THE ROLE OF ENVIRONMENTAL BUFFERS IN POTABLE WATER REUSE

by

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ABSTRACT

XUEYING OLIVIA BROWN. The Role of Environmental Buffers in Potable Water Reuse. (Under the direction of DR. OLYA KEEN).

Conventional drinking water resources are not a sustainable solution to supply the rapidly growing populations in traditionally arid states. These areas, as well as others around the world, are increasingly reliant on recycling treated municipal effluent to supplement water resources. In most instances, treated effluent is discharged into a body of water or a groundwater system that supplies the influent for a downstream drinking water treatment facility. It is debatable whether the process of utilizing aquatic environmental buffers enhances or contaminates the treated effluent. This study evaluated the ability of different types of environmental buffers (groundwater recharge, riverbank filtration, wetland treatment, river and lake discharge) to attenuate contaminants representative of different classes and different environmental fate by measuring conventional water quality parameters as well as unregulated constituents of concern in several field studies representative of different types of environmental buffers.

DEDICATION

This dissertation is dedicated to my loving husband Adam, for whom I would not have been able to do this without.

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LIST OF ABBREVIATIONS

Advanced Oxidation Process	AOP
Aurora Prairie Waters	APW
Antibiotic Resistance Gene	ARG
Aquifer Recharge and Recovery	ARR
American Society for Testing and Materials	ASTM
Advanced Water Purification Facility	AWPF
Benzo[a]pyrene	BaP
Below Detection Limit	BDL
5-Day Biochemical oxygen demand	BOD_5
Contaminants of Emerging Concern	CECs
Charlotte Mecklenburg Utilities	CMU
Chemical Oxygen Demand	COD
Charlotte Water	CW
Disinfection By-Product	DBP
Dissolved Oxygen	DO
Direct Potable Reuse	DPR
Drinking Water Treatment Plant	DWTP
Environmental Protection Agency	EPA
Flame Ionization Detector	FID
Fluorenylmethyloxycarbonyl Chloride	FMOC
Free Water Surface	FWS

Franklin Water Treatment Plant	FWTP
Gas Chromatography	GC
Groundwater Replenishment System	GWRS
High-density polyethylene	HDPE
Inductively Coupled Plasma-Atomic Emission Spectrometry	ICP-OES
Indirect Potable Reuse	IPR
High Performance Liquid Chromatography–Mass Spectrometry	HPLC-MS
Horizontal Subsurface Flow	HSSF
Lysine Iron Agar	LIA
Limit of Detection	LOD
Maximum Contaminant Levels	MCL
Maximum Contaminant Level Goal	MCLG
McDowell Creek	MDC
Method Detection Limit	MDL
Microfiltration	MF
Million Gallons per Day	MGD
Mountain Island Lake	MIL
Most Probable Number	MPN
4-Methylumbelliferyl-β-D-Glucuronide	MUG
Not Applicable	NA
National Pollutant Discharge Elimination System	NPDES
Orange County Sanitation District	OCSD
Orange County Water District	OCWD

Ortho-Nitrophenyl-β-galactoside	ONPG
Polycyclic Aromatic Hydrocarbons	PAH
Polymeraze Chain Reaction	PCR
Prado Constructed Wetlands	PCW
Polytetrafluoroethylene	PTFE
Quality Assurance/Quality Control	QA/QC
Quantitative Polymerase Chain Reaction	qPCR
Riverbank Filtration	RBF
Reverse Osmosis	RO
Robert W. Hite Treatment Facility	RWHTF
Solid Phase Extraction	SPE
Total Organic Carbon	TOC
Triple Sugar Iron Agar	TSI
Total Suspended Solids	TSS
Ultra-high Purity	UHP
United States Drug Administration	USDA
United States Geological Survey	USGS
Ultraviolet Advanced Oxidation Process	UV-AOP
UV AOP product water at OCWD AWPF	UVP
Ultraviolet - Visible Spectroscopy	UV-VIS
Vertical Flow	VF
Volatile Suspended Solids	VSS
Wastewater Treatment Plant	WWTP

CHAPTER 1. INTRODUCTION

According to the US National Drought Monitor Center, in 2018, an estimated 24% of the US population resided in arid or semi-arid areas.[1] Coincidentally, those same regions have undergone some of the highest population expansion in the past decade, with 7 of the top 10 of fastest growing states now categorized as 'drought prone'.[2] Even so, some long-established metro-areas are also plagued by endemic drought conditions characterized by abnormal climate patterns brough about by global warming in addition to historically low annual rainfalls. Los Angeles, CA, for example, the second largest city in the US by population, received an average of 4.79 inches of precipitation in 2018 as compared to the 57.8 inches Charlotte NC received that year.[3] And despite the availability of local ground water, the city of Los Angeles is still reliant on imported water to sustain its current population as the overall groundwater availability only represents less than 10% of the city's water demands. As such, naturally occurring water resources are no longer considered a viable option to sustain current and projected populations for drought-laden US cities which, consequently, prompts those afflicted regions to consider the practice of potable water reuse, or the practice of reusing treated wastewater for drinking water purposes. Expounding upon that, potable water reuse can be further classified into two categories: direct and indirect reuse. As its namesake suggests, direct reuse, in theory, is a closed loop system that purifies municipal wastewater to drinking water standards and directly distributes it for commercial, residential, or industrial use. Indirect reuse on the other hand, discharges treated wastewater effluent into the environment before any drinking water treatment occurs. In this instance, specific bodies of water, aka environmental buffers, may be utilized to

provide a gap between wastewater and drinking water treatment. Environmental buffers also may exhibit chemical or biological properties that can break down certain contaminants or at least expedite their attenuation.

The goal of this study was to examine the role of the different environmental buffers in potable reuse by evaluating their ability to attenuate various aquatic contaminants. The hypothesis of the study is that in potable water reuse, environmental buffers serve primarily to improve public perception of water reuse but are less impactful in terms of water quality improvement when compared to direct potable reuse practices.

A comprehensive analysis was conducted to determine the fate and transport of contaminants in individually selected buffers as well as potable reuse systems as a whole. This includes the analysis of weather–impacted samples by comparing the concentration of contaminants between wet and dry weather samples.

1.1 ENVIRONMENTAL BUFFERS

In most instances, unacknowledged, or *de facto*, potable water reuse occurs when treated wastewater effluent is discharged into a body of water, including but not limited to rivers, wetlands, lakes, and aquifers, which then serves as a drinking water source for another downstream entity. In the case of acknowledged reuse, the treated effluent is intentionally discharged into carefully implemented potable water reuse systems, also known as environmental buffers, that functionally imitate engineered water treatment technology. In both acknowledged and unacknowledged potable reuse, as shown in Figure 1, water purification occurs via common environmental processes such as dilution, photolysis, hydrolysis, biodegradation, and sorption that occurs naturally within said buffers with the intent to attenuate aquatic contaminants. In addition to the myriad of

physiological benefits a buffer may have as a contaminant, another function environmental buffers' serve is the improvement of public perception of water reuse, whether it is justified or not. The notion of direct recycling wastewater into drinking water may discourage the public from supporting such practices, thus the "yuck factor" is an important consideration in potable water reuse implementation projects.[4]

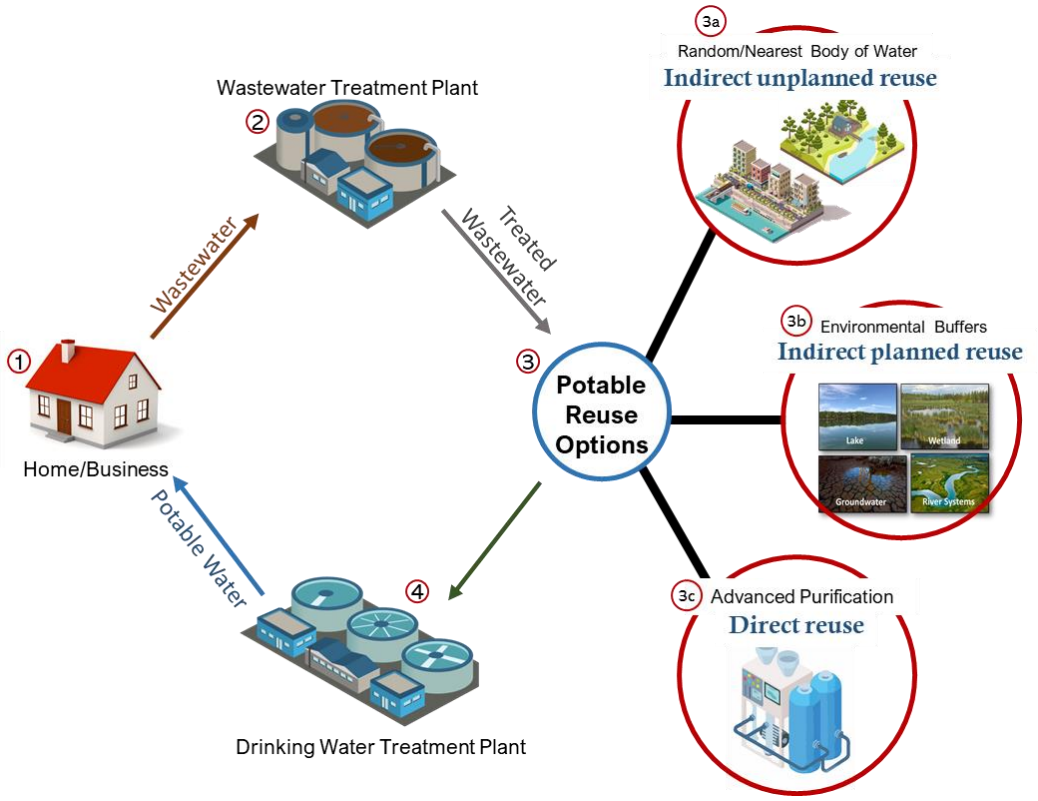


FIGURE 1. Potable water reuse cycle.

On the other hand, recent developments in direct acknowledged potable reuse offers an alternative to environmental processes by using advanced water purification technology. Although indirect reuse practices may also employ advance purification methods on either the wastewater or drinking water ends of the operation, the primary differentiation is that direct reuse operates within a closed-circuited system that minimizes, if not eliminates, exposure to the environment. One of the main benefits of

this design is to eliminate any anthropogenic recontamination in the potable water reuse cycle by bypassing any environmental contact. However, currently, this method of water treatment is not a financially feasible option for all treatment plants to adopt. Moreover, although the purified water has been treated to the highest industry standards, the public's aversion to the "pipe-to-pipe" method means utilities must take into account the "yuck factor" in the implementation of direct potable water reuse projects.[5] In an effort to mitigate the public's perception, water that is destined for potable reuse applications is often not transported directly to a drinking water treatment facility, but rather is diverged into a subsurface aquifer or a surface reservoir instead.

However, not all environmental buffers, whether natural or constructed, are fully equipped to process the entire contaminant load discharged by wastewater treatment plants (WWTP), especially regarding particulate matter, microorganisms, and contaminants of emerging concern (CECs) such as pharmaceuticals, plasticizers, personal care products, and other trace-level contaminants. Conversely, environmental buffers can also introduce unregulated emerging contaminants, often associated with urban and agricultural runoff, to treated wastewater effluents that are discharged into local water systems.

While these contaminants are presently unregulated, multiple studies have established their relevance to aquatic health. For example, chronic exposure to trace levels of pharmaceuticals has been demonstrated to cause disruption of predator avoidance patterns,[6] feminization of male fish,[7] and other endocrine disrupting effects.[8] Additionally, interactions between the native microorganism population and sub-

inhibitory levels of antibiotics and pharmaceuticals present in treated wastewater could lead to developments in antibiotic resistance in the natural environment.[9-13]

Research studies in the past delved into an individual environmental buffer's ability to retain conventional contaminants such as nutrients and microbial contaminants, but no comprehensive assessment has been be done on the various environmental buffers' ability to attenuate xenobiotic compounds such as pharmaceuticals, insecticides, herbicides, and constituents of automotive fluids in addition to the aforementioned conventional contaminants as a whole. Though the fate and transport of CECs could be surmised through laboratory bench tests and known chemical properties of those compounds, the exact behavior of CECs in the environmental buffers is yet to be determined. Additionally, recontamination of treated water as a result of the release into an environmental buffer has not been studied before.

