (°C)	23.4	21.6	17.8	20.8
TOC (mg/L)	6.0	3.2	2.7	2.9
Aluminum (mg/L)	0.072	<0.005	<0.005	0.037
Antimony (mg/L)	<0.0006	<0.0006	<0.0006	<0.0006
Arsenic (mg/L)	0.0069	0.0028	0.0022	0.0023
Barium (mg/L)	0.066	0.13	0.15	0.13
Beryllium (mg/L)	<0.0004	<0.0004	<0.0004	<0.0004
Cadmium (mg/L)	<0.0005	<0.0005	<0.0005	<0.0005
Chromium (mg/L)	<0.005	<0.005	<0.005	<0.005
Copper (mg/L)	0.025	<0.005	0.012	<0.005
Lead (mg/L)	<0.001	<0.001	<0.001	<0.001
Manganese (mg/L)	0.04	<0.005	<0.005	<0.005
Mercury (mg/L)	<0.0002	0.0002	<0.0002	<0.0002
Molybdenum (mg/L)	0.02	0.0062	0.0051	<0.005
Nickel (mg/L)	0.0057	<0.005	<0.005	<0.005
Selenium (mg/L)	0.0036	0.0024	0.0022	0.0022
Silver (mg/L)	<0.005	<0.005	<0.005	<0.005
Thallium (mg/L)	<0.0002	<0.0002	<0.0002	<0.0002
Vanadium (mg/L)	<0.005	<0.005	<0.005	<0.005
Zinc (mg/L)	0.037	<0.005	0.029	<0.005
TSS (mg/L)	<5	<5	<5	<5
Turbidity (NTU)	2.68	0.51	0.33	2.66
UV_{254} (cm^{-})	0.1000	0.0404	0.0417	0.0511

Table A.2. Background concentrations in Las Vegas Wash before spike mix.

RAW DATA - SAMPLES BEFORE SPIKE		
Date Collected	**10/21/2008 9:05**	**10/23/2008 15:00**
Location	**LV Wash**	**LV Wash**
Sub Location		**T=Oa**
Tap Location		
Sulfamethoxazole	990	1000
Atenolol	630	670
Trimethoprim	58	59
Iopromide	25	<10
Caffeine	14	14
Fluoxetine	12	9.1
Meprobamate	580	560
Dilantin	130	180
Carbamazepine	160	190
Atrazine	0.68	<.25
Diazepam	4.4	2.9
Atorvastatin	6.1	6.6
Benzophenone	83	90
Primidone	150	140
TCPP	1700	1400
DEET	120	96
TCEP	540	490
Gemfibrozil	110	120
Bisphenol A	<5	<5
Diclofenac	39	69
Naproxen	79	88
Triclosan	25	15
Octylphenol	<25	<25
BHA	3.9	<1
Musk Ketone	<25	40
Ibuprofen	6.3	6
Testosterone	<.5	<.5
Progesterone	<.5	<.5
Estrone	<.2	<.2
Estradiol	<.5	<.5
Ethynylestradiol	<1	<1

Table A.3. Background concentrations in Drinking Water Intake before spike mix.

RAW DATA - SAMPLES BEFORE SPIKE		
Date Collected	**10/21/2008 9:27**	**10/23/2008 15:00**
Location	**IPS 2**	**IPS 2**
Sub Location		**T=Oa**
Tap Location		
Sulfamethoxazole	18	20
Atenolol	<1	1.1
Trimethoprim	0.4	0.71
Iopromide	<10	<10
Caffeine	<5	5.4
Fluoxetine	<.5	<.5
Meprobamate	10	10
Dilantin	2.6	2.5
Carbamazepine	3.4	2.8
Atrazine	1.1	1.1
Diazepam	<.25	<.25
Atorvastatin	<.5	<.5
Benzophenone	<50	<50
Primidone	2.8	3
TCPP	<100	<100
DEET	5.2	5.1
TCEP	<10	<10
Gemfibrozil	0.26	<.25
Bisphenol A	<5	<5
Diclofenac	<.5	<.5
Naproxen	<.5	<.5
Triclosan	<1	1.1
Octylphenol	<25	<25
BHA	<1	<1
Musk Ketone	<25	<25
Ibuprofen	<1	<1
Testosterone	<.5	<.5
Progesterone	<.5	<.5
Estrone	<.2	<.2
Estradiol	<.5	<.5
Ethynylestradiol	<1	<1

Table A.4. Background concentrations in Colorado River before spike mix.

RAW DATA - SAMPLES BEFORE SPIKE		
Date Collected	**10/22/2008 11:00**	**10/23/2008 15:00**
Location	**Up Co. Riv.**	**Up Co. Riv.**
Sub Location		**T=Oa**
Tap Location		
Sulfamethoxazole	6.8	7
Atenolol	<1	<1
Trimethoprim	<.25	<.25
Iopromide	<10	<10
Caffeine	6	5.2
Fluoxetine	<.5	<.5
Meprobamate	2.4	2.3
Dilantin	<1	1.6
Carbamazepine	0.96	1.1
Atrazine	1.2	0.98
Diazepam	<.25	<.25
Atorvastatin	<.5	<.5
Benzophenone	<50	<50
Primidone	0.77	0.57
TCPP	<100	<100
DEET	15	3.6
TCEP	<10	<10
Gemfibrozil	<.25	<.25
Bisphenol A	<5	<5
Diclofenac	<.5	<.5
Naproxen	<.5	<.5
Triclosan	12	1.9
Octylphenol	<25	<25
BHA	<1	<1
Musk Ketone	<25	<25
Ibuprofen	<1	<1
Testosterone	<.5	<.5
Progesterone	<.5	<.5
Estrone	<.2	<.2
Estradiol	<.5	<.5
Ethynylestradiol	<1	<1

Table A.5. Background concentrations in Las Vegas Bay before spike mix.

RAW DATA - SAMPLES BEFORE SPIKE		
	10/23/2008 9:30	**10/23/2008 15:00**
	LAKE MEAD/BB	**LVB 6.7**
	LVB6.7	**T=Oa**
Tap Location	**0M**	
Sulfamethoxazole	38	37
Atenolol	5.6	6.1
Trimethoprim	<.25	<.25
Iopromide	<10	<10
Caffeine	51	140
Fluoxetine	0.52	<.5
Meprobamate	26	28
Dilantin	5.4	6
Carbamazepine	6.3	6.5
Atrazine	0.32	<.25
Diazepam	<.25	<.25
Atorvastatin	<.5	<.5
Benzophenone	<50	<50
Primidone	6.3	6.7
TCPP	<100	<100
DEET	22	9.3
TCEP	<10	<10
Gemfibrozil	<.25	<.25
Bisphenol A	<5	19
Diclofenac	<.5	<.5
Naproxen	<.5	0.5
Triclosan	<1	<1
Octylphenol	<25	<25
BHA	<1	<1
Musk Ketone	<25	<25
Ibuprofen	<1	<1
Testosterone	<.5	<.5
Progesterone	<.5	<.5
Estrone	<.2	<.2
Estradiol	<.5	<.5
Ethynylestradiol	<1	<1

Table A.6. Raw data values for compound concentrations at each site after spike was added for Las Vegas Wash.

	Total Concentrations							
day	0	1	4	7	14	29	56	120
Sulfameth.	1200	1300	1300	1400	1400	1400	33	58
Atenolol	800	850	640	340	<4	<4	<4	<4
Trimethoprim	310	340	320	310	290	81	32	13
Iopromide	200	580	430	300	100	<40	<40	<40
Caffeine	460	490	450	500	530	360	220	64
Fluoxetine	190	210	200	190	210	170	140	100
Meprobamate	840	790	840	830	600	53	41	49
Dilantin	580	500	460	410	410	360	360	81
Carbamazepine	410	440	420	430	390	350	370	310
Atrazine	240	300	250	260	230	220	200	200
Diazepam	340	350	310	350	370	340	320	280
Atorvastatin	230	210	240	250	210	70	32	18
Primidone	420	390	380	400	400	400	380	400
DEET	210	270	220	210	200	9.9	10	23
TCEP	670	640	670	670	610	580	570	580
Gemfibrozil	340	350	350	350	340	290	200	15
Bisphenol A	360	340	210	180	30	21	<5	27
Diclofenac	370	410	370	370	260	250	220	110
Naproxen	350	380	360	360	350	180	85	<2
Triclosan	80	94	66	49	39	<4	<1	<4
BHA	140	170	93	66	31	15	<1	<4
Ibuprofen	310	340	300	250	210	100	<1	<4
Testosterone	296	20	<2	<2	<2	<2	<2	<2
Progesterone	27	<2	<2	<2	<2	<2	<2	<2
Estrone	22	26	28	9.3	<.8	<.8	<.8	<.8
Estradiol	60	39	5.7	<2	<2	<2	<2	<2
Ethynylestradiol	148	138	131	128	94	108	70	48
Total ng/L	8404	8666	7744	7212	5904	3958	3250	2318
Total % removal	**0**	**-3**	**8**	**14**	**30**	**53**	**61**	**72**

Table A.7. Raw data values for compound concentrations at each site after spike was added for Las Vegas Bay.

	Total Concentrations								
day	0	1	2	4	7	14	29	56	120
Sulfameth.	340	360	390	410	350	400	380	390	310
Atenolol	260	250	240	280	250	260	210	77	8.9
Trimethoprim	270	300	310	300	320	290	280	260	220
Iopromide	380	320	380	330	360	410	350	210	340
Caffeine	560	580	590	560	510	710	600	150	<20
Fluoxetine	250	230	240	230	210	210	170	110	51
Meprobamate	330	350	350	370	370	350	350	360	330
Dilantin	230	250	320	290	340	280	290	290	280
Carbamazepine	270	240	300	290	270	230	250	210	230
Atrazine	210	240	240	260	250	250	220	180	160
Diazepam	340	310	360	360	340	350	300	280	220
Atorvastatin	230	210	220	230	240	170	150	160	110
Primidone	240	290	240	250	320	260	260	290	260
DEET	140	160	140	150	140	160	160	170	130
TCEP	170	190	170	210	220	110	120	94	140
Gemfibrozil	240	260	240	240	260	230	270	240	230
Bisphenol A	320	330	310	330	320	290	290	260	<20
Diclofenac	420	430	450	490	510	280	270	320	110
Naproxen	270	270	280	290	310	280	280	250	<2
Triclosan	76	74	67	65	67	65	60	38	26
BHA	340	340	290	300	290	180	78	36	6.5
Ibuprofen	330	300	320	300	320	340	230	<1	<4
Testosterone	115	12.9	<2	<2	<2	<2	<2	<2	<2
Progesterone	32.6	10.1	<2	<2	4.38	<2	<2	<2	<2
Estrone	61.9	83.3	86.8	72.8	45.3	26.3	<.8	<.8	<.8
Estradiol	23.6	5.51	<2	<2	<2	<2	<2	<2	<2
Ethynylestradiol	140	157	160	136	125	115	119	109	85.6
Total ng/L	6589	6553	6694	6744	6742	6246	5687	4484	3248
Total % removal	**0**	**1**	**-2**	**-2**	**-2**	**5**	**14**	**32**	**51**

Table A.8. Raw data values for compound concentrations at each site after spike was added for Las Vegas Bay Sterile Control.

	Total Concentrations								
day	0	1	2	4	7	14	29	56	120
Sulfameth.	380	340	360	390	350	400	410	370	310
Atenolol	270	310	260	280	260	270	270	270	220
Trimethoprim	280	270	290	280	330	260	260	250	230
Iopromide	290	320	210	450	550	290	320	280	430
Caffeine	550	540	560	540	570	680	680	580	600
Fluoxetine	250	250	230	240	260	260	220	190	160
Meprobamate	350	320	360	360	350	340	370	350	370
Dilantin	320	280	260	240	310	270	320	310	270
Carbamazepine	290	290	270	270	270	220	230	230	200
Atrazine	260	220	220	250	250	250	210	180	160
Diazepam	330	330	330	350	360	340	310	280	250
Atorvastatin	220	210	210	220	230	190	220	160	160
Primidone	310	270	260	260	350	290	270	270	300
DEET	140	150	140	140	140	170	170	160	150
TCEP	210	210	200	300	220	150	130	110	140
Gemfibrozil	250	250	250	280	250	270	230	250	250
Bisphenol A	300	340	280	340	320	340	270	280	270
Diclofenac	490	440	460	430	430	290	270	280	260
Naproxen	290	280	300	290	280	280	280	250	270
Triclosan	76	70	68	70	71	84	74	63	54
BHA	310	320	310	270	200	100	22	<1	<4
Ibuprofen	300	320	310	310	310	280	300	310	310
Testosterone	141	166	190	177	108	<2	<2	<2	<2
Progesterone	31.7	29.5	30.1	26.2	24.9	<2	16	<2	<2
Estrone	49.1	36.2	33.1	29.5	38.7	56.1	82.7	71.9	63
Estradiol	28.8	35.9	42.3	37.9	33.1	16.3	<2	<2	2.84
Ethynylestradiol	162	153	170	133	151	117	130	105	100
Total ng/L	6879	6751	6604	6964	7017	6213	6065	5600	5530
Total % removal	**0**	**2**	**4**	**-1**	**-2**	**10**	**12**	**19**	**20**

Table A.9. Raw data values for compound concentrations at each site after spike was added for Drinking Water Intake.

	Total Concentrations								
day	0	1	2	4	7	14	29	56	120
Sulfameth.	320	310	340	320	340	360	370	340	360
Atenolol	270	240	260	270	260	270	260	190	120
Trimethoprim	290	280	280	300	280	280	290	270	240
Iopromide	210	320	380	390	400	300	290	310	400
Caffeine	460	430	460	380	430	420	490	380	390
Fluoxetine	240	250	250	240	240	180	91	31	7.4
Meprobamate	320	330	350	340	340	400	380	290	330
Dilantin	310	360	270	320	400	330	330	300	230
Carbamazepine	190	250	230	280	250	240	250	200	200
Atrazine	220	250	240	240	250	210	210	190	150
Diazepam	340	410	370	370	330	310	320	260	160
Atorvastatin	210	220	190	170	170	120	75	53	35
Primidone	260	290	270	280	270	300	280	270	280
DEET	140	130	150	140	130	150	140	130	120
TCEP	190	190	200	210	270	140	140	100	100
Gemfibrozil	250	240	240	250	250	240	230	240	250
Bisphenol A	290	290	250	250	88	42	<20	<5	27
Diclofenac	310	330	310	330	330	230	200	260	260
Naproxen	270	290	270	270	270	260	260	230	69
Triclosan	72	68	74	74	73	68	60	44	32
BHA	320	330	250	220	200	120	45	15	<4
Ibuprofen	310	300	280	300	300	280	200	14	4.4
Testosterone	319	140	26	<2	<2	<2	<2	<2	<2
Progesterone	32	12	<2	<2	<2	<2	<2	<2	<2
Estrone	12	14	15	15	14	17	29	15	<.8
Estradiol	70	69	68	41	36	23	3.9	<2	<2
Ethynylestradiol	158	145	149	129	135	108	126	104	96
Total ng/L	6384	6488	6172	6129	6056	5398	5070	4236	3860
Total % removal	**0**	**-2**	**3**	**4**	**5**	**15**	**21**	**34**	**40**

Table A.10. Raw data values for compound concentrations at each site after spike was added for the Colorado River.

day	0	1	2	4	7	14	29	56	120
Sulfameth.	310	390	310	330	330	320	320	330	360
Atenolol	270	270	260	240	260	220	190	87	24
Trimethoprim	280	300	260	290	280	270	280	240	140
Iopromide	410	430	470	500	320	350	270	150	290
Caffeine	460	430	430	520	500	520	550	520	480
Fluoxetine	250	250	230	220	220	180	180	98	55
Meprobamate	310	350	300	320	320	340	340	350	330
Dilantin	250	300	230	410	320	340	290	310	260
Carbamazepine	250	280	260	260	260	230	230	220	220
Atrazine	250	250	240	230	260	230	220	210	180
Diazepam	370	330	340	320	330	320	280	260	200
Atorvastatin	220	250	220	210	210	160	170	150	100
Primidone	280	270	270	270	220	280	290	250	270
DEET	130	120	130	150	150	140	140	120	150
TCEP	180	230	190	190	200	160	160	76	150
Gemfibrozil	250	270	220	240	260	240	240	230	250
Bisphenol A	290	310	310	270	280	290	290	300	220
Diclofenac	350	330	310	350	310	230	210	260	270
Naproxen	290	260	270	260	270	260	260	150	<2
Triclosan	80	70	72	75	67	54	42	39	27
BHA	230	230	200	190	170	100	67	35	4.9
Ibuprofen	310	310	290	290	290	250	260	160	27
Testosterone	242	4.7	<2	<2	<2	<2	<2	<2	<2
Progesterone	26	<2	<2	<2	<2	8.5	<2	<2	<2
Estrone	31	38	17	1.3	<.8	<.8	<.8	<.8	<.8
Estradiol	42	11	<2	<2	<2	<2	<2	<2	<2
Ethynylestradiol	143	156	133	147	134	106	125	90	68
Total ng/L	6504	6440	5962	6283	5961	5598	5404	4635	4076
Total % removal	**0**	**1**	**8**	**3**	**8**	**14**	**17**	**29**	**37**