1.2 WETLANDS

Known as "the kidneys of the environment", the high rates of biogeochemical cycling in wetlands with constant recycling of macro and micro nutrients allows it to be a self-sustaining ecosystem. [14] The unique composition of various physical, chemical, and biological attributes of a wetland contributes to its high biodiversity of both plants and animals by providing a wide variety of wildlife habitats, all the while maintaining a complex food web. Wetlands are also essential for maintaining nutrient balance in aquatic systems by removing excess nutrients that otherwise may cause eutrophication in the environment.[15] Anthropogenic greenhouse gasses, such as carbon dioxide, are significantly retained by wetlands as well through plant respiration and humic biomass.[16]

Wetlands are also an important factor for the preservation of a diverse species of aquatic, amphibious, and avian organisms.[17]

In terms of water reuse, wetlands, specifically constructed wetlands, are often viewed as a holistic solution for additional treatments to further purify treated wastewater effluents and stormwater runoffs. Specifically, they've been shown to be effective at removing aquatic microbes, attenuation of nutrients, and inorganic contaminants as well.[18-20] Variations of constructed wetlands designs are available, including free water surface (FWS) wetlands, horizontal subsurface flow (HSSF) wetlands, and vertical flow (VF) wetlands. The selection of wetlands is dependent on multiple factors such as treatment goal, influent characteristics, local environment, and social factors. [21] For example, water in FWS wetlands are usually open water zone with a high depth, the water flows above the surface of the vegetative substrate and in essence, mimics natural marshes.[22, 23] The primary process of contaminant degradation in FWS wetlands occur via sedimentation, biochemical oxidation, UV disinfection, and bioassimilation. HSSF and VF wetlands on the other hand, introduce a vertical hydraulic flow by incorporating groundwater flow through porous bed material without directly exposing the water to the environment, and in these instances, the primary processes that occur include filtration, microbial respiration, fermentation and methanogenesis, denitrification, and sorption.[21, 22]

A review conducted by Tao et. al (2017) found that amongst the 30 full scale constructed wetlands monitored, almost all of them met the US Environmental Protection Agency (EPA) guidelines for water reuse in effluent pH values. Approximately 84% of those systems had TSS and BOD_5 levels that met low-quality reuse guidelines; such as

industrial cooling, restricted urban and agriculture, and environmental reuse; and 50% of these systems had TSS and BOD_5 values that met the quality for high-quality reuse; such as indirect potable reuse (IPR), and unrestricted urban and agriculture reuse.[21, 24, 25] However, one caveat associated with using wetlands is the high amount of microbial background, specifically fecal coliforms, in the range of 10 – 5000 CFU/100 mL, which exceeds most, if not all, categories of water reuse guidelines. [21, 26]

Aside from its physiochemical benefits in water reuse, wetlands also functionally imitate equalization basins by reducing hydraulic flow velocity, thus minimizing the adverse effects of water erosion and stormwater runoff.

1.3 GROUNDWATER

Groundwater and aquifer recharges are essential for maintaining hydraulic balance in the aquatic system as they indirectly support freshwater ecosystems in addition to mitigating pollutants in the aquatic system. Environmental buffers, such as wetlands and surface water/hyporheic zones, are heavily dependent on groundwater for replenishment as several aquatic ecosystems are reliant on groundwater as their main supply of source water.[27, 28] Degradation of contaminants in aquifers can occur through microbial uptake via aerobic, anaerobic, and anoxic conditions where subterrestrial microbes will oxidize inorganic compounds, such as sulfate and nitrate as a replacement for oxygen.[29] But non-biotic mechanisms such as dilution, adsorption via soil filtration, and retardation of advection transport are more likely to occur.[30] And unlike surface water, groundwater is less vulnerable to pathogens infiltration and contaminants more

prevalent in terrestrial landscapes, making it a more reliable source of high-quality drinking water.

In potable water reuse, in most cases, treated effluent is diverted into percolation basins where the water is allowed to infiltrate into the aquifer, also known as soil aquifer treatment (SAT) before being extracted for drinking water purposes. Other alternative methods of SAT include injection wells, infiltration trenches, or riverbank filtration (RBF). The quality of the feedwater has a strong impact on overall treatment efficacy, but it is still considered the most economical potable reuse alternative.[31] In cases of aquifer storage and recovery (ASR), purified water is reintroduced to local aquifers in an attempt to replenish, or recharge, the depleted supply. While SATs can supplement water availability in regions prone to water shortages and over extraction, the blending of advanced purified effluent into natural aquifers can ultimately reduce the quality of the highly treated feedwater by exposing it to preexisting impurities, such as municipal and agricultural contaminants, that were already present in the aquifer, thus requiring more treatment once the water is extracted for potable reuse.

According to Drewes et. al. (2002), aquifers have been shown to effectively remove the majority of pharmaceuticals and personal care products that were chosen to be monitored in the study. They found that via percolation with an average retention time of less than six months, stimulants and analgesic drugs and blood lipid regulators were reduced to a concentration near or below the study's detection limit.[32] However, although aquifer recharge buffers are effective in removing those compounds, the long-term integrity of aquifers can be compromised via adsorption of pharmaceuticals and personal

care products which may not readily degradable in subterrestrial environments. That aside, wastewater effluents post aquifer infiltration were shown to have considerably lower levels of suspended solids, BOD_5, microbial contaminants, metals, and nutrients, most notably nitrate.[33]

1.4 LAKES, RIVERS, AND RIPARIAN ZONES

From microhabitats to large stream systems, moving surface waters are essential for the allocation of nutrients, primary productivity, and the transportation and deposition of inorganic solids in the environment. The riparian transition zones of rivers and lakes are often inundated with nutrients and organic matter from runoffs, providing river channels with riverine plants and microorganisms which can minimize hydraulic erosions.[34] In lake systems, the detrital matter and suspended organics support a wide variety of benthic macroinvertebrates that are essential in the terrestrial food chain.[35, 36] Large rivers often carry suspended organic matter to provide food matter for non-photosynthetic organisms and are home to numerous species of freshwater fish species. In rivers, the main bulk of nutrients are carried by lotic systems to nutrient sinks, such as floodplains and wetlands if one is available nearby and is connected to the system.

As an environmental buffer, the main role of surface water systems is to mitigate response time between wastewater and drinking water treatment in case of facility failure. It is also the most commonly utilized buffer in IPR practices and can often be the limiting factor in implement IPR for a facility must be constructed within a viable location to be readily accessible to this buffer. Aside from its infrastructural benefits, the diverse physical habitat of rivers can also support a wide variety of biological communities

responsible for primary productivity that can accelerate the uptake and attenuation of certain nutrients as well as outcompete some microbial contaminants and reduce their presence.[36] In areas of high-turbulence flows with high dissolved oxygen, open canopy, and shallow water, photolytic degradation of contaminants can occur while simultaneously supporting photosynthetic organisms that may otherwise metabolize other contaminants as well. However, in most cases, the water purification characteristics of surface water buffers are often dependent on the local environment and the quality of the effluent being discharged into it.

For riparian zones, it has similar purification properties as the aquifer recharge such as filtration and adsorption. However, as aquifers are subterrestrial, riparian zones have the added benefit of contaminant attenuation via plants and microbes though it is more susceptible to environmental pollution from runoff, animals, and anthropogenic activities than groundwater buffers.

1.5 CONTAMINANTS

The buffers were evaluated on their ability to attenuate conventional aquatic contaminants, including nutrients, metals, and microbes in conjunction with emerging contaminants of concern such as pharmaceuticals and herbicides. The CECs selected for analysis in this study are known to be consistently detected in wastewater-impacted streams. The list also includes the most prescribed antibiotics: azithromycin, amoxicillin, cephalexin, ciprofloxacin, sulfamethoxazole-trimethoprim, doxycycline, levofloxacin, clindamycin, and penicillin V.

Apart from the antibiotics listed above, the following compounds have been selected for monitoring as well: carbamazepine, sucralose, sulfamethoxazole, ibuprofen, glyphosate, atrazine, and benzo[a]pyrene (BaP). The selected compounds will be used as surrogates for other chemicals that have similar environmental fate with Table 1 below listing their dominant pathway of decay.

TABLE 1. Environmental attenuation pathways for selected contaminants.

Compound	Biodegradation	Adsorption	Photolysis
Sucralose	No	No	No
Ibuprofen	**Yes**	No	No
Carbamazepine	No	**Yes**	No
Sulfamethoxazole	No	No	**Yes**

Glyphosate, atrazine, and benzo[a]pyrene served as indicators of agricultural and urban runoff pollution. Glyphosate is the active ingredient in Round-up, one of the highest volume herbicides used in both agriculture and urban lawn and roadway maintenance (270–290 million pounds of active ingredient used in 2012).[37] Atrazine is a commonly used agricultural pesticide and a second highest volume herbicide used in the US (64-74 million pounds of active ingredient in 2012).[37] Benzo[a]pyrene is a carcinogenic product of incomplete fuel combustion indicative of the impact from urban traffic and coal-burning power plants.[38]

Additionally, contaminants with specific degradation properties and known sources were selected as indirect tracers to help identify the possible sources of contamination. For example, the presence of metals is usually indicative of urban and industrial pollutions. Nutrients, anions, and microbial indicators may be indicative of local municipal wastewater input as well as urban pollution and anthropogenic activity.

Antibiotic resistance genes (ARGs) along with *giardia* and *cryptosporidium* were monitored via PCR method.

CHAPTER 2. METERIALS AND METHODS

2.1 FIELD SAMPLING SITES

Three separate water reuse systems were evaluated separately for their efficacy in removing aquatic contaminants. Each location contained at least two buffers that work in conjunction with one another, as seen in Table 2 below. Though the buffers are interconnected, they were evaluated individually for contaminant removal.

TABLE 2. Summary of individual buffers studied per location.

Orange County Water District	**Aurora Prairie Waters**	**Charlotte Water Utilities**
Constructed Wetland (IPR)	River (IPR)	Lake/River (IPR)
Groundwater replenishment	Riverbank Filtration (IPR)	

2.1.1 Orange County Water District (OCWD)

Traditionally, the residents of Orange County have been reliant on the aquifers beneath the northern and central parts of the county as its source of drinking water. However, as the demand for water increased with the rise in population, the groundwater system was no longer a sustainable option. The natural recharge process, mainly provided by the Santa Ana River (SAR), was unable to keep up with the rate of depletion. In an effort to offset the imbalance, Orange County Water District (OCWD) partnered with the Orange County Sanitation District (OCSD) to implement the Groundwater Replenishment System (GWRS) project in 2008. This goal of this project was to provide adequate amounts of clean water to the 850,000 residents of Orange County (OC) through potable reuse practices and technology.

Raw wastewater is pre-treated by the OCSD before supplying it to the Advanced Water Purification Facility (AWPF) as feedwater. The wastewater influent received by OCSD is first screened to remove large debris to prevent clogging during later treatment

processes. The screened influent goes through preliminary treatment whereby heavy solids are removed via settling and floatables are skimmed in primary clarifiers. Organics are then removed with biological treatments such as activated sludge and trickling filters before settling again to remove the microorganisms. The resulting secondary effluent is then either sent to OCWD for purification or discharged into the ocean.

The OCWD AWPF receives the secondary effluent from OCSD and purifies the water with a three-steps state-of-the-art treatment process. Currently, OCWD operates at 100 million gallons per day (MGD). First, particulates up to 0.2 µm such as suspended colloids and large microorganisms are removed using microfiltration. Then, the water is pressurized and further filtered using reverse osmosis (RO) whereby inorganic contaminants such as salts, metals, and anthropogenic compounds are removed. Lastly, ultraviolet advanced oxidation process (UV-AOP) inactivates or breaks down any remaining organic compounds before the purified water is re-mineralized and sent to recharge basins.

The following were the samples provided by OCWD: (1) SAR right before a portion of the water was diverted to enter Prado Constructed Wetlands (PCW); (2) a location approximately halfway through PCW[a]; (3) a blend of activated sludge and trickling filter effluent from the OCWD that feeds into the OCWD AWPF; (4) product water from AWPF after microfiltration, RO, and UV/advanced oxidation treatment; (5)

[a] Due to the high volume of precipitation at the start of this project in winter 2017, OCWD was only able to sample midway through Prado Wetlands since the outlet was under water. To keep consistency, sampling was conducted at the same location throughout the study.

sample water from a monitoring well near the groundwater recharge basin that only receives OCWD AWPF finished product water. The well has a depth of 155.0 feet and 1-month travel time with little impact from recharge from the SAR. The sampling locations and schematics are presented in Figures 2 and 3.

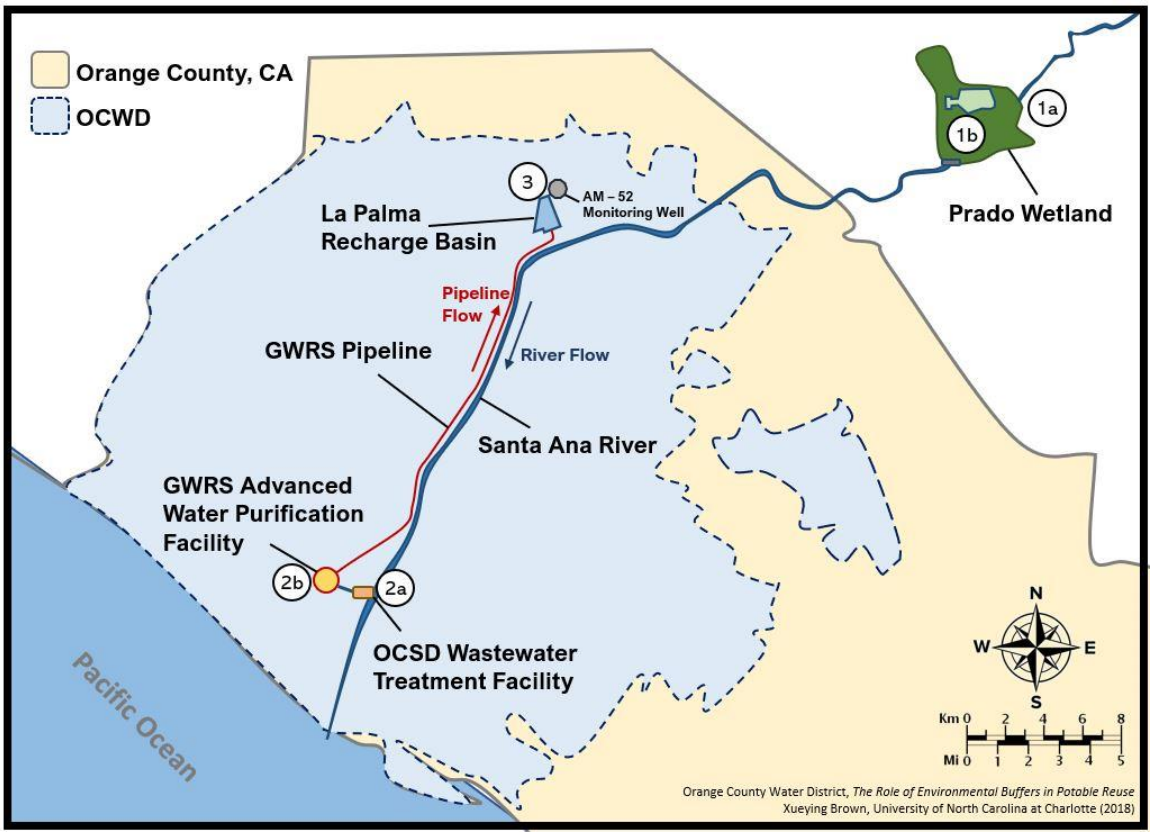


FIGURE 2. OCWD sampling locations as designated by numbers 1a - 3.

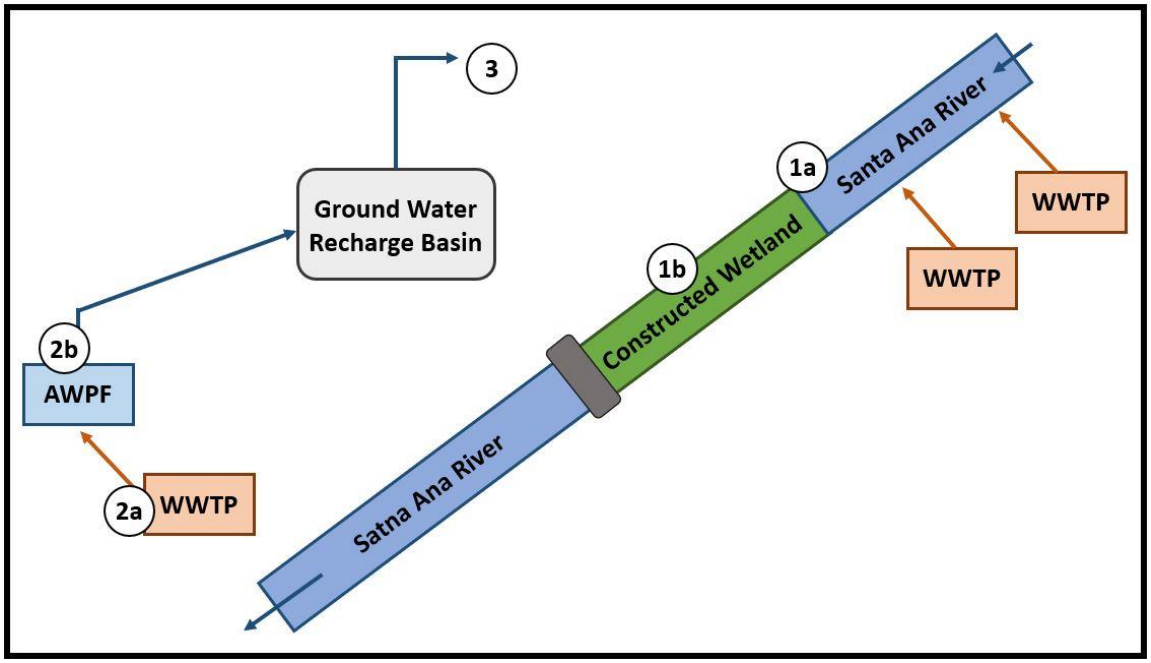


FIGURE 3. Flow schematic of OCWD sample sites.

2.1.2 Aurora Prairie Waters (APW)

Aurora Prairie Waters (APW) drinking water treatment plant (DWTP) is located in Colorado. The plant is another example of acknowledged potable water reuse and is located on South Platte River downstream from the Denver Metro area WWTPs. Major discharges into South Platte River come from Denver Metro North, Denver Metro South and Littleton/Englewood WWTPs. The three plants together report a discharge of approximately 100 million gallons per day (MGD). With the base flow in the river around 10-15 MGD during the dry season (6 months of the year), WWTP effluent can constitute as much as 90% of flow in the river by the time it reaches APW intake. Even in high flow, South Platte River is approximately 40% treated wastewater. By acknowledging the source of its water as WWTP effluent, APW considers itself as a water reuse facility and has a suite of treatment processes to address the potential unregulated CECs in its source water. The treatment processes, similarly, to the OCWD

GWRS, include advanced processes such as riverbank filtration, UV/H_2O_2 AOP and activated carbon adsorption.

The sampling schedule was based on staff availability at the utility providing samples. The following were the samples provided by APW, Denver Metro, and Englewood/Littleton wastewater treatment plants: (1a) effluent from Englewood-Littleton WWTP; (1b) effluent from south Denver Metro WWTP; (1c) effluent from north Denver Metro WWTP; (2a) treated wastewater that has traveled through SPR and served as influent into the recharge storage basin via riverbank filtration; (2b) effluent from the recharge storage basin (influent into the advanced purification treatment works). The plant has also provided the results of internal monitoring of advanced purification system performance for a variety of emerging contaminants. The diagram in Figure 4 and 5 illustrates the sampling locations. The samples for this site did not have enough wet-weather and dry-weather samples to conclusively conduct any statistical analysis, therefore any impact of precipitation could not be analyzed.

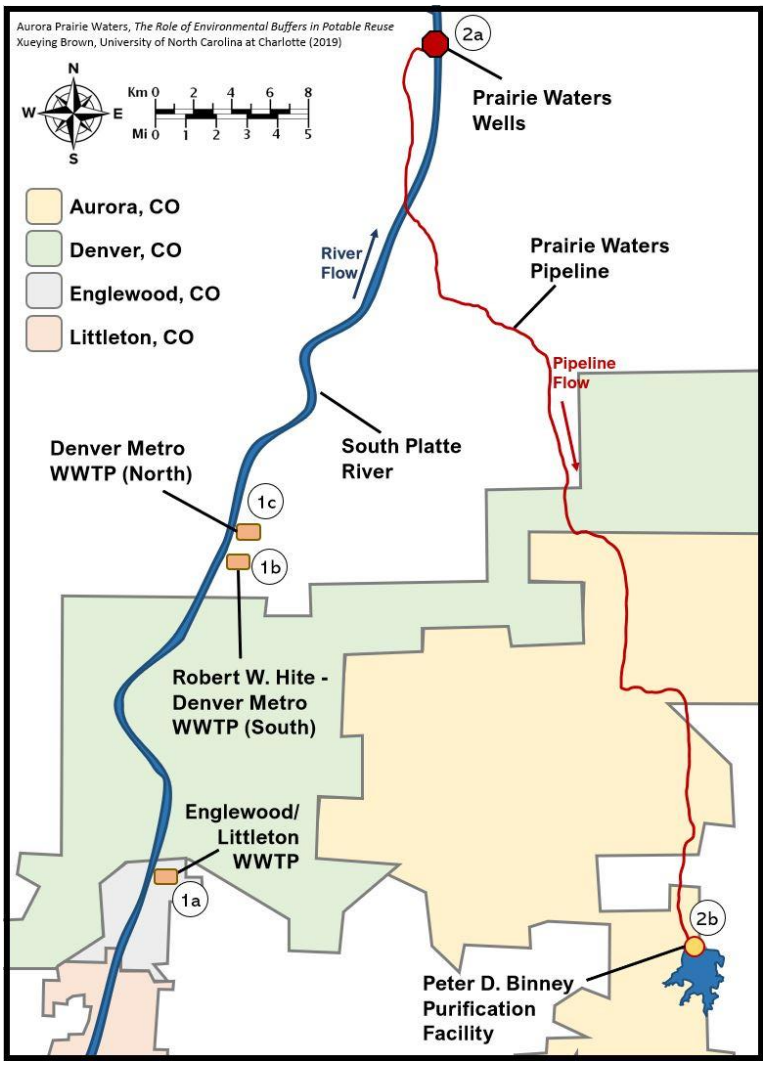


FIGURE 4. APW sampling locations as designated by number 1a - 2b.

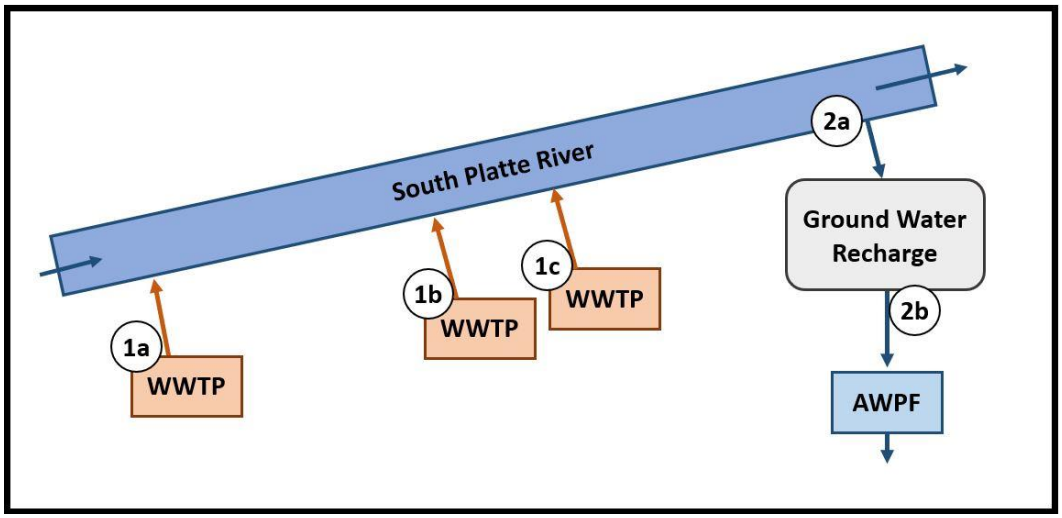


FIGURE 5. Flow schematic of APW sampling sites

2.1.3 Charlotte Mecklenburg Utilities (CMU)

Charlotte Metro area utilities are one of the many examples of *de facto* potable reuse. McDowell WWTP treats wastewater to a high standard because it discharges it into Catawba River, which flows into Mountain Island Lake (MIL) – Charlotte's drinking water supply reservoir. Upstream of MIL, Catawba River dam forms Lake Norman, which is surrounded by residential development and is used for recreational boating, power plant cooling, and other activities, each of which can introduce environmental contaminants. For details see Figures 6 and 7.

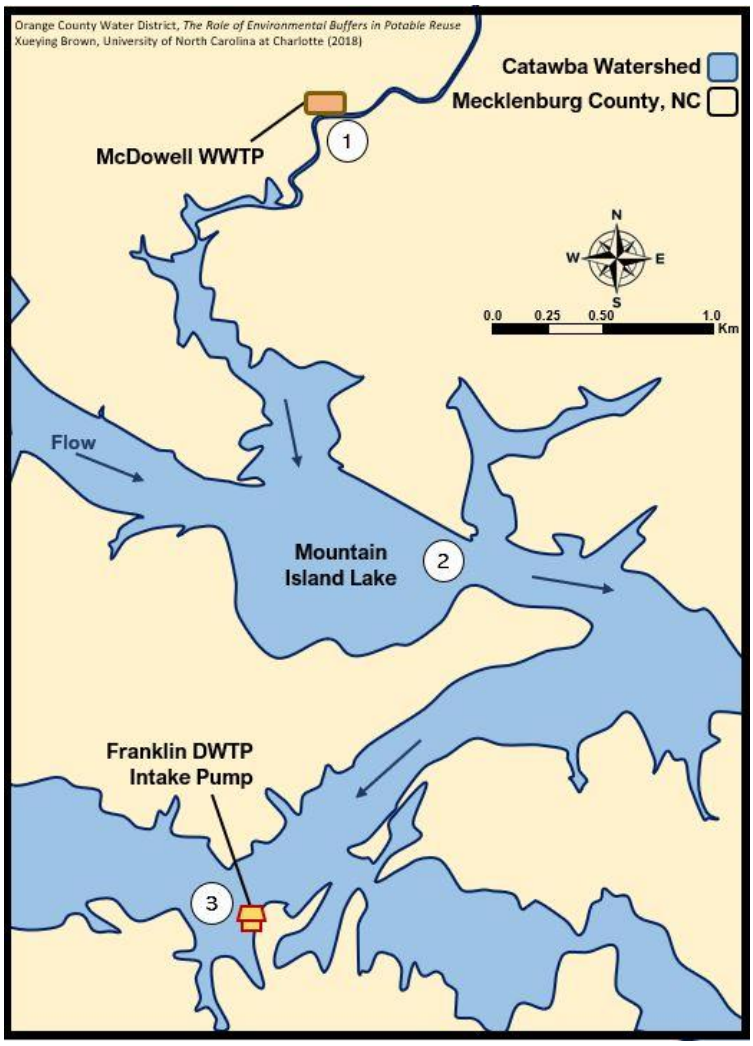


FIGURE 6. CMU sampling locations as designated by number 1 - 3.