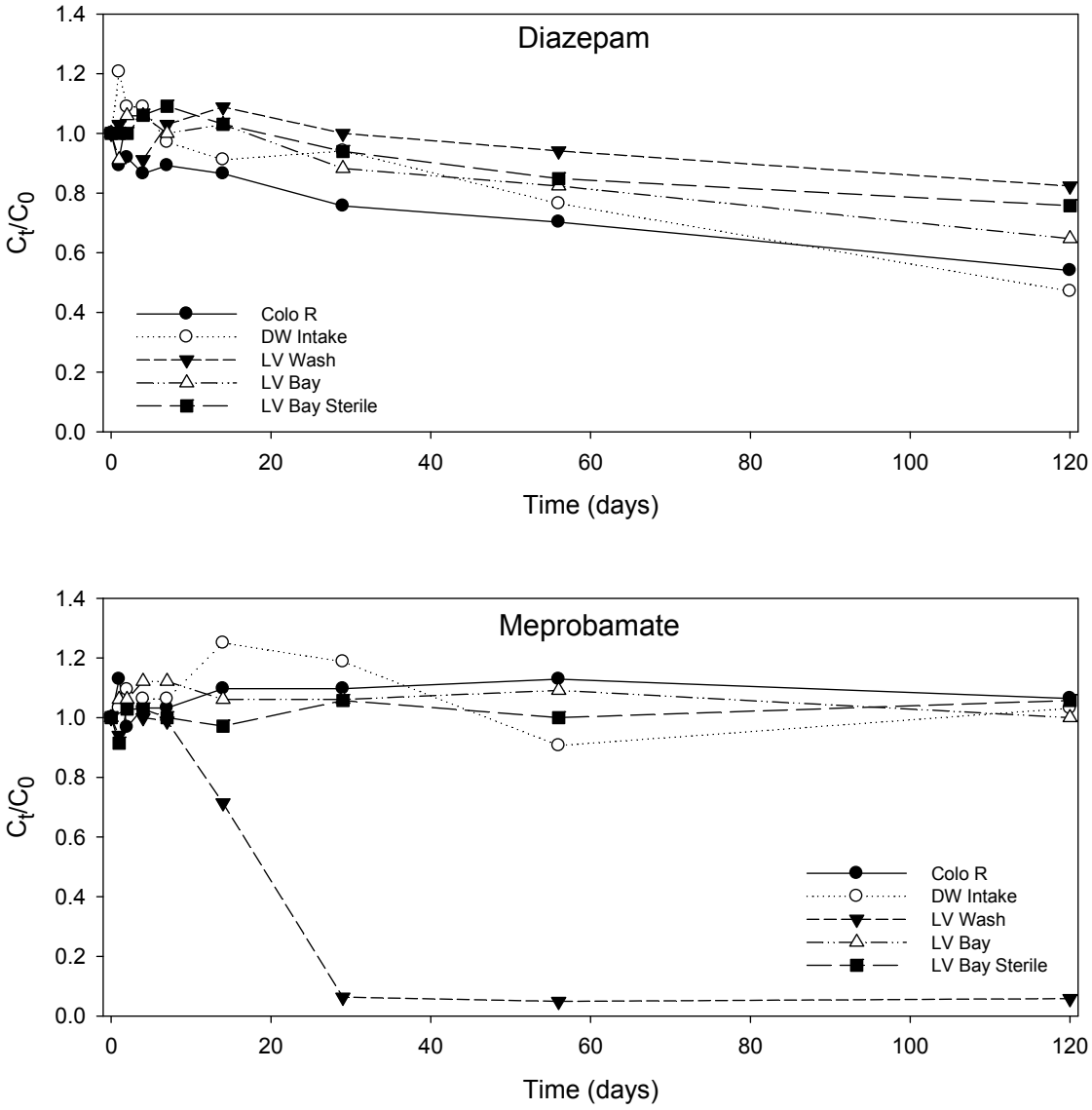


Figure A.1. Degradation curves at each site organized according to chemical compound

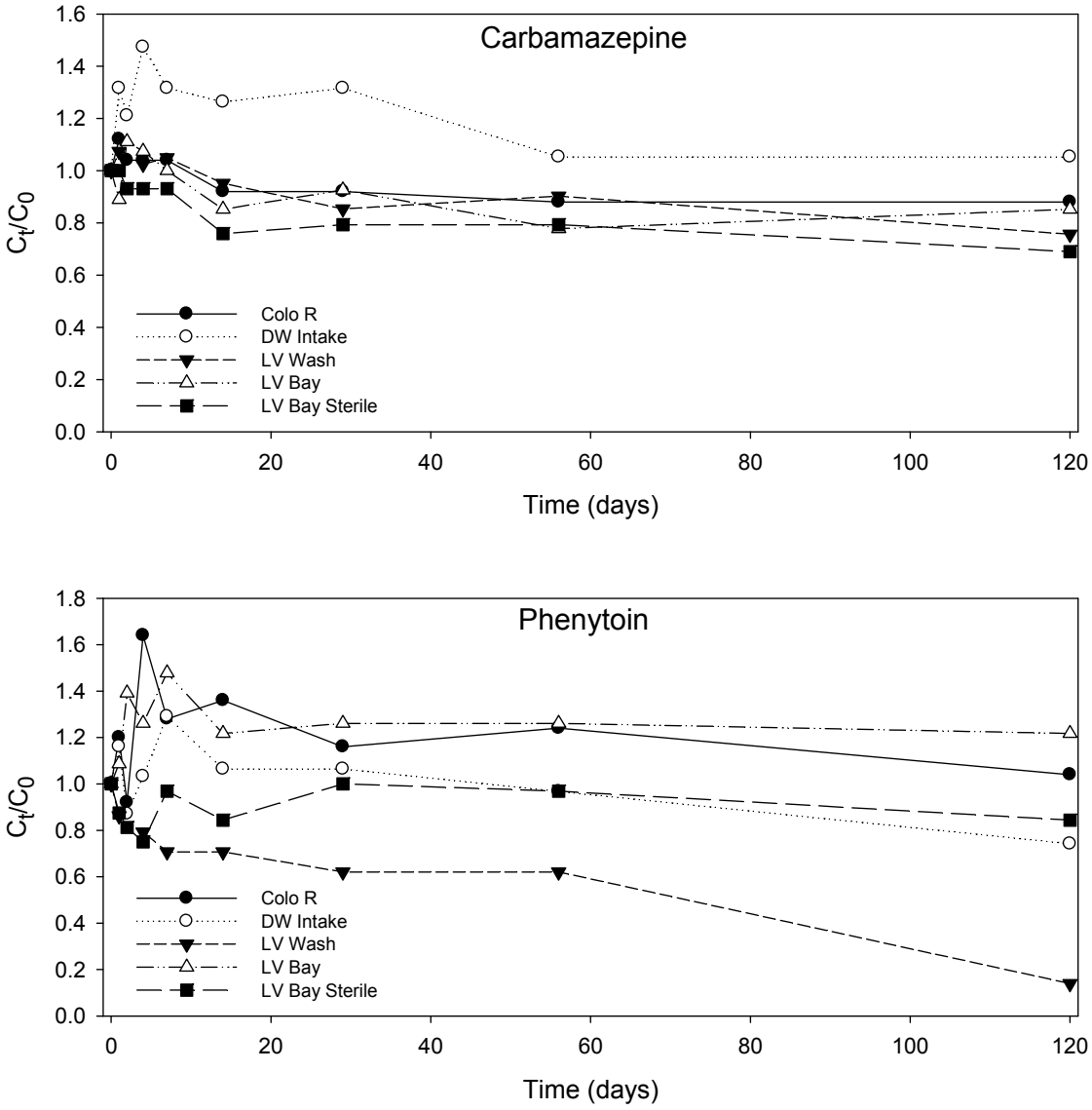


Figure A.2. Degradation curves at each site organized according to chemical compound