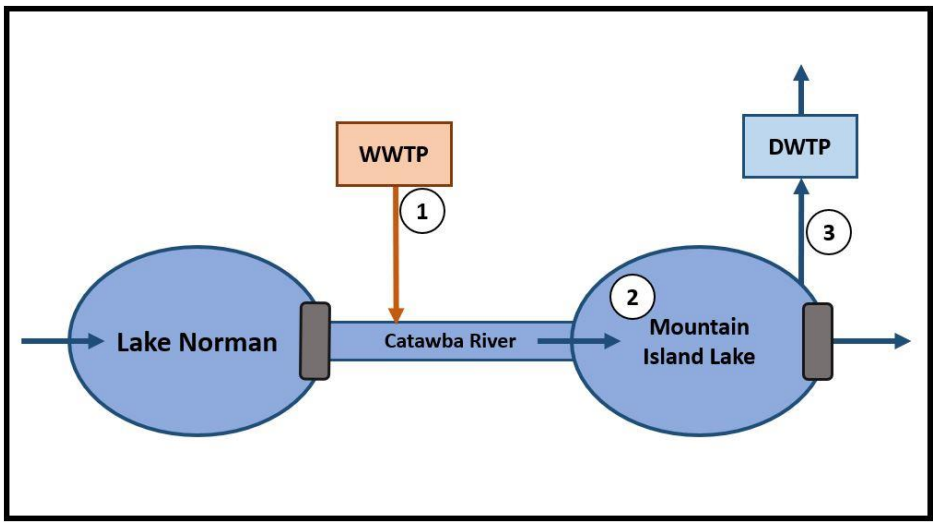


FIGURE 7. Flow schematic of CMU sampling sites.

The following locations were sampled: (1) treated wastewater from McDowell WWTP collected after disinfection and prior to reaeration; (2) influent into the MIL consisting of the mixture of Lake Norman flow and effluent from McDowell WWTP; and (3) influent to Franklin DWTP downstream MIL.

2.1.4 Sample Logistics

The original goal was to collect samples of the effluent released into the environmental buffer and the samples of hydrologically paired influent to DWTPs involved in acknowledged or unacknowledged potable water reuse. The intent was to collect samples that are hydrologically paired by taking into account the approximate time it takes for a parcel of water to traverse the environmental buffer based on the flow rates through the buffer as measured by the real-time United States Geological Survey (USGS) gages or by the treatment facility (a.k.a Lagrangian samples). However, due to the necessity to coordinate with several facilities for each of the field sites, and the availability of the operators to collect samples at the specific days and times, it proved to

be logistically challenging to collect samples that were exactly hydrologically paired. While the sampled systems do not exhibit continuous steady-state conditions, quasi-steady-state can be observed within short snapshots of time. All of the sampled systems can be considered well mixed and therefore no major fluctuations in system inputs and processes were anticipated within the timeframe of sample collection. As such, samples were not analyzed as individual paired sets but rather an analysis was performed of the overall means with unequal variances in quasi-steady-state systems.

However, wet weather events can result in major upsets in hydrologic systems, therefore, samples identified as “wet weather” were analyzed both together and separately with the “dry weather” samples. Samples were designated as “wet weather” if a major storm event was considered to likely impact the system being sampled. Table 3 below lists the sampling dates by location and wet/dry sample designation. Real-time USGS precipitation and streamflow gages (where applicable), as shown in Table 4, and reports from the participating facilities on their flow conditions were used to identify samples as “dry weather” or “wet weather”. Estimated travel time for the water parcel between the sampling points is also listed, where applicable, as shown in Table 5.

TABLE 3. Sample collection time and dates. Wet weather samples are designated by bolded entries.

OCWD									
1a		**1b**		**2a**		**2b**		**3**	
Date	Time	Date	Time	Date	Time	Date	Time	Date	Time
2/28/2017	**9:30 AM**	**2/28/2017**	**9:40 AM**	**3/7/2017**	**10:20 AM**	**3/7/2017**	**11:25 AM**	**2/21/2017**	**9:40 AM**
3/23/2017	**8:39 AM**	**3/23/2017**	**8:53 AM**	**3/28/2017**	**9:05 AM**	**3/28/2017**	**9:29 AM**	3/13/2017	9:33 AM
6/29/2017	9:05 AM	6/29/2017	9:16 AM	6/22/2017	8:41 AM	6/22/2017	9:35 AM	6/19/2017	10:10 AM
8/8/2017	9:15 AM	8/8/2017	9:27 AM	7/25/2017	9:46 AM	7/25/2017	9:23 AM	7/17/2017	9:45 AM
8/28/2017	9:26 AM	8/28/2017	9:38 AM	9/19/2017	7:04 AM	9/19/2017	7:30 AM	8/21/2017	9:08 AM
3/1/2018	**9:16 AM**	**3/1/2018**	**9:33 AM**	**3/15/2018**	**7:40 AM**	**3/15/2018**	**8:21 AM**	4/16/2018	9:21 AM
APW									
1a		**1b**		**1c**		**2a**		**2b**	
Date	Time	Date	Time	Date	Time	Date	Time	Date	Time
5/11/2017	**8:00 AM**	5/15/2017	9:00 AM	**5/11/2017**	**1:10 PM**	5/15/2017	11:59 AM	5/15/2017	8:27 AM
5/24/2017	7:46 AM	**6/7/2017**	**12:30 AM**	**6/7/2017**	**1:00 PM**	6/19/2017	12:43 PM	6/19/2017	9:48 AM
6/14/2017	7:33 AM	**8/16/2017**	**12:45 PM**	**8/16/2017**	**1:15 PM**	7/10/2017	11:28 AM	7/10/2017	8:52 AM
7/13/2017	**8:00 AM**	**1/11/2018**	**10:40 AM**	**1/11/2018**	**11:10 AM**	1/10/2018	10:45 AM	1/10/2018	8:55 AM
8/15/2017	**7:32 AM**								
CMU									
1		**2**		**3**					
Date	Time	Date	Time	Date	Time				
4/13/2017	11:05 AM	4/13/2017	12:35 PM	4/13/2017	2:16 PM				
4/21/2017	11:00 AM	4/21/2017	12:03 PM	4/21/2017	1:14 PM				
4/26/2017	**10:50 AM**	**4/26/2017**	**12:11 PM**	**4/26/2017**	**1:23 PM**				
5/25/2017	**10:26 AM**	**5/25/2017**	**3:05 AM**	**5/25/2017**	**11:52 AM**				
6/1/2017	**9:57 AM**	**6/1/2017**	**11:43 AM**	**6/1/2017**	**12:52 PM**				
10/4/2017	10:30 AM	10/5/2017	1:41 PM	10/4/2017	11:20 AM				

TABLE 4. USGS stream gages

Location		**Gage No.**	**Hydrologic Unit**	**Latitude**	**Longitude**
OCWD	SANTA ANA R BL PRADO DAM CA	11074000	18070203	33°53'00"	117°38'40"
	SANTA ANA R A SANTA ANA CA	11078000	18070203	33°45'04"	117°54'27"
APW	SOUTH PLATTE RIVER BELOW UNION AVE, AT ENGLEWOOD, CO	06710247	10190002	39°37'57"	105°00'52"
	SOUTH PLATTE RIVER AT ENGLEWOOD, CO.	06711565	10190002	39°39'54"	105°00'13"
	SOUTH PLATTE R AT 64TH AVE. COMMERCE CITY, CO.	06714215	10190003	39°48'44"	104°57'28"
CW	MCDOWELL CREEK NR CHARLOTTE, NC	0214266000	03050101	35°23'22"	80°55'15"

TABLE 5. Estimated travel time and flow rate

Sample Location			Average Hydraulic Residence Time or Flow Rate
OCWD	1a – 1b	Prado Wetland	5 – 7 days[15]
	2a – 2b	AWPF	100 MGD[39]
	3	La Palma Recharge Basin	1 month (depth of 155.0 ft)
APW	1a – 2a	South Platte River	26.1 hrs[b] [40]
	1b/1c – 2a	South Platte River	17.0 hrs[40]
CMU	2a – 2b	SPR to AWPF	50 MGD[41]
	1 – 2	McDowell Creek to MIL	15 MGD (USGS)
	2 – 3	MIL to DW intake	12 days[42]

[b] Rough estimation based on the average low flow velocity of 1.124 ft/s as reported by USGS in October 1985. SP-100, SP-200, SP-300, SP-400, SP-500, SP-600, SP-700, SP-800, SP-900, SP-1000, SP-1100, SP-1100A, SP-1200, SP-1300, SP-1400, SP1500, SP-1600, and SP-1700. Distance: APW 1a-2a ≈ 20 miles, APW 1b/1c-2a ≈ 13 miles.

2.2 CONTAMINANTS OF INTEREST

The aquatic contaminants in Tables 6 and 7 were selected for analysis. The contaminants were chosen based on their individual toxicity as well as their potential to identify possible sources of pollution. The presence of metals contaminants indicates urban, agricultural, or industrial pollution; anions and nutrients also indicate urban, agricultural or industrial pollution, but specifically from National Pollutant Discharge Elimination System (NPDES); and the detection of aquatic microorganisms indicate anthropogenic activity and NPDES discharge.

TABLE 6. Conventional aquatic contaminants of interest for this study.

Metals/Cations	**Nutrients/Anions**	**Microorganisms**	**Aggregate Water Quality**
Boron (B)	Chloride (Cl^-)	Total Coliform	Total Organic Carbon (TOC)
Calcium (Ca)	Bromide (Br^-)	Fecal Coliform	Total Suspended Solids
Cadmium (Cd)	Sulfate (SO_4^{2-})	*Escherichia coli*	Conductivity
Copper (Cu)	Iodine (I^-)	*Enterococci*	pH
Iron (Fe)	Nitrite (NO_2^-)	*Salmonella spp.*	Alkalinity
Mercury (Hg)	Nitrate (NO_3^-)	*Cryptosporidium spp.*	Chemical Oxygen Demand (COD)
Magnesium (Mg)	Total Phosphate	*Giardia spp.*	5-Day Biochemical Oxygen Demand (BOD-5)
Manganese (Mn)	Total Phosphorous		
Sodium (Na)	Total Nitrogen		
Lead (Pb)			

TABLE 7. Selected emerging contaminants of concern for analysis

Pharmaceuticals	**Anthropogenic Compounds**	**ARGs**
Carbamazepine	Glyphosate	tetA
Azithromycin	Sucralose	tetW
Amoxicillin	Atrazine	sulI
Cephalexin	Benzo[a]pyrene	sulII
Ciprofloxacin		sulIII
Sulfamethoxazole-Trimethoprim		qnrA
Doxycycline		qnrB
Levofloxacin		ereA
Clindamycin		blaCTX-M
Penicillin V		blaSHV
Ibuprofen		16S rRNA

Sucralose, ibuprofen, carbamazepine, and sulfamethoxazole were selected to serve as surrogate compounds for their dominant attenuation pathways: conservative control, biodegradation, adsorption, and photolysis respectively.

2.3 FIELD SAMPLING PROTOCOL

All water quality parameters were analyzed in triplicate to establish the confidence intervals of the analytical methods.

The utilities were provided with containers specifically prepared for the analysis of trace contaminants. Borosilicate amber bottles were acid-washed, rinsed with distilled deionized water, dried and cleaned in the furnace at 550 °C for >2 hrs to decompose any organic matter. Polytetrafluoroethylene (PTFE) lined caps were used. Aliquots needed for microbiological analysis were collected in autoclaved Nalgene bottles. Bottles were shipped to utilities with ice packs, prepaid shipping labels and chain of custody forms with instructions to chill the collected samples and ship them with ice packs using overnight shipping method with morning delivery. The samples were immediately processed upon receipt. If immediate processing is not possible, samples for microbiological analysis were frozen at – 80 °C. The samples for non-microbial analysis were kept at 4 °C until analysis within holding time limits specified by the specific method. Aliquots for specific analyses were acidified for storage as required by the Standard Methods as presented in Table 8.

TABLE 8. Protocols for the preparation and preservation of samples and containers.

Analyte	Container	Preparation	Preservation	Hold Time
Total P, COD, NO^{3-}, NO^{2-}	Glass	Ashed 550 °C	n/a	2 d
Total N, Anions, Characterization	Nalgene	n/a	n/a	1 d
Metals	Nalgene	Rinsed with HNO_3	HNO_3, pH<2	180 d

PO_4^-	Glass	Ashed 550 °C, Rinsed with HNO_3	Filter, 0.45 μm	2 d
ECC[4] and TOC	Glass	Ashed 550 °C	HCl, pH<2[1]	1 d[2]
Microbial and BOD	Nalgene	Autoclave	n/a	1 d[3]

[1] qPCR samples stored at -80 °C after concentration
[2] indefinite hold time after SPE
[3] indefinite hold time after extracted samples were concentrated via SPE and evaporation
[4] ECC: emerging contaminants of concern

2.4 COST ANALYSIS

This study compared the before and after buffer contaminant concentrations to the current EPA drinking water maximum contaminant level (MCL) and maximum contaminant level goal (MCLG) standards. Cost evaluation was only considered for contaminants that were:

regulated by the EPA,

above the MCL or MCLG in the buffer influent,

below the MCL or MCLG in the buffer effluent, and

statistically different between in the buffer influent and effluent.

2.5 ANALYTICAL METHODS

2.5.1 Microbiology

All experimental negative controls and blanks were autoclaved sterile 18 MΩ·cm H_2O. All equipment was disinfected via autoclave, UV, or 70% ethanol.

DNA extractions and PCR reactions were done in triplicates. Primers used for Giardia were tagged against β-Giardin P241 and for Cryptosporidium against COWP P 702. Initial ARGs screening was performed by polymerase chain reaction (PCR) procedure followed by visualizing of the PCR product on gel. Based on the presence of a particular antibiotic resistant gene in each sample, further quantification were completed using real-time PCR.

2.5.1.1 Salmonella

Membrane filtration for salmonella concentration and microbial enrichment method were 1999 Standard Methods 9260B-1d, 9260D, 9221C and 9260B 2-a.

The method reduced the sample volume to a manageable size by physically separating the bacteria from the sample water via size exclusion. Sample water was filtered, volume as indicated in Table 9, without interrupting the filtration process through a sterile 142-mm (0.45 µm) membrane filter. The filter was placed in 100 mL of sterile peptone broth (Hardy Diagnostics) and homogenized at 33000 rpm for 1 min with a handheld VWR Bio-Gen 200 homogenizer. The homogenate was then diluted with double-strength selenite cysteine broth (Becton, Dickson and Company) via serial dilution as shown in Table 10, then incubated for 48h at 35-37°C.

TABLE 9. Volume filtered for salmonella experiment.

OCWD									
1a		**1b**		**2a**		**2b**		**3**	
Date	vol. (L)	Date	vol. (L)	Date	vol. (L)	Date	vol. (L)	Date	vol. (L)
2/28/2017	4.0	2/28/2017	4.0	3/7/2017	4.0	3/7/2017	4.0	2/21/2017	2.0
3/23/2017	4.0	3/23/2017	4.0	3/28/2017	2.0	3/28/2017	4.0	3/13/2017	4.0
6/29/2017	4.0	6/29/2017	2.0	6/22/2017	3.5	6/22/2017	3.4	6/19/2017	4.0
8/8/2017	3.0	8/8/2017	3.0	7/25/2017	2.0	7/25/2017	3.0	7/17/2017	3.0
8/28/2017	3.0	8/28/2017	2.0	9/19/2017	1.0	9/19/2017	4.0	8/21/2017	5.0
3/1/2018	1.5	3/1/2018	1.5	3/15/2018	2.0	3/15/2018	5.0	4/16/2018	3.0
APW									
1a		**1b**		**1c**		**2a**		**2b**	
Date	vol. (L)	Date	vol. (L)	Date	vol. (L)	Date	vol. (L)	Date	vol. (L)
5/11/2017	4.0	5/15/2017	2.0	5/11/2017	4.0	5/15/2017	3.0	5/15/2017	4.0
5/24/2017	1.0	6/7/2017	4.0	6/7/2017	4.0	6/19/2017	1.5	6/19/2017	3.0
6/14/2017	1.73	8/16/2017	4.0	8/16/2017	4.0	7/10/2017	2.0	7/10/2017	3.0
7/13/2017	2.0	1/11/2018	3.0	1/11/2018	3.0	1/10/2018	4.0	1/10/2018	3.0
8/15/2017	2.0								
CW									
1		**2**		**3**					
Date	vol. (L)	Date	vol. (L)	Date	vol. (L)				
4/13/2017	4.0	4/13/2017	2.0	4/13/2017	2.0				
4/21/2017	4.0	4/21/2017	2.0	4/21/2017	2.0				
4/26/2017	2.0	4/26/2017	2.0	4/26/2017	2.0				
5/25/2017	3.0	5/25/2017	2.0	5/25/2017	1.0				
6/1/2017	2.0	6/1/2017	2.0	6/1/2017	1.0				
10/4/2017	1.0	10/5/2017	1.0	10/4/2017	2.0				

TABLE 10. Salmonella selenite cysteine broth serial dilution

Dilution	Homogenate: selenite cysteine broth (mL/100mL)	Volume of stock homogenate added (mL)	Selenite cysteine broth added (mL)	Final concentration of homogenate to selenite cysteine broth (mL/L)
0.1x	n/a	2.5 mL	22.5 mL	0.1
0.01x	10:100	2.5 mL	22.5 mL	0.01
0.001x	1:100	2.5 mL	22.5 mL	0.001

Individual plates of xylose lysine deoxycholate agar (Millipore) were inoculated with the incubated dilution. The plates were then incubated upside down, to prevent condensation from falling onto the plates, for 24 hrs at 35 °C. After incubation, if present, white or black opaque salmonella colonies were selected for triple sugar iron agar (TSI) & lysine iron agar (LIA) slant (Carolina Biological Supply) tube inoculation using a sterile inoculation loop. The slant tubes were then incubated for 24 hrs at 35 °C then observed for indicator in accordance to the standard method: TSI slant produces red slants and acid yellow butt with/without gas bubbles, and blackening or pink slant and yellow butt; LIA slant produces black butt with red slant. Bacterial density was estimated following Environmental Protection Agency (EPA) Table 9221:IV and 9221C in units of most probable number/100 mL.

2.5.1.2 Coliforms and E. coli

IDEXX Colilert-18; WP100I-18, was used for the detection of coliform bacteria. Ortho-Nitrophenyl-β-galactoside (ONPG) and 4-Methylumbelliferyl-β-D-Glucuronide (MUG) were used as nutrient indicators, followed by Quanti-Tray Enumeration Procedure for most probable number (MPN) estimation.

The enzyme β-galactosidase is produced by *E. coli* to breakdown lactose into glucose and galactose. In the presence of β-galactosidase, ONPG is hydrolyzed to

produce ortho-nitrophenol. The hydrolyzed compound produces a yellowish color and were used to quantify E. coli via colorimetric assay as shown in Table 11.

TABLE 11. Interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

Appearance of Vessel	**Result**
Less yellow than the comparator1 when incubated at 35±0.5 °C or 44.5±0.2 °C	Negative for total coliforms and E. coli; Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 35±0.5 °C	Positive for total coliforms
Yellow equal to or greater than the comparator when incubated at 44.5±0.2 °C	Positive for fecal coliforms
Yellow and fluorescence equal to or greater than the comparator when incubated at 35±0.5 °C	Positive for E. coli

2.5.1.3 Enterococci

Enterococci followed the IDEXX procedure as section 2.4.2, but with nutrient broth (WENT200) and an incubation time and temperature of 24 hrs at 41 °C ± 0.5 °C. Sterile 18 MΩ·cm water served as negative control and *Enterococcus faecalis* was used as positive control. Results were interpreted according to Table 12.

TABLE 12. Interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

Appearance of Vessel	**Result**
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Negative for enterococci

2.5.2 Anions and Nutrients

2.5.2.1 Total Phosphorous, Total Nitrogen, Phosphate, Nitrate, and Nitrite

Total phosphate samples were immediately processed with 0.45 µm syringe filters upon collection. Total phosphate and total phosphorous was determined via the ascorbic acid method with acid persulfate digestion (2018, Standard Methods 4500-PE). Experiments were performed using the HACH Test ‘N Tube™ (TNT) for Phosphorus

(Total) TNT Reagent Set, Low Range (HACH, Cat. #2742645). Samples were heated according to the method and kit instructions (HACH DRB200 Digital Reactor Block) and analyzed with spectrophotometer (HACH DR6000 Benchtop UV-VIS, Program 535 and 536). 18 MΩ·cm H_2O served as method blank and QA/QC was assessed with known concentrations of sodium phosphate (Fisher Scientific, Cat. # S472-500) and glyphosate (Sigma-Aldrich).

Total nitrate was determined using the dimethylphenol method (HACH 10206 compliant under the U.S. EPA list of approved methods 40 CFR part 141.23, 2011) using HACH TNTplus Vial Test, Low Range with barcode recognition (HACH, Cat. #TNT835). Total nitrite was determined using the diazotization method (HACH 10237 compliant under the U.S. EPA list of approved methods 40 CFR part 141.23, 2011) using HACH TNTplus Vial Test, Low Range with barcode recognition (HACH, Cat. #TNT839). Samples were analyzed with spectrophotometer (HACH DR6000 Benchtop UV-VIS). 18 MΩ·cm H_2O served as method blank. Total nitrogen was determined using the persulfate digestion method (HACH 10072 compliant under the U.S. EPA list of approved methods 40 CFR part 141.23, 2011). Total Nitrogen Reagent Set (HACH) was used and samples were heated in the HACH DRB200 Digital Reactor Block and quantified using the spectrophotometer (HACH DR6000 Benchtop UV-VIS program 394). 18 MΩ·cm H_2O served as method blank.

2.5.2.2 Sulfate, Chloride, Iodide, and Bromide

Determination of inorganic anions by ion chromatography (1997, EPA Method 300.1A). Water samples were collected in 100 mL high-density polyethylene (HDPE) bottles with no additional preservatives. Forty mL of samples were immediately

prefiltered (Sigma-Aldrich Milipore glass-fiber filters AP series, Cat. #F5911) with vacuum filtration apparatus then transferred to sample vials (Thermo-Fisher Dionex Autoselect Polyvial, Cat. #055058) and stored at 4 °C until analysis.

Samples were analyzed using the Dionex ICS-3000 system; Dionex IonPAc™ AS 22 4 X 250 mm capillary column (Thermo Scientific, Cat. #64141); Dionex IonPAc™ AG22 4 X 50 mm guard column (Thermo Scientific, Cat #.064139); and Chromeleon® 7 Chromatograph Data System (v.7.2.1.5537) for data acquisition and quantification. A solution mixture of sodium bicarbonate and carbonate, 1.7 mM and 1.8 mM, respectively, in 18 MΩ·cm H_2O served as the eluent. Ultrapure 18 MΩ·cm H_2O was used as method blank, and internal and external standards were diluted from purchased standard solutions to verify QA/QC: chloride (Sigma-Aldrich, Cat. #BCBR6357V); bromide (Sigma-Aldrich, Cat. #BCBR6356V); iodide (Sigma-Aldrich, Cat. #BCBR6362V), and sulfate (Sigma-Aldrich, Cat. #BCBR7884V).

2.5.3 Metals

Determination of metals by inductively coupled plasma-atomic emission spectrometry (ICP-OES) was performed using EPA Method 200.7 (1994). Samples were collected in 100 mL high-density polyethylene (HDPE) bottles with 1 mL of 70% HNO_3 (Fisher Chemical). Samples were stored at 4 °C until processing. To process, samples were prefiltered (Sigma-Aldrich Milipore glass-fiber filters AP series, Cat. #F5911) with vacuum filtration apparatus then filtered again with 0.45 µm cellulose acetate membrane filter (Whatman) then transferred to 10 mL sampling tubes (SARSTEDT). The filtered samples were analyzed with Agilent Technologies 5100 series™ ICP-OES using UHP carrier grade argon (Roberts Oxygen Company) with the Agilent SPS 4 autosampler and

Agilent G8481A Recirculating Chiller. The ICP Expert software© (v.7.3.0.8799) was used for data acquisition and quantification. Ultrapure 18 MΩ·cm H_2O was used as method blank and internal and external standards were diluted from purchased standard solutions to verify QA/QC: cadmium (Sigma-Aldrich, Cat. #BCBS3572V), lead (Sigma-Aldrich, Cat. #BCBP7738V), copper (Sigma-Aldrich, Cat. #BCBS0410V), calcium (Sigma-Aldrich, Cat. #BCBR3915V), magnesium (Sigma-Aldrich, Cat. #BCBR3922V), manganese (Sigma-Aldrich, Cat. #BCBN7651), iron (Sigma-Aldrich, Cat. #BCBR8249V), boron (Inorganic Ventures), mercury (Inorganic Ventures), and sodium (Inorganic Ventures).

2.5.4 Water Characterization

2.5.4.1 Total Suspended Solids

EPA Method 160.2 (Gravimetric, dried at 103-105 °C) was used. Glass fiber filters (Millipore) were rinsed in ultra-pure water ≈ 3 times by holding the filters with a clean pair of forceps and dipping it in 200 mL of 18 MΩ·cm H_2O. The rinsed filters were then dried on clean metal pans or aluminum sheets at 120 °C for 1 hr. The dried filters were cooled in desiccators and the weight of the dried filter and aluminum pan was recorded (W_1). A vacuum funnel filtration was set up to filter the samples. After filtration, the filter was then replaced onto the sample aluminum pan and dried in the oven at 100 °C for 30 min. The dried filters were cooled in desiccators and the weight of the dried filter and aluminum pan was recorded (W_2).

Final total suspended solids (TSS) was calculated using Equation 1 with the recorded weights and volume filtered.

$$\left(TSS = \frac{(W_2 - W_1)}{volume\ of\ filtered\ sample\ (mL)} \times 1000\right)$$

TSS = Total suspended solids (g/L)
W_1 = Weight of aluminum pan + filter (g)
W_2 = Weight of dried aluminum pan + filter + filtrate (g)

Equation 1. TSS

2.5.4.2 Conductivity, Alkalinity, and pH

Samples were analyzed for conductivity, alkalinity, and pH at room temperature using the H-Series H280G laboratory pH, conductivity, dissolved oxygen (DO) & Ion Specific meter. The pH probe was calibrated with buffer solutions (Fisher Chemicals) at pH 4, 7, and 10 immediately before sample measurement. The conductivity probe was calibrated with a conductivity standard of 1000 µS/cm at 25 °C (HACH) prior to sample measurement. The pH and conductivity probes were rinsed thoroughly with 18 MΩ·cm H_2O from a wash bottle between each measurement. To measure, the probes were submerged in 500 mL of sample water that was agitated by a stir bar. 18 MΩ·cm H_2O served as blanks.

2.5.4.3 Alkalinity

Alkalinity was measured via titration using the HACH Alkalinity Test Kit, Model AL-DT 2063700 (1999, Standard Method 2320B). 18 MΩ·cm H_2O served as blanks.