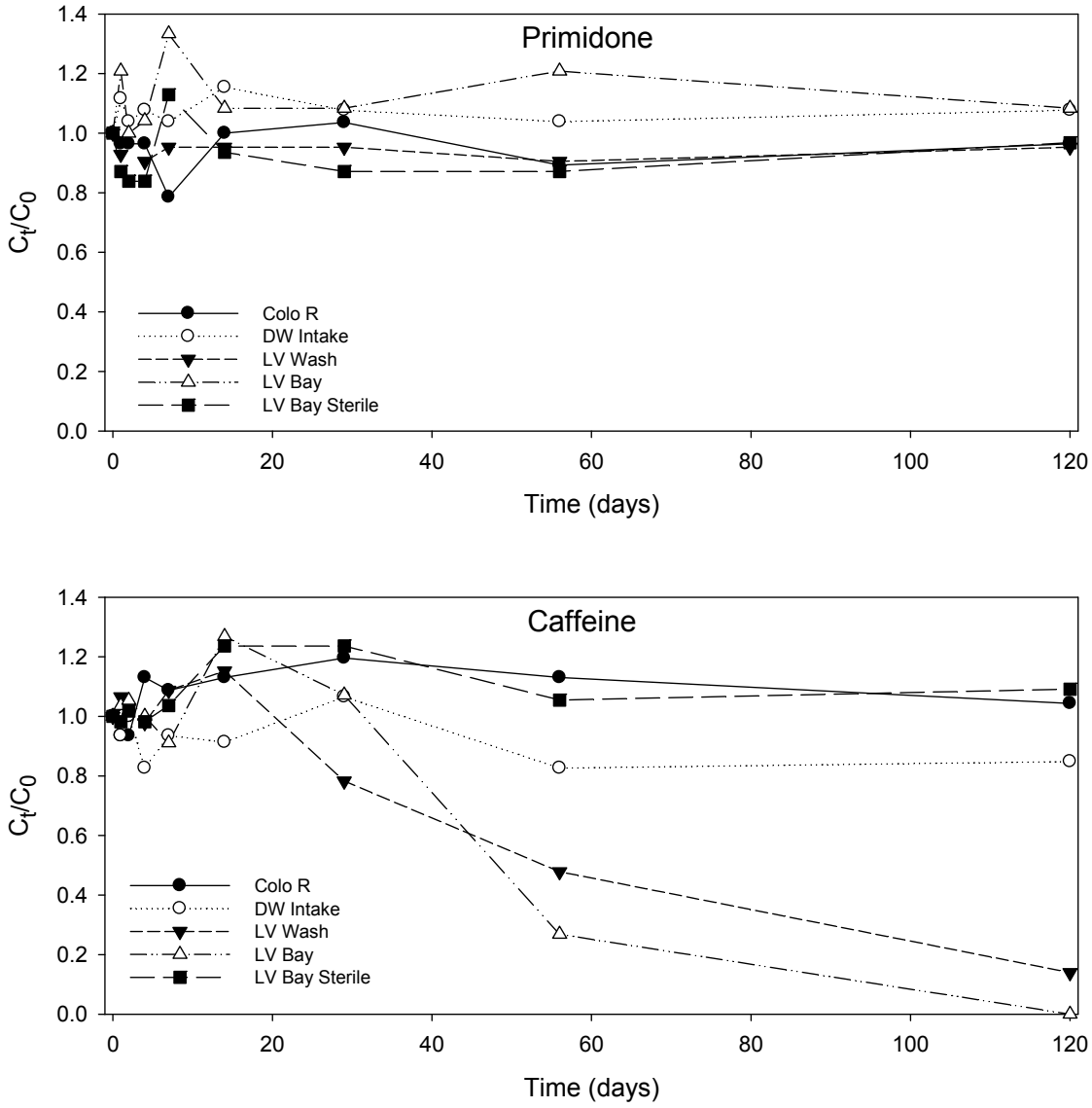


Figure A.3. Degradation curves at each site organized according to chemical compound

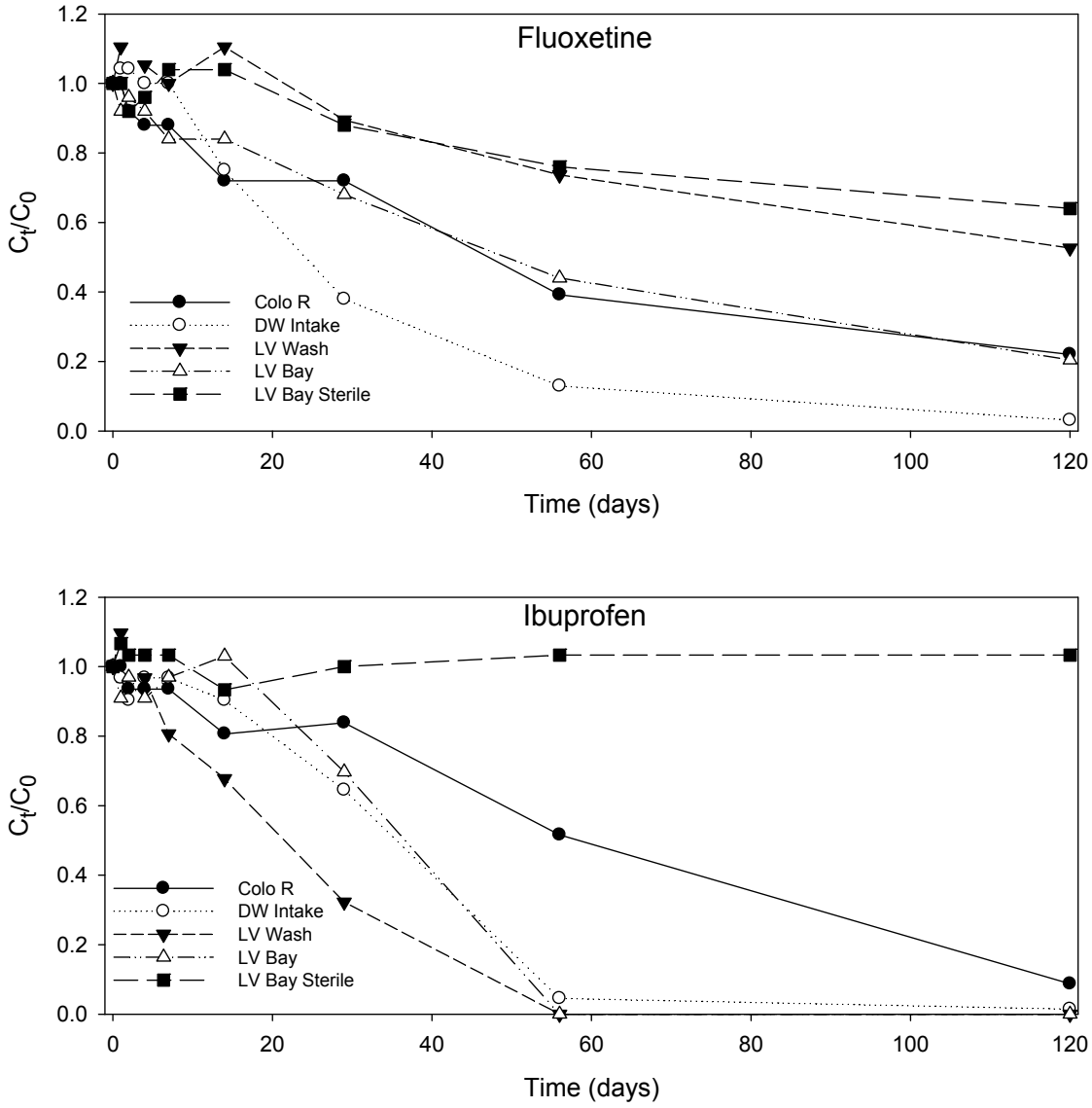


Figure A.4. Degradation curves at each site organized according to chemical compound

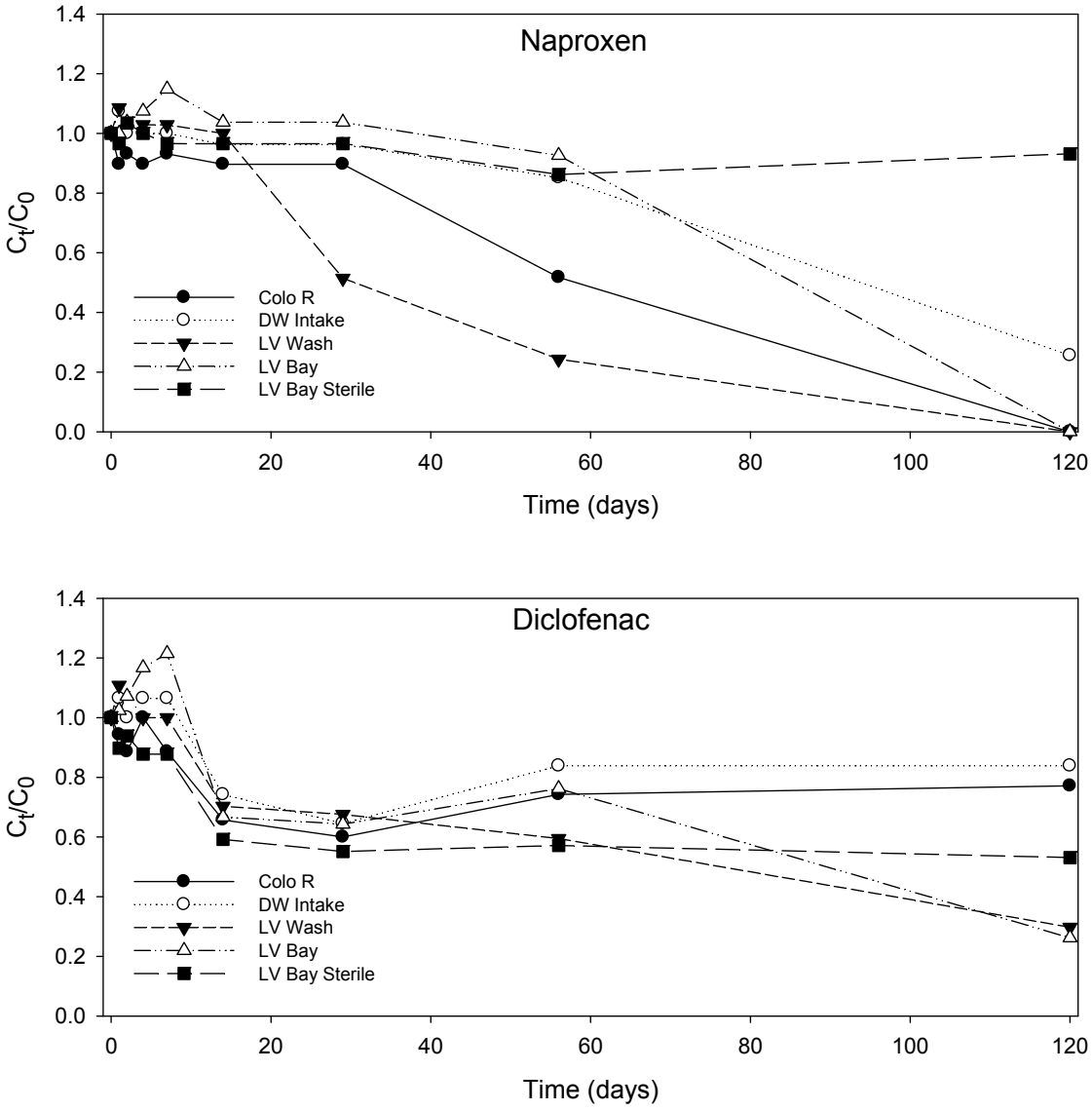


Figure A.5. Degradation curves at each site organized according to chemical compound

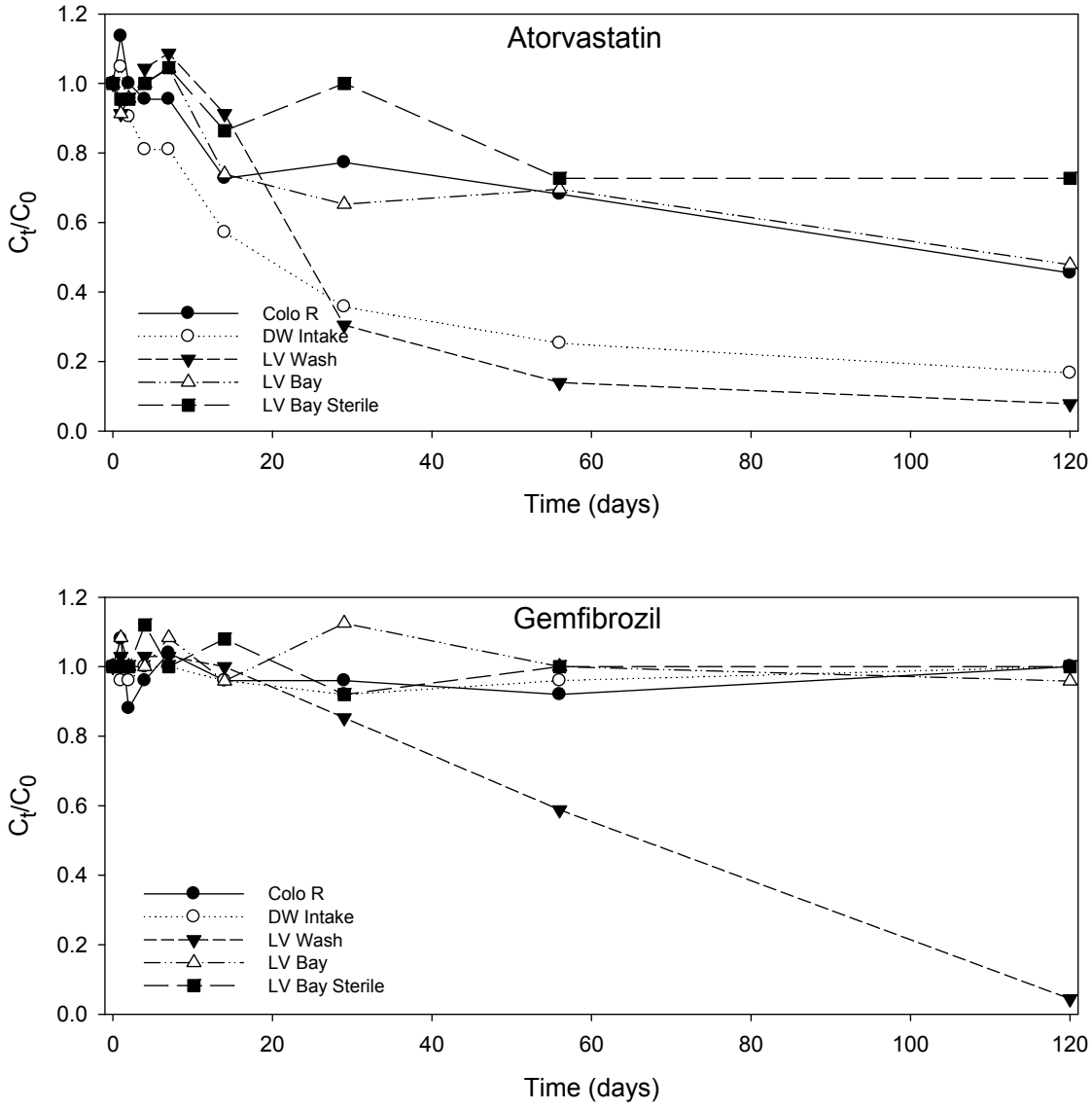


Figure A.6. Degradation curves at each site organized according to chemical compound

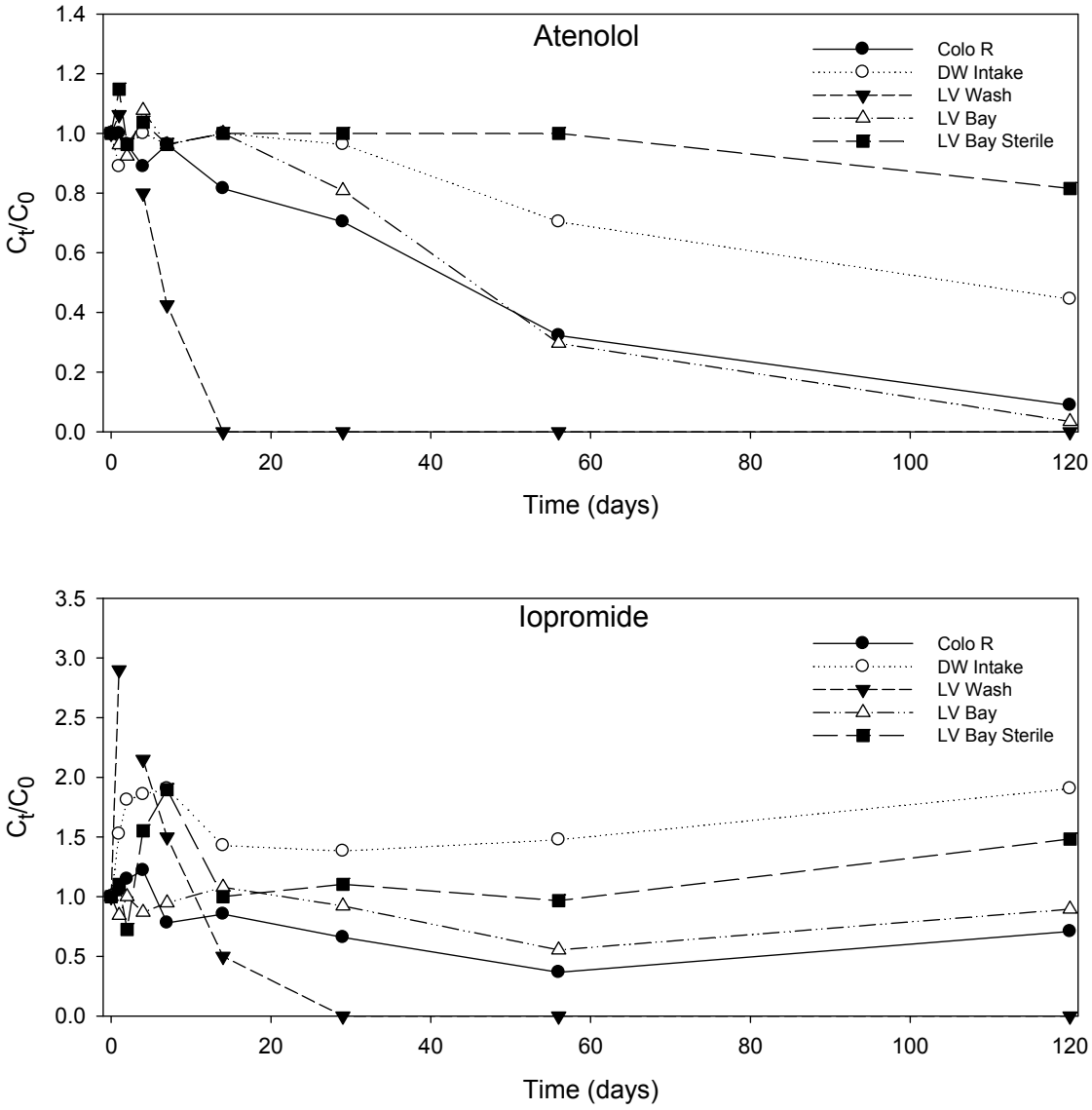


Figure A.7. Degradation curves at each site organized according to chemical compound