2.5.4.4 Total Organic Carbon (TOC)

Shimadzu TOC-LCPN instrument (720 °C combustion) was used (Standard Method 5310-B 2000). The samples were acidified to pH < 2 with HCl immediately upon collection and cooled to ≤ 6 °C without freezing. All TOC sample vials were

pretreated by baking them at 500 °C for at least 1 hr. The hold time at ≤6 °C is 28 days from collection. The samples were stored in tightly closed vials to minimize exposure to light and atmosphere. The samples were introduced into the instrument via an autosampler. The autosampler is equipped with a magnetic stirring mechanism, and the samples were mixed by a stir bar throughout the analysis to prevent the settling of particulate matter. The instrument performed automatic sample acidification and nitrogen gas sparging to eliminate inorganic carbon.

2.5.4.5 Chemical Oxygen Demand (COD)

COD was determined using the reactor digestion method (1999, Standard Method 5220D). Experiments were conducted using the COD 2125915 Digestion Vials, High Range (HACH)[c] and COD K-7366 Digestion Vials, High Range (CHEMetrics)[d]. The vials were heated using the HACH DRB200 Digital Reactor Block and quantified using the spectrophotometer (HACH DR6000 Benchtop UV-VIS program 435. 18 MΩ·cm H_2O served as method blank.

2.5.4.6 5-Day Biochemical Oxygen Demand (BOD_5)

Standard Method 5210B (1999) was used. Dilution water was prepared by autoclaving de-ionized (DI) water in polypropylene container(s). HACH 1486266 BOD buffer nutrient solution was made and autoclaved immediately prior to sample analysis to prevent unwanted microbial growth. Twin bottles were made, diluted according to Table 13. The initial dissolved oxygen (DO) was recorded for bottles 1 – 4. Bottles 5 – 8 were

[c] OCWD samples 1a and 1b (02/28/17)
[d] CHEMetrics COD HR (0 – 1500 ppm) is equivalent to the HACH COD HR (20 – 1500 ppm) digestion vials (620 nm). Therefore, HACH Method 8000 and HACH instruments were used to process the CHEMetrics COD vials. No additional adapters necessary for HACH models DR6000 and DRB200.

stoppered, parafilmed, and placed in a dark incubator at room temperature. After day 5, the DO of the incubated bottles was recorded, and mg/L of BOD was calculated using Equation 2.

Table 13. BOD dilution chart, 300 mL total per bottle.

Bottle #	Dilution water volume (mL)	Sample water volume (mL)
1, 4	50	250
2, 5	150	150
3, 6	200	100
4*, 7*	300	0

*Quality control

$$\left(BOD_5 = \frac{(DO_{in} - DO_f)}{\frac{volume\ of\ sample\ (mL)}{volume\ of\ BOD\ bottle\ (mL)}} \right)$$

$\mathrm{BOD_5}$ = 5 Day biochemical oxygen demand (mg/L)
$\mathrm{DO_{in}}$ = Inital dissolved oxygen (mg/L)
$\mathrm{DO_f}$ = Final dissolved oxygen (mg/L)

Equation 2. BOD-5

2.5.5 Emerging Contaminants

Waters samples were collected in 2 L borosilicate glass bottles that were pre-baked at 500 °C for 1 hr. The samples were acidified immediately to pH<2.0 with 5.0 mL of 10 N HCl (RICCA) upon collection and chilled to <6.0 °C. The liquid chromatography–mass spectrometry (LCMS) samples were extracted within 24 hr using the solid phase extraction method detailed below and stored in the dark at 4.0 °C until analysis. The following compounds were chosen for analysis: carbamazepine, azithromycin, sucralose, amoxicillin, atrazine, cephalexin, ciprofloxacin, sulfamethoxazole-trimethoprim, doxycycline, levofloxacin, clindamycin, penicillin V, and benzo[a]pyrene. Glyphosate samples were collected in the same manner, in 500 mL borosilicate glass bottles, without the acidification process.

2.5.5.1 Pharmaceuticals and Pesticides

Solid-phase extraction (SPE)

All samples were filtered with glass filter (EMD) and 0.45 µm cellulose acetate membrane filter (Whatman) in an acid washed and baked (500 °C for 1 hr) gravity filter. The filtered samples were then transferred to 1 L flasks (500 °C for 1 hr) and spiked with 1mL of 1 µg/L deuterated carbamazepine prior to extraction (D-10). SPE was performed

using the Resprep 12-port SPE manifold (Restek). Hydrophilic-Lipophilic-Balanced (HLB) cartridges (SupelCo, 500 mg/12 mL) were conditioned with 5 mL of methanol (HPLC grade, Fisher Chemical) and 5 mL of solvent grade water (HPLC grade, EMD) sequentially at a rate of 1 – 2 mL/min. The filtered samples were transferred from the flasks to the cartridges with a SPE cartridge adaptor. The samples were loaded at an average rate of 4.0 mL/min. The cartridges were then eluted with 2.5 mL of methanol then 2.5 mL of acetonitrile (HPLC grade, EMD) and the eluents were collected in glass test tubes (VWR) that were rinsed 3x with 18 MΩ·cm H_2O then baked at 500 °C for 1 hr prior to sample collection. The samples were then heated at a constant 80 °C in an OA-HEAT™ Model 5085 water bath and evaporated with ultra-high purity (UHP) carrier grade nitrogen (Roberts Oxygen Company) using a N-EVAP™111 nitrogen evaporator system until <1 mL remained. The condensed eluent was then reconstituted with a 50/50 mixture of methanol and acetonitrile to 1mL. The reconstituted eluent was then transferred to 1.8 mL autosampler vials (VWR) and stored in -20.0 °C until LCMS analysis.

Liquid chromatography–mass spectrometry (LCMS)

The extracted samples were analyzed using Thermo Fisher Accela/Velos HPLC-MS Ion Trap with Diode Array Detector; Hypersil GOLD™ VANQUISH™ C18 UHPLC Column 100 X 2.1 (Thermo Scientific), and Velos Pro ™ 2 LTQ Tune Plus (v.2.7.0.126 SP4) and Xcalibur (v.3.1.66.10) software for data acquisition and quantification. Mobile phase A was 1% formic acid in HPLC grade water and mobile phase B was HPLC grade acetonitrile. The LC/MS/MSMS gradient conditions are described in Table 14.

TABLE 14. Summary of LCMS conditions for mobile phase gradients and flow rate.

Time (min)	Flow Rate (mL/min)	Mobile Phase B (percent)
0.0	0.250	10.0
2.0	0.250	10.0
17.5	0.250	60.0
20.0	0.250	100.0
23.0	0.250	100.0
24.0	0.250	10.0
29.0	0.250	10.0

The analytes of interest were identified and validated by comparing their retention times, primary ions, and daughter ions to external standards as shown in Table 15. Concentrations were calculated by determining the ratio of known quantitation of standard to peak area.

TABLE 15. Summary of LCMS retention times, ions, and calibration curves.

Compound and Analysis Method	Retention Time (min)	Exact Mass/ Electrospray Ionization Mode	Primary Fragment (m/z)	Calibration curve (µg/L) and R^2 value
Carbamazepine (MSMS)	14.14	237.0 [M + H]	194.0	2.5 – 100.0 $R^2 = 0.9968$
Carbamazepine D-10 (MSMS)	14.02	247.0 [M + H]	195.0	2.5 – 100.0 $R^2 = 0.9965$
Azithromycin (MSMS)	11.70	749.6 [M + H]	591.3	2.5 – 1000 $R^2 = 09998$
Sucralose (MS)	5.01	395.1 [M - H]	441.0 (Formate Ion)	2.5 – 250.0 $R^2 = 0.9997$
Amoxicillin (MS)	1.41	364.1 [M - H]	349.0	2.5 – 1000 $R^2 = 0.9995$
Atrazine (MS)	14.97	216.1 [M + H]	173.9	2.5 – 50.0 $R^2 = 0.9987$
Cephalexin (MS)	4.60	348.1 [M + H]	158.0	2.5 – 250.0 $R^2 = 0.9999$
Ciprofloxacin (MSMS)	6.59	332.3 [M + H]	314.3	2.5 – 50.0 $R^2 = 0.9999$
Sulfamethoxazole (MSMS)	9.85	264.3 [M + H]	155.9	2.5 – 100.0 $R^2 = 0.9982$
Trimethoprim (MSMS)	4.22	291.1 [M + H]	230.0	2.5 – 50.0 $R^2 = 0.9991$
Doxycycline (MS)	11.76	443.4 [M - H]	428.0	2.5 – 100.0 $R^2 = 0.9991$
Levofloxacin (MS)	5.65	362.1 [M + H]	318.1	2.5 – 100.0 $R^2 = 0.9998$
Clindamycin	11.82	425.9 [M + H]	377.1	2.5 – 100.0

(MSMS)				$R^2 = 0.9998$
Penicillin V (MS)	15.07	349.3 [M - H]	160.0	2.5 – 250.0 $R^2 = 0.9999$

QA/QC was determined with internal and external standards, HPLC grade water was used for blank, and percent SPE recovery was assessed with external standards and carbamazepine D-10 internal standards.

2.5.6 Benzo[a]pyrene

EPA Method 525.2 was followed for analysis of BaP.

2.5.6.1 Solid-phase extraction (SPE)

All samples were filtered with glass filter (EMD) and 0.45 µm cellulose acetate membrane filter (Whatman) in an acid washed and baked (500 °C for 1 hr) gravity filter. Samples were extracted with 14 days of collection. The filtered samples were then transferred to 1 L flasks (500 °C for 1hr). Duplicate samples were filtered, and one of the duplicates was spiked with 0.25 mL of 10 mg/L benzo[a]pyrene standard (Restek). SPE was performed using the Resprep 12-port SPE manifold (Restek). Hydrophilic-Lipophilic-Balanced (HLB) cartridges (SupelCo, 500 mg/12 mL) were conditioned sequentially with 4 mL ethyl acetate (Sigma-Aldrich) and 4 mL of dichloromethane (Thermo Fisher Scientific), 4 mL HPLC grade methanol, and 4 mL of HPLC grade water at a rate of 1 – 2 mL/min. The filtered samples were transferred from the flasks to the cartridges with SPE cartridge adaptor. The samples were eluted at an average rate of 4.0 mL/min, elution rate determined using. The cartridges were then eluted with 4 mL of ethyl acetate and 4 mL of dichloromethane and the eluents were collected in glass test tubes (VWR) that were rinsed 3x with 18 MΩ·cm H_2O then baked at 500 °C for 1hr). The samples were then heated at a constant 50 °C in an OA-HEAT™ Model 5085 water

bath and evaporated with UHP carrier grade nitrogen (Roberts Oxygen Company) using a N-EVAP™111 nitrogen evaporator system until <1mL remained. The condensed eluent was then reconstituted with a 50/50 mixture of ethyl acetate/dichloromethane to 1mL. The reconstituted eluent was then transferred to 1.8 mL autosampler vials (VWR) and stored in -20.0 °C until GC analysis.

2.5.6.2 Gas Chromatography (GC)

The extracted samples were analyzed with flame ionization detector (FID) Shimadzu GC 2014 with AOC-20i auto injector; Column, Rxi-17Sil, MS 15m-long x 0.25 mm-internal diameter, 0.25 µm, and Labsolutions (v.5.85) software for data acquisition and quantification.

GC operation conditions followed 1999 Standard Method 6440B-3c. Carrier gases were ultra-high purity (UHP) grade helium gas (Roberts Oxygen Company), UHP grade hydrogen gas (Roberts Oxygen Company), and zero grade air (Roberts Oxygen Company). Injection conditions were as follows: volume: 0.5 µL, hold: splitless; detector temperature: 320 °C; purge flow: 75 mL/min. Oven/column temperature was ramped from 80.0 °C (hold 2.0 min) to 297.5 °C at 15 °C/min then 320 °C at 10 °C/min and held for 20 minutes.

2.5.7 Glyphosate

2.5.7.1 Fluorenylmethyloxycarbonyl Chloride (FMOC) Derivatization (USGS Method 5-A10)

Samples were derivatized within 5 days of collection. All samples were filtered with glass filter (EMD) and 0.45 µm cellulose acetate membrane filter (Whatman) in an

acid washed and baked (500 °C for 1hr) gravity filter. 10 mL of the filtered samples were dispensed into 15 mL conical centrifuge vials. Each 10 mL sample was spiked with 0.1 mL of 100 µg/L of glyphosate-2-13C enriched standard (Sigma-Aldrich). 0.5 mL of 5% sodium tetraborate buffer solution (Sigma-Aldrich) was added to each sample tube, then vortexed to mix. FMOC (Indofine) 2.5-mM in acetonitrile (ACN) (EMD) was added to the vortexed sample then inverted 3 times to mix. The samples were then capped and placed in a 40 °C water bath in the dark for 24 ± 1 hours. After the incubation, 0.6 mL of 2% phosphoric acid (Fisher-Scientific) in HPLC grade water was added to the samples and inverted 3 times to mix. The acid quenched samples were then stored at 4 °C until SPE.

2.5.7.2 Solid-phase extraction (SPE)

USGS Method 5-A10 was followed for glyphosate extraction. SPE was performed using the Resprep 12-port SPE manifold (Restek). Hydrophilic-Lipophilic-Balanced (HLB) cartridges (SupelCo, 30 mg/1 mL) were conditioned sequentially with 2 mL of HPLC grade methanol and 2 mL HPLC grade water. The samples were eluted at an average rate of 2.0 mL/min. The cartridges were then washed with 1 mL of HPLC grade water and eluted with 5 mL of 5 µM ammonia acetate in HPLC grade water then 5 mL of acetonitrile (HPLC grade, EMD). The eluents were collected in glass test tubes (VWR). The samples were then heated at a constant 80 °C in an OA-HEAT™ Model 5085 water bath and evaporated with UHP carrier grade nitrogen (Roberts Oxygen Company) using a N-EVAP™111 nitrogen evaporator system until <1 mL remains. The condensed eluent was then reconstituted with a 50/50 mixture of 5 µM ammonia acetate

and acetonitrile until 1 mL. The reconstituted eluent was then transferred to 1.8 mL autosampler vials (VWR) and stored in -20.0 °C until LCMS analysis.