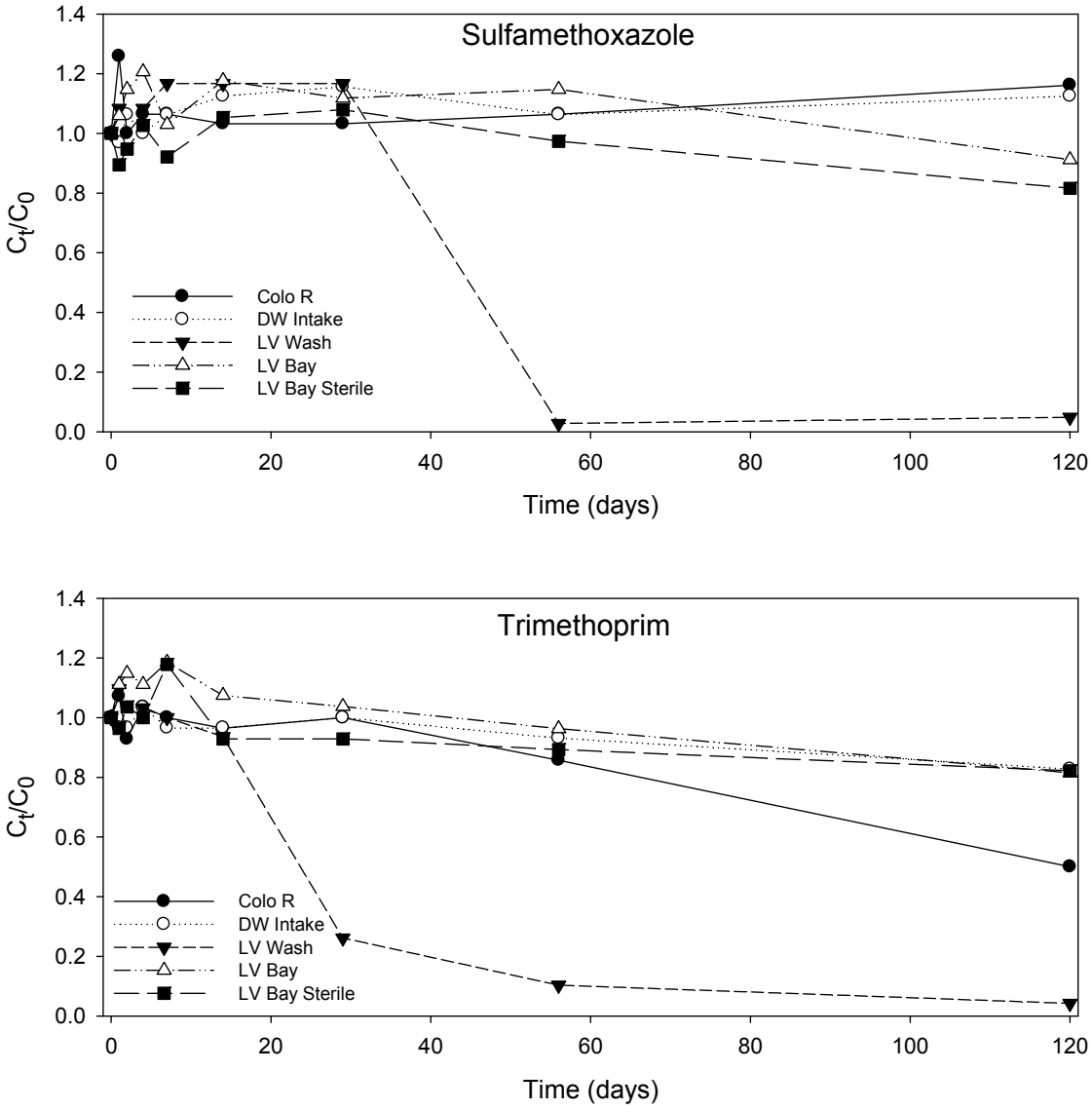


Figure A.8. Degradation curves at each site organized according to chemical compound

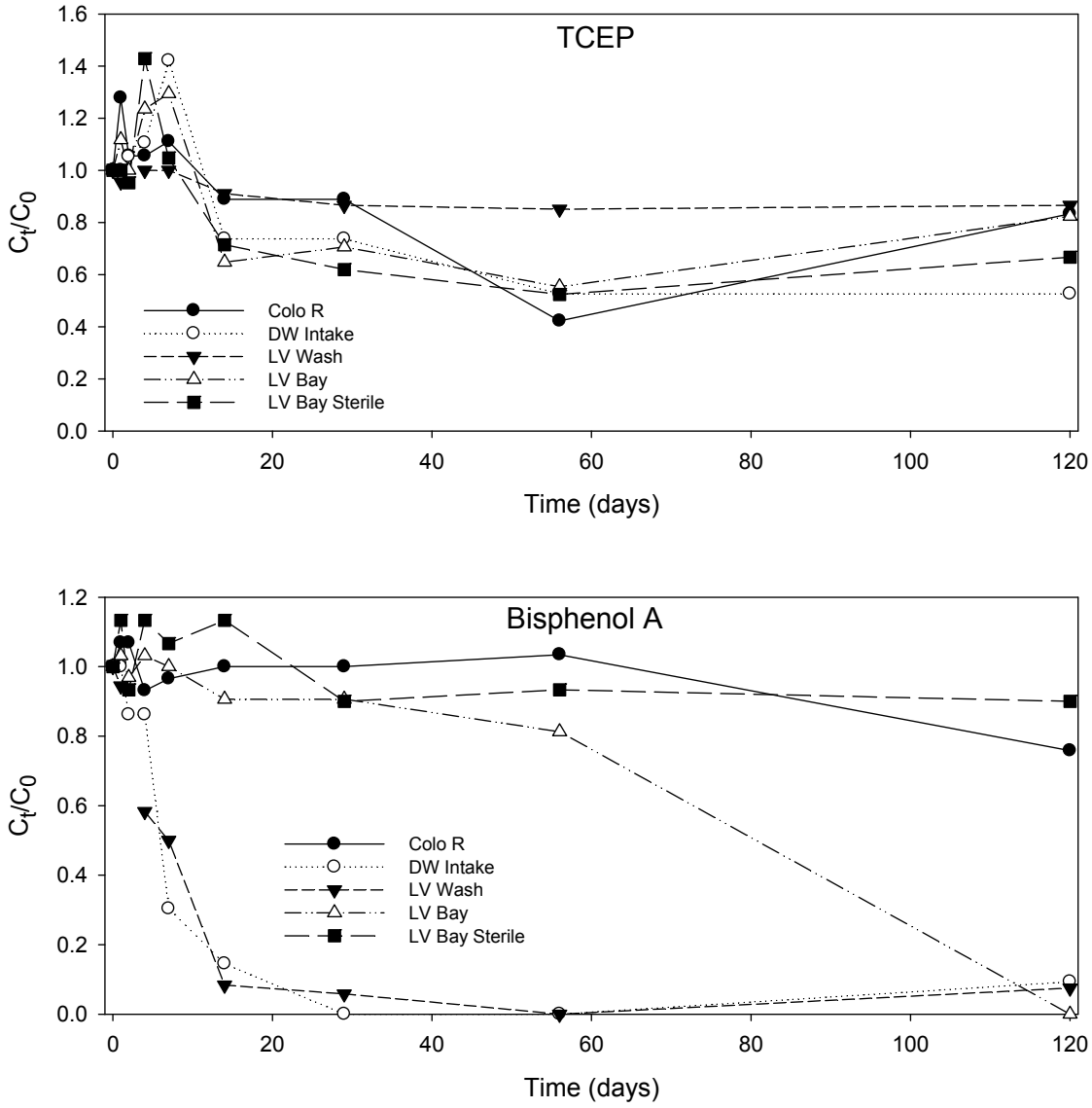


Figure A.9. Degradation curves at each site organized according to chemical compound

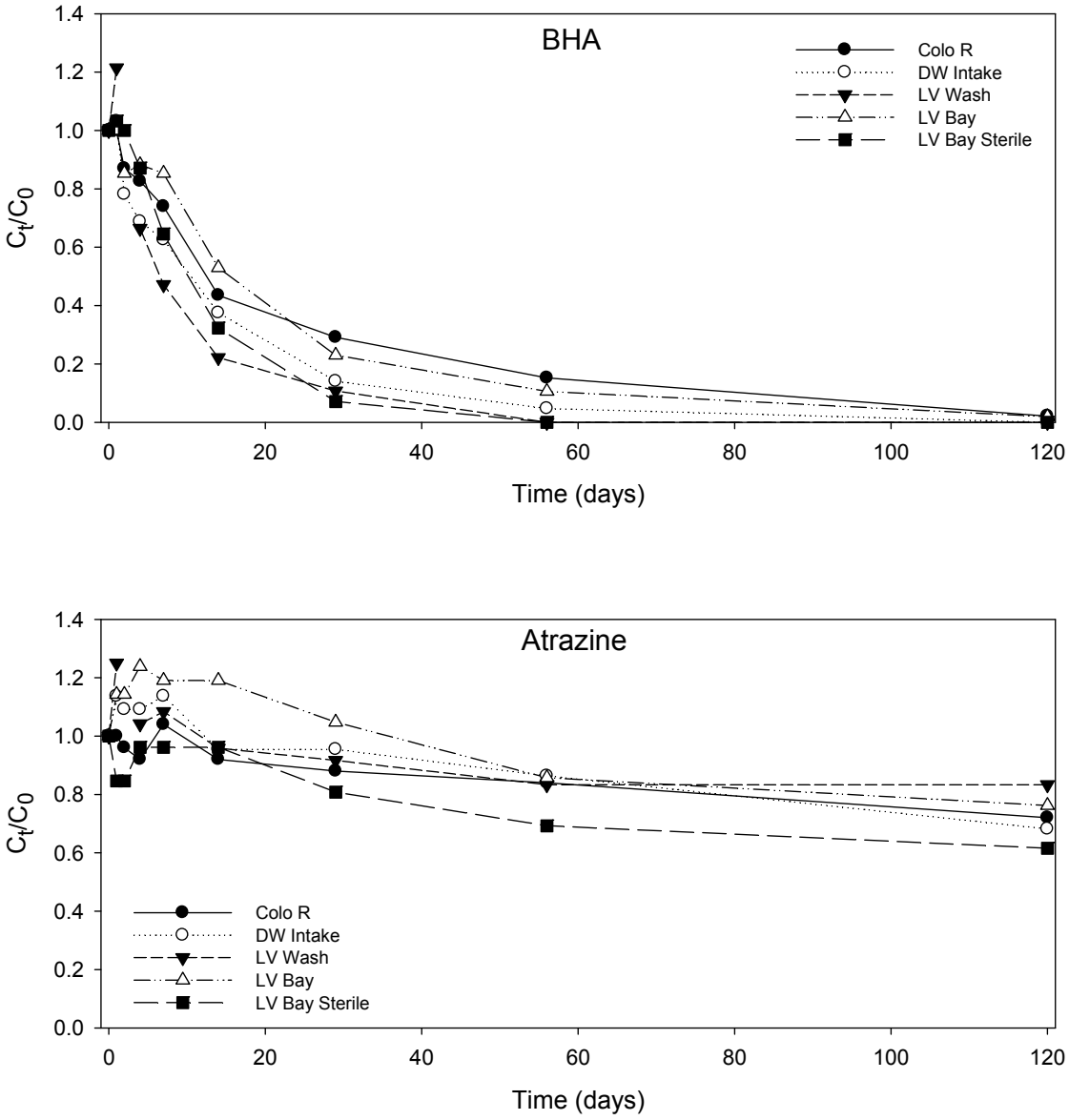


Figure A.10. Degradation curves at each site organized according to chemical compound

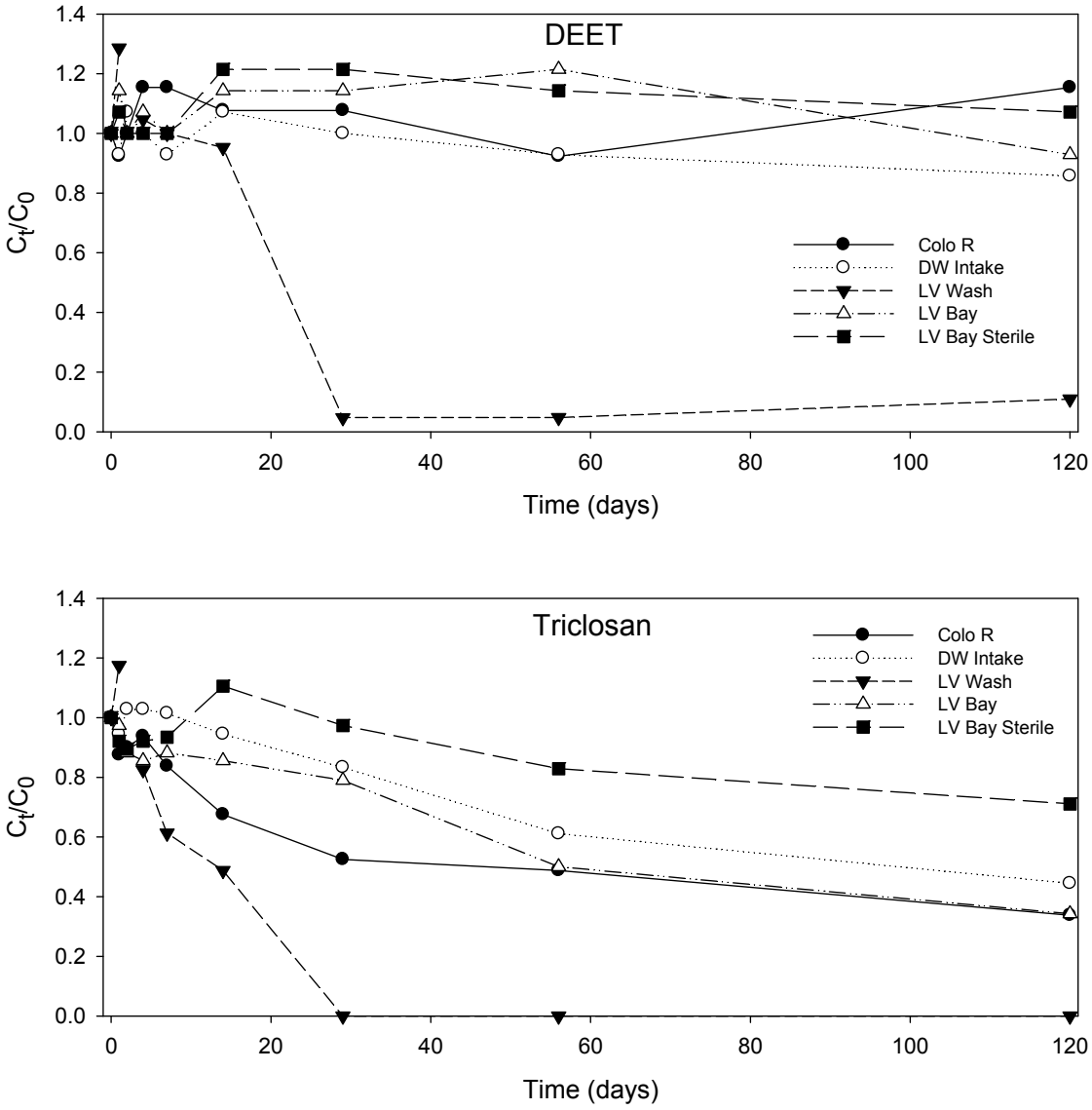


Figure A.11. Degradation curves at each site organized according to chemical compound