2.5.7.3 Liquid chromatography–mass spectrometry (LCMS)

The extracted samples were analyzed using Thermo Fisher Accela/Velos HPLC-MS Ion Trap with Diode Array Detector; Hypersil GOLD™ VANQUISH™ C18 UHPLC Column 100 X 2.1 (Thermo Scientific), and Velos Pro ™ 2 LTQ Tune Plus (v.2.7.0.126 SP4) and Xcalibur (v.3.1.66.10) software for data acquisition and quantification. Mobile phase A was 5 µM ammonium acetate in HPLC grade water and mobile phase B was HPLC grade acetonitrile. The LC/MS/MSMS gradient conditions are described in Table 16.

Glyphosate peaks were identified and validated by comparing its retention times, primary ions, and daughter ions to external standards as shown in Table 16. Calibration standards were derivatized and extracted.

TABLE 16. Summary of glyphosate LCMS conditions and calibration curve.

Time (min)	Flow Rate (mL/min)	Mobile Phase B (percent)
0.0	0.250	10.0
2.0	0.250	10.0
8.0	0.250	35.0
9.0	0.250	100.0
12.0	0.250	100.0
13.0	0.250	10.0
18.0	0.250	10.0

Compound and Analysis Method	Retention Time (min)	Exact Mass/ Electrospray Ionization Mode	Primary Fragment (m/z)	Calibration curve (µg/L) and R^2 value
Glyphosate FMOC (MSMS)	6.60	392.0 [M + H]	214.1	10.0 – 750.0 $R^2 = 0.9964$
Glyphosate-2-C13 FMOC (MSMS)	6.60	393.0 [M + H]	215.1	10.0 – 750.0 $R^2 = 0.9995$

2.5.8 Antibiotic Resistant Genes (ARGs), Cryptosporidium, and Giardia.

2.5.8.1 Sample Processing and DNA Extraction

Samples were immediately processed after receiving in the lab. Membrane filtration using 0.45 µm pore size was used to concentrate the samples, which were then frozen at -80 °C for future analysis. Membrane filter after filtration of a certain volume of the original sample (1–10 L) was eluted in 1.5–5 mL of tris buffer solution as follows: the filter paper and buffer were placed in a 50 mL centrifuge tube and agitated with a vortex mixer for 3–5 minutes to suspend the biomass entrained on the filter paper, and then centrifuged at 4000 rpm for 3 min to generate a pellet of the suspended solids in the solution. The supernatant was carefully discarded leaving the pellet, which was saved for DNA extraction at -80 °C. The volume of the sample used in the analysis was taken into account in final calculations. Total DNA was extracted using a Qiagen DNA isolation kit (QIAmp DNA Mini Kit) and the automated QIACUBE extraction system using a preprogrammed protocol for pelleted bacterial DNA extraction. The DNA quality was screened by A260/A280 and A260/A230 values, and DNA concentration was determined in ng/µL using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc.). The DNA extracts were stored at -20°C.

*2.5.8.2 **Quantification of ARGs***

Quantification of ARGs was done using qPCR (Biorad) from the extracted DNA. Different ARGs targets that were identified are presented in Table 17 below. Positive and negative controls were included for quality control of the samples in each of the qPCR run. Positive controls were cloned plasmid DNA with target gene (process described in the next

section), and for negative controls nuclease-free water was used instead of DNA extract of the sample. Each sample was run in triplicate to ensure reproducibility. The 16S rRNA gene was also analyzed for total microbial population and for normalization of the target resistant genes. Each 96-well plate had 20 µL volume of reaction mixture which contained 0.5 part of 2X SYBR green master mix (Biorad), 0.02 part of10 µM of forward primer, 0.02 part of 10 µM of reverse primer, 0.36 part of nuclease-free distilled water and 0.1 part of DNA template from the samples. The sample reading values obtained based on the standard curves were calculated and reported as the number of gene copies per 100 mL of original water sample.

TABLE 17. Target gene and corresponding primers characteristics.

Target gene	**Primer**	**Primer sequence (5′—3′)**	**Product size (base pairs)**	**Annealing temp. (°C)**
tetA	tetA-F	GCTACATCCTGCTTGCCTTC	210	55
	tetA-R	CATAGATCGCCGTGAAGAGG		
tetW	tetW-F	GAGAGCCTGCTATATGCCAGC	168	60
	tetW-R	GGGCGTATCCACAATGTTAAC		
sulI	sulI-F	CGCACCGGAAACATCGCTGCAC	163	55.9
	sulI-R	TGAAGTTCCGCCGCAAGGCTCG		
sulII	sulII-F	TCCGGTGGAGGCCGGTATATGG	191	60.8
	sulII-R	CGGGAATGCCATCTGCCTTGAG		
sulIII	sulIII-F	TCCGTTCAGCGAATTGGTGCAG	128	60
	sulIII-R	TTCGTTCACGCCTTACACCAGC		
qnrA	qnrA-F	TCAGCAAGAGGATTTCTCA	516	50
	qnrA-R	GGCAGCACTATGACTCCCA		
qnrB	qnrB-F	TCGGCTGTCAGTTCTATGATCG	469	54
	qnrB-R	TCCATGAGCAACGATGCCT		
ereA	ereA-F	AACACCCTGAACCCAAGGGACG	420	52
	ereA-R	CTTCACATCCGGATTCGCTCG		
blaCTX-M	blaCTX-M-F	ATGTGCAGYACCAGTAARGT	593	50
	blaCTX-M-R	TGGGTRAARTARGTSACCAGA		
blaSHV	blaSHV-F	TTTATCGGCCYTCACTCAAGG	930	58
	blaSHV-R	GCTGCGGGCCGGATAACG		
16S rRNA	1369F	CGGTGAATACGTTCYCGG	123	56
	1492R	GGWTACCTTGTTACGACTT		

2.5.8.3 ***qPCR standard development (positive controls)***

Positive controls were used to construct the standards for qPCR by transforming gene bearing plasmids into the *E. coli* using TOPO Cloning kit (Invitrogen™). Fresh regular PCR product from the samples with confirmed presence of the target gene was mixed with the cloning solution containing the TOPO Cloning *E.coli* vector. This mixture was then transformed into the competent *E. coli* cells (designed to accept foreign DNA and duplicate the genomic information while it is multiplying) followed by growth of these cells on agarose gel with lysogeny broth media. Culture suspension was prepared using the transformed colonies, screened by PCR again to verify cloning of the target gene. Plasmid was extracted according to the QlAprep® Spin Miniprep Kit (QIAGEN). The concentration of the purified plasmid DNA was determined using NanoDrop Spectrophotometer. Standards with different range of copies per mL were prepared by serial dilutions of purified plasmid extracts. Absolute quantification was done using qPCR assay. The CP (Crossing Point) value in the quantification graphs for each respective concentration was used to generate the final standard curve.

2.6 GRAPHS AND CALCULATIONS

2.6.1 Mass Balance

Contaminant concentrations in CW, which consisted of multiple substantial sources of influent, were determined by percent volume composition as shown in Equation 3.

$$\left(C_{inT} = \frac{(C_{in1} \times Q_{in1}) + (C_{in2} \times Q_{in2}) + \dots (C_{inx} \times Q_{inx})}{(Q_{in1} + Q_{in2} + \dots Q_{inx})}\right)$$

C_{inT} = Total contaminant concentration of blended influent
C_{in1-x} = Influent contaminant concentrations
Q_{in1-x} = Influent Flow

Equation 3. Mass balance of flow and contaminant concentration

2.6.2 Percent Removal

All environmental buffer systems were assumed to be in steady state with the only variating parameter being hydraulic flow volume, which were categorized by rain events and the local climate. The sites were paired, collected, and evaluated based on the buffer of interest, as shown in Table 18.

TABLE 18. Field study pairs and environmental buffers

	Wetland	Aquifer Recharge	River/Lake	Advanced Purification
OCWD	OCWD 1a / 1b	OCWD 2b / 3		OCWD 2a / 2b
APW		APW 4a / 4b	APW 1, 2, 3 / 4a	
CMU			CMU 1 / 2 / 3	

Table 2 provides additional information on sampling dates and times. The steady state assumption provides a simplified buffer dynamic system without the need to account for contaminant residence time, spatial distribution, or rate of spontaneous decay.

Attenuation was determined by subtracting the average effluent concentration from the average influent concentration as shown in Equation 4. Contaminants with negative concentrations were considered "re-contaminated", and positive concentrations to be "attenuated". All calculations were conducted using Microsoft Excel (Microsoft Office 365 ProPlus, v.16.0.11029.20045).

$$\left(Percent_{Recontamination} = \frac{C_{out} - C_{in}}{C_{out}}\right) \qquad \left(Percent_{Attenuation} = \frac{C_{in} - C_{out}}{C_{in}}\right)$$

C_{out} = final contaminant concentration / buffer effluent
C_{in} = initial contaminant concentration / buffer influent

Equation 4. Percent recontamination and attenuation equations

2.6.3 Statistical Analysis

2.6.3.1 T-Test

Percent removal significance was determined using a 2 tailed t-tests analysis at α = 0.05. The test was performed under the assumption of normal distribution and heteroskedastic unequal variance. $H_0 = \mu_0$ and $H_a \neq \mu_0$, reject H_0 if p-value ≤ 0.05. All statistical calculations were conducted using Microsoft Excel (v.1812).

2.6.3.2 Values Below Detection Limit (BDL)

Guidance for Data Quality Assessment – Practical Methods for Data Analysis, Section 4.7 was consulted. [43]

1. < 15% non-detects, substitution method. EPA suggested replacement method of detection limit divided by 2 (LOD/2).
2. < 25% non-detects, the substitution method was utilized. Croghan and Egeghy recommends replacement method of detection limit divided by square root of 2

(LOD/√2). appears to be the best choice of replacement values with overall smaller error rate than option a. [44]

3. 15% - 50% non-detects, maximum likelihood estimation of the mean and variance. Cohen's Method, provides adjusted estimates of the sample mean and standard deviation that accounts for data below the detection level to be used in statistical analyses.[e]

   i. Compute the sample mean $\bar{X}_d$ from the data above the detection limit (DL)[f]:

   $$\bar{X}_d = \frac{1}{m} \sum_{i=1}^{m} X_i$$

   ii. Compute the sample variance s^2_d from the data above the detection limit:

   $$s_d^2 = \frac{\sum_{i=1}^{m} X_i{}^2 - \frac{1}{m}(\sum_{i=1}^{m} X_i)^2}{m-1}$$

   iii. Compute h and γ, and estimate λ.[g]

   $$h = \frac{n-m}{n} \qquad\qquad \gamma = \frac{s_d^2}{(\bar{X}_d - DL)^2}$$

   iv. Estimate the corrected sample mean, $\bar{X}$, and sample variance, s^2 to account for the data below the detection limit, as follows:

   $$\bar{X} = \bar{X}_d - \lambda(\bar{X}_d - DL) \qquad s^2 = s_d^2 + \lambda\,(\bar{X}_d - DL)^2$$

4. 15% - 50% non-detects, Trimmed Mean. For environmental data, nondetects usually occur in the left tail of the data so trimming the data can be used to adjust the data set to account for nondetects when estimating a mean.

[e] Requires that the data without the non-detects be normally distributed and the detection limit is always the same.
[f] Let $X_1, X_2, \ldots, X_n$ represent the n data points with the first m values representing the data points above the DL.
[g] If exact value of h and γ do not appear in the table, use double linear interpolation to estimate.

i. Let t represent the integer part of the product np. For example, if p = .25 and n = 17, np = (.25)(17) = 4.25, so t = 4[h].

$$t = np$$

ii. Delete the t smallest values of the data set and the t largest values of the data set. For example, delete 4 of the largest and smallest value from the data set.

iii. Compute the arithmetic mean of the remaining n - 2t values:

$$\bar{X} = \frac{1}{n-2t} \sum_{i=1}^{n-2t} X_i$$

5. 15% - 50% non-detects, Winsorized Mean and Standard Deviation, replaces data in the tails of a data set with the next most extreme data value For environmental data, non-detects usually occur in the left tail of the data. Therefore, winsorizing can be used to adjust the data set to account for non-detects.

i. List data from smallest to largest (including non-detects). Label $X_{(1)}$, $X_{(2)}$,…, $X_{(n)}$ so that$X_{(1)}$ is the smallest value and $X_{(n)}$ the largest[i].

ii. Replace the n-m nondetects with $X_{(m + 1)}$ and replace the n-m largest values with $X_{(n - m)}$.

iii. Using the revised data set, compute the sample mean, $\bar{X}_w$, and the sample standard deviation, s_w:

$$\bar{X}_w = \frac{1}{n} \sum_{i=1}^{n} X_i \qquad s = \sqrt{\frac{\left(\sum_{i=1}^{n} X_i^2\right) - n\bar{X}^2}{n-1}} \qquad s_w = \frac{s(n-1)}{(2m-n-1)}$$

[h] Let $X_1, X_2, \ldots, X_n$ represent the n data points. ($0 < p < 0.5$), suggest for environmental samples p = 0.15.
[i] Let X1, X2, . . . , Xn represent the n data points and m represent the number of data points above the detection limit (DL), and hence n-m below the DL.