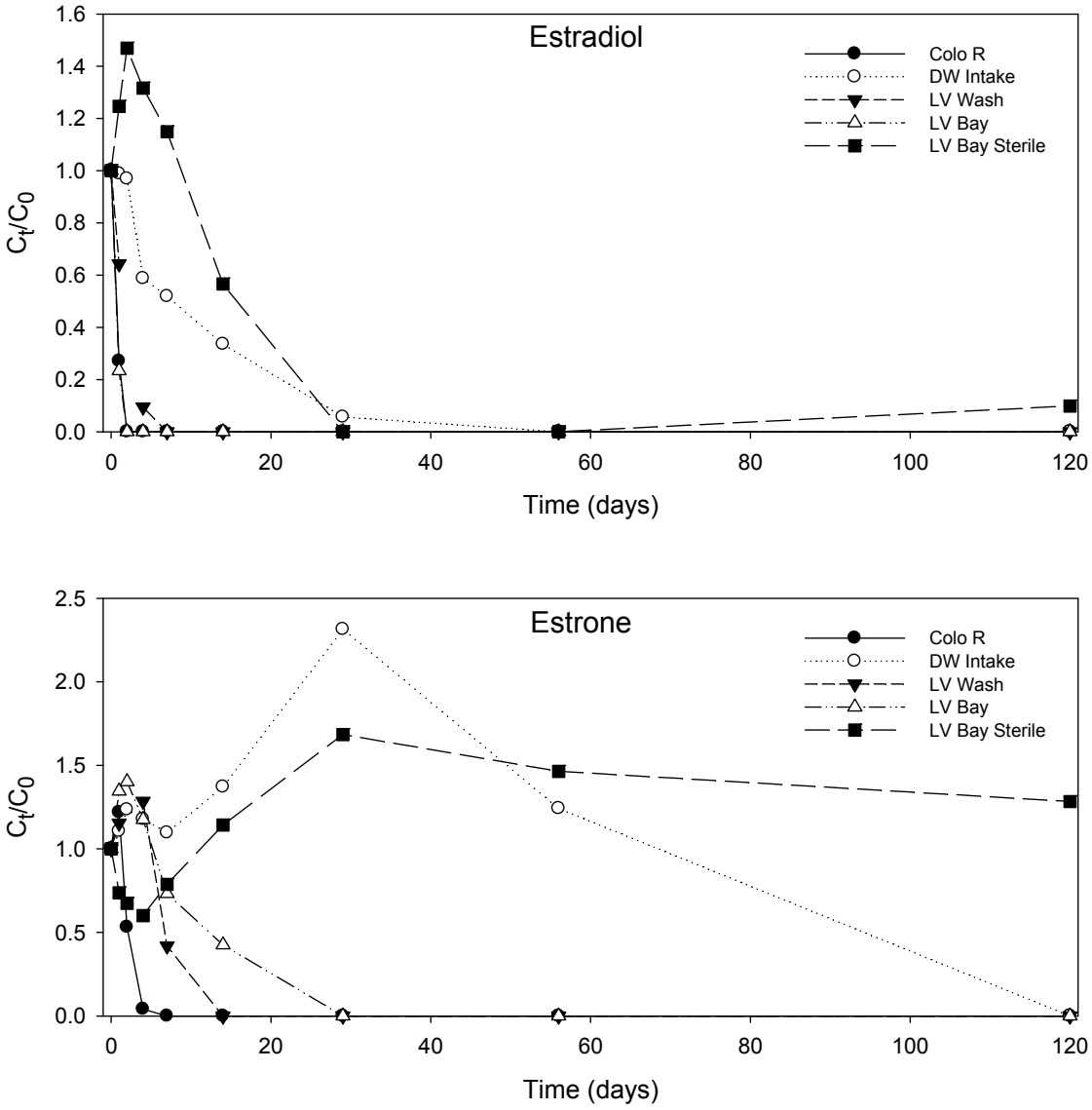


Figure A.12. Degradation curves at each site organized according to chemical compound

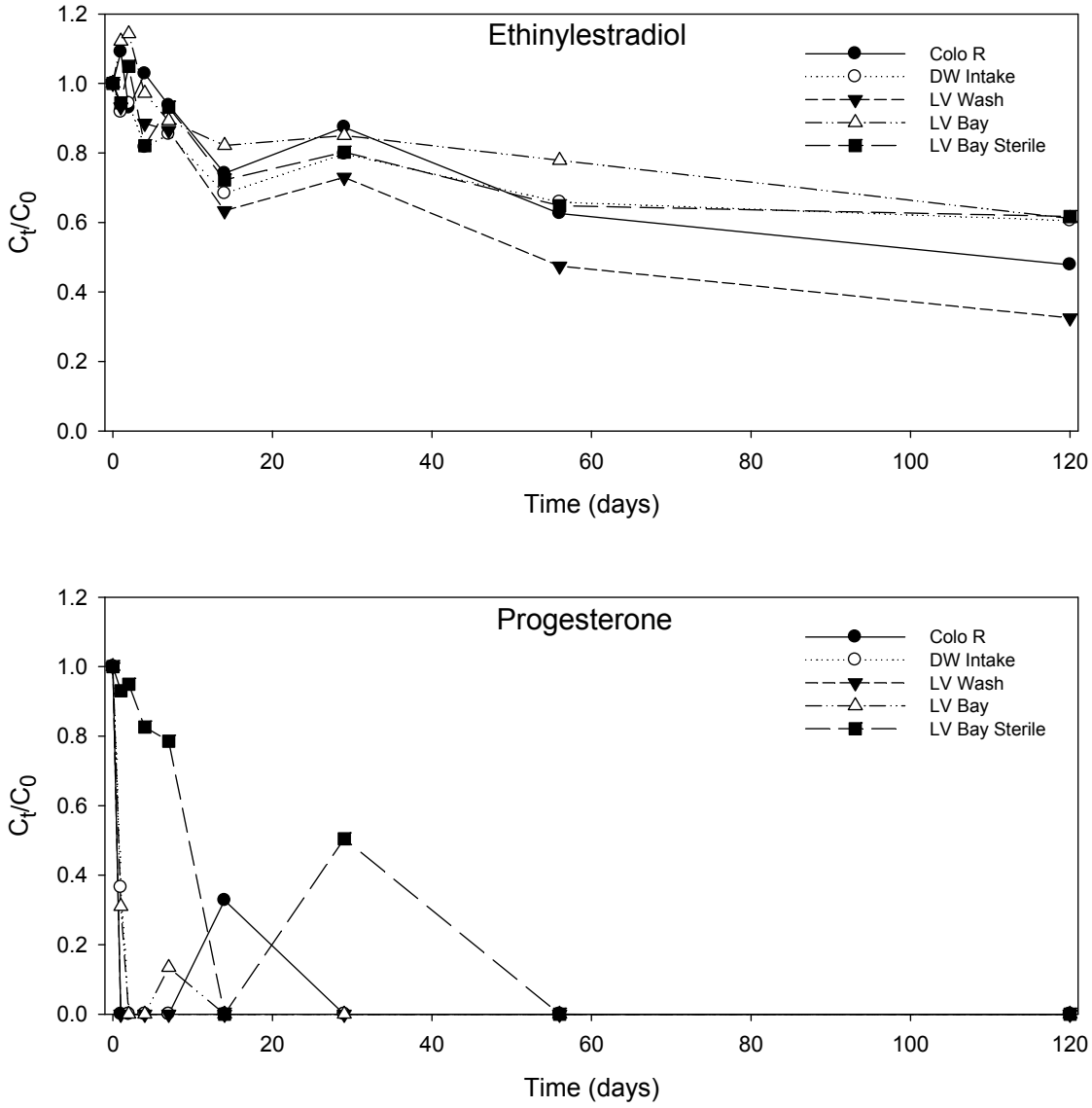


Figure A.13. Degradation curves at each site organized according to chemical compound

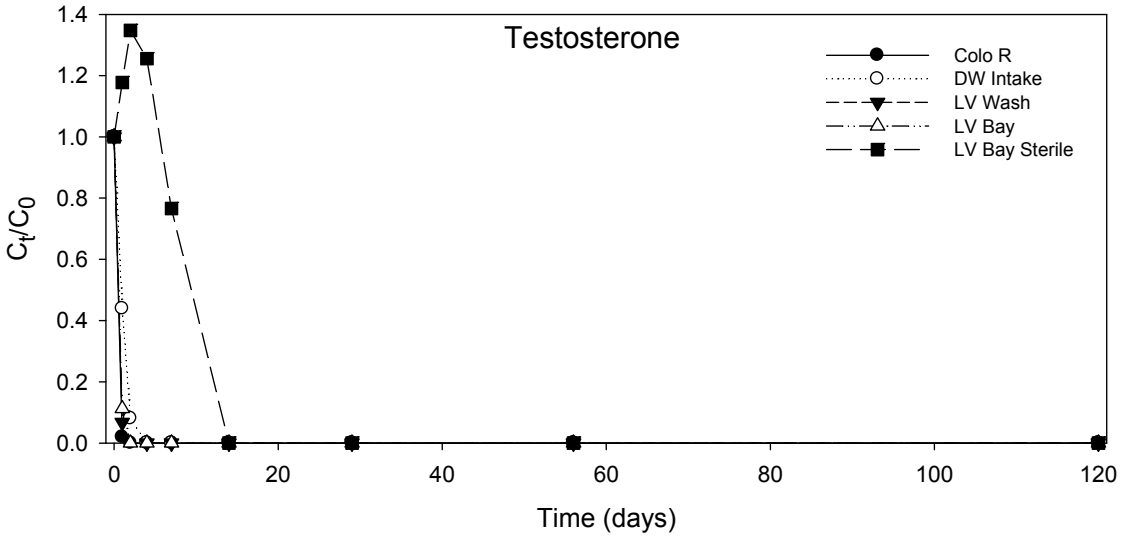


Figure A.14. Degradation curves at each site organized according to chemical compound

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