6. > 50% non-detects, Test of Proportions, Z-test (only if more than 50% of the data are below the detection limit but at least 10% of the observations are quantified). H_0: $p_1 = p_2$ versus H_A: $p_1 \neq p_2$. However, due to low sample size, cannot efficiently use this method.

2.6.4 Data Presentation

Paired sample box plots were generated in Microsoft Excel. The non-standardized scale was not intended to create visual biasness. The main function of the plots was to compare the before and after concentration of an analyte within one specific buffer system. Any variability was still captured on a non-zero scale axis since it applied to the compared analytes equally.

Boxplots that show concentrations at two points being analyzed combine wet and dry weather samples. Wet and dry weather sets for each field study location were analyzed separately as well, and if any significant influences of the weather were captured, they are discussed in text. Boxplots are intended as a visual representation of the range and the variability of values observed for each analyte at each sampling location. The diamond symbol on the boxplots represents the averages for the given data sets (each set consisting of 6-5 values). In some instances, the averages may be influenced by outliers in which case they will appear on the “whisker” portion of the boxplot. The boxplots were made using Microsoft Excel (Microsoft Office 365 ProPlus, v.16.0.11029.20045), see Figure 8.

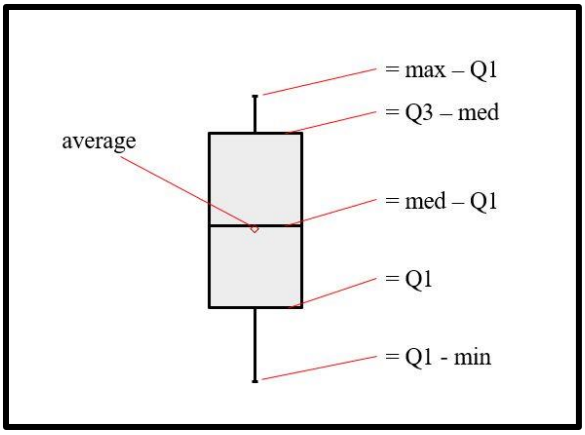


FIGURE 8. Description of boxplot

the smallest value (min), first quartile (Q1), median (med), third quartile (Q3), and the highest value (max) was calculated with excel functions: =MIN(), = QUARTILE(,,1), =MEDIAN(), =QUARTILE(,,3), and =MAX(). The plot areas were determined by calculating the area under the lower bound = Q1, lower bound = med – Q1, and upper bound = Q3 – med. The top whisker was determined by the first standard deviation = max – Q3, and the bottom whisker = Q1 – min.

An additional figure for each class of contaminants shows the average percent change in concentration between two locations under comparison.

When contaminants show 100% removal in those figures, the effluent value was below the method limit of detection (MLD) and was set as zero to obtain a numeric value for removal. However, non-detect samples may have non-zero concentrations of up to detection limit. The percent removal reported in the discussion section accounts for the MDL and factors the lowest possible concentration detectable into the percent calculation. For example, if the MDL is 5 mg/L, the influent measures 100 mg/L and the effluent is below detection limit, the removal is considered to be >95% rather than 100%. MDL values for each method can be found in the appendices.

2.7 CALIBRATION AND QAQC

2.7.1 Microbiology

2.7.1.1 IDEXX

Live *Escherichia coli* and *Enterococcus faecalis* cultures were kept suspended in Lauria-/Bertani nutrient broth in individual 1.5 mL microtubes (nutrient broth and microtubes were pre-sterilized via autoclaving at 121 °C for 1 hr at 15psi). The inoculated microtubes were incubated at 35 °C until use. To inoculate for positive control, 1 mL of the live culture was extracted from the microtubes and dispensed into the IDEXX enumeration broth and processed along with the field samples. One positive and negative control was processed per batch of samples. (-) control results measure < 1.0 MPN/100 mL and (+) control results measure > 2419.6 MPN/100 mL. Details of which can be seen in Table 19.

2.7.1.2 Salmonella spp.

(-) control results measure < 1.8 MPN/100 mL.

TABLE 19. Summary of QAQC objectives for the verification of methods.

	(-) Control/Blank[j]	(+) Control	Lab Replicates	Method Detection Limit (MDL)
Salmonella spp.	18 MΩ·cm H_2O	n/a	1[e]	1.8 – 1600.0 MPN/100 mL
Total coliform	18 MΩ·cm H_2O	*Escherichia coli*	1[k]	1.0 – 2419.6 MPN/100 mL
Fecal coliform	18 MΩ·cm H_2O	*Escherichia coli*	1[e]	1.0 – 2419.6 MPN/100 mL
Escherichia coli	18 MΩ·cm H_2O	*Escherichia coli*	1[e]	1.0 – 2419.6 MPN/100 mL
Enterococci spp.	18 MΩ·cm H_2O	*Enterococcus faecalis*	1[e]	1.0 – 2419.6 MPN/100 mL
ARGs	18 MΩ·cm H_2O			
Cryptosporidium.	18 MΩ·cm H_2O			
Giardia	18 MΩ·cm H_2O			

[j] Sterilized via autoclave – 121 °C for 1 hr at 15psi
[k] Triplicates built in IDEXX and salmonella quantification method

2.7.2 Oxygen Demand

2.7.2.1 COD

HACH - laboratory blanks read < 20.0 mg/L and laboratory duplicate samples were within ± 20% accuracy. CHEMetrics - laboratory blanks read 0.0 ± 12 mg/L (CHEMetrics allowable for 0 mg/L standard using a spectrophotometer) and laboratory duplicate samples within ± 20% accuracy. See Table 20 for details

2.7.2.2 BOD-5

The field samples should have a minimum DO depletion of 2.0 mg/L and a residual DO of 1.0 mg/L. The control sample should not have a DO depletion of more than 0.20 mg/L. See Table 20 for details.

TABLE 20. Summary of QAQC and quality objectives for testing oxygen demand.

	(-) Control/Blank	(+) Control	Lab Replicates	Method Detection Limit
COD	18 MΩ·cm H_2O	n/a	3	20 mg/L
BOD-5	18 MΩ·cm H_2O[d]	n/a	1	1 mg/L residual DO

2.7.3 Anions and Nutrients

Primary standard solutions were prepared by diluting (+) control standards listed in Table 21 in HPLC grade water and stored at 4 °C.

2.7.3.1 HACH Kits

Working standard solutions were prepared the day of QA/QC evaluation via dilution in HPLC grade water. QA/QC was performed with 5 working laboratory standards for each parameter, concentrations ranged from the minimum detection limit to the maximum detection limit. Acceptance limits for laboratory standards and laboratory replicates is within ± 20% accuracy. Laboratory blanks or (-) control measure < MDL.

All samples were evaluated for possible interferences and if necessary, pH was adjusted accordingly to the specific method of analysis.

TABLE 21. Summary of QAQC and quality objectives for testing anion and nutrients.

	(-) Control/Blank	(+) Control Standards	Lab replicates	Method Detection Limit
Total P	18 MΩ·cm H_2O	Glyphosate	3	0.02 mg/L – P
Total N	18 MΩ·cm H_2O	Ammonium acetate	3	2.0 mg/L – N
Phosphate	18 MΩ·cm H_2O	Potassium phosphate	3	0.06 mg/L – PO_4^{3-}
Nitrate	18 MΩ·cm H_2O	Potassium nitrate	3	0.23 mg/L NO_3^- – N
Nitrite	18 MΩ·cm H_2O	Sodium nitrite	3	0.015 mg/L NO_2 – N
Sulfate	18 MΩ·cm H_2O	Sodium sulfate	3	0.5 mg/L – SO_4^{2-}
Chloride	18 MΩ·cm H_2O	Sodium chloride	3	0.5 mg/L – Cl^-
Iodide	18 MΩ·cm H_2O	Potassium iodide	3	0.05 mg/L – I^-
Bromide	18 MΩ·cm H_2O	Sodium bromide	3	0.05 mg/L – Br^-
COD	18 MΩ·cm H_2O	Potassium hydrogen phthalate	3	0.0 mg/L COD

2.7.3.2 Ion Chromatography

The Chromeleon® 7 Chromatograph Data System (v.7.2.1.5537) software was used to develop the calibration curves for each standard. Minimum R^2 value for linear calibration fit > 0.9990 at 95% confidence level. Calibration standards were prepared fresh with HPLC grade water for each batch run. QA/QC was assessed per batch using initial calibrating check standards, laboratory replicates, matrix spikes, and laboratory blanks, all of which fall within ± 15% accuracy and < MDL for blanks. Continuing calibration standards and laboratory reagent blanks were analyzed per sample and fall within ± 15% accuracy and < MDL respectively. Nine standards analyzed per calibration curve; sulfate and chloride ranged from 0.5 mg/L – 100 mg/L; and iodide and bromide ranged from 0.05 mg/L – 10 mg/L.

2.7.4 Metals

Inductively Coupled Plasma-Atomic Emission Spectrometry

The ICP Expert software© (v.7.3.0.8799) software was used to develop the calibration curves for each standard. Six standards were analyzed per calibration curve and the lowest standard dictates the MDL of the metal as described in Table 22. Minimum R^2 value for linear calibration fit was > 0.9995 at 90% confidence level. Calibration standards were prepared fresh with HPLC grade water for each batch run. QA/QC was assessed per batch using initial calibration standards, laboratory replicates (3 replicates), and laboratory blanks, all of which fall within ± 10% accuracy and < MDL for blanks. Continuing calibration standards and laboratory reagent blanks were analyzed between 10 sample intervals and fall within ± 10% accuracy and < MDL respectively.

TABLE 22. Summary of quality objectives and calibration range for metals.

	Blank[1]	Wavelength (nm)	Calibration Range
Boron	2.0 % HNO_3	249.772	0.01 – 0.20 mg/L
Cadmium	2.0 % HNO_3	214.439	0.01 – 0.20 mg/L
Copper	2.0 % HNO_3	327.395	0.01 – 0.20 mg/L
Lead	2.0 % HNO_3	220.347	0.01 – 0.20 mg/L
Calcium	2.0 % HNO_3	442.673	0.75 – 75.0 mg/L
Iron	2.0 % HNO_3	273.358	0.03 – 0.50 mg/L
Magnesium	2.0 % HNO_3	279.800	0.50 – 25.0 mg/L
Manganese	2.0 % HNO_3	280.108	0.03 – 0.50 mg/L
Sodium	2.0 % HNO_3	589.920	1.00 – 200 mg/L
Mercury	2.0 % HNO_3	194.164	0.01 – 0.20 mg/L

2.7.5 Water Characterization

2.7.5.1 Total Suspended Solids

Laboratory blanks measured < 0.1 mg/L and laboratory sample replicates were within ± 20% accuracy.

2.7.5.2 Conductivity

Laboratory reagent blanks measured < 0.05 µS/cm (ASTM D1125); initial calibration check standard was within ± 5% of calibration standard; and laboratory sample replicates were within ± 5% accuracy.

2.7.5.3 pH:

Initial calibration check standards measured within ± 0.5% of calibration standards at pH 4, 7, and 10; and laboratory sample replicates were within ± 5% accuracy.

[1] HNO_3 solution prepared in HPLC grade water

2.7.5.4 Alkalinity:

Laboratory blanks measured < 10 mg/L as $CaCO_3$ and laboratory sample replicates were within ± 5% accuracy.

2.7.5.5 Total Organic Carbon:

Shimadzu TOC-Control software was used to develop the calibration curve with a minimum R^2 value for linear calibration fit > 0.9995 at 95% confidence level. QA/QC was assessed per batch using initial calibration standards, laboratory replicates (3 replicates), laboratory sample matrix spikes, inorganic and organic quality control standards, and laboratory blanks, all of which fall within ± 25% accuracy and < MDL for blanks. Continuing calibration standards and laboratory reagent blanks were analyzed with each sample. The standards must fall ± 25% to the expected concentrations and blanks < MDL, as indicated in Table 23.

TABLE 23. Summary of QAQC objectives and MDL for aggregate water quality assessment.

	(-) Control/Blank	(+) Control	Lab Replicates	Method Detection Limit (MDL)
TSS	18 MΩ·cm H_2O	N/A	3	0.1 mg/L
Conductivity	18 MΩ·cm H_2O	1000 µS/cm	3	0.0 µS/cm[m]
pH	18 MΩ·cm H_2O	pH standard solutions	3	-2.0 – 19.9[g]
Alkalinity	18 MΩ·cm H_2O	NA	3	10 mg/L as $CaCO_3$
TOC	18 MΩ·cm H_2O	Potassium hydrogen phthalate	3	0.5 mg/L - C

2.7.6 Emerging Contaminants

2.7.6.1 Pharmaceuticals and Pesticides:

[m] Instrument detection limit

All calibration stock solutions were stored at 4 ℃ until use and working calibration and standard solutions were prepared with each batch run. SPE QA/QC was assessed with laboratory blanks; laboratory sample matrixes were spiked with the surrogate analyte deuterated carbamazepine D-10 plus non-deuterated standards of known concentrations for checking extraction efficiency and to determine recovery rate; and laboratory replicates. LCMS QA/QC was assessed by running initial and continuing calibration standards, laboratory reagent and blanks (18 MΩ·cm H_2O), in addition to the SPE QA/QC blanks, spikes, replicates, and standards. Laboratory replicates, initial calibration, and continuing standards fall within ± 25% accuracy; reagent and blanks read < MDL, and recovery fall ± 25% of the average carbamazepine D-10 recovery per sample location (e.g. OCWD 1a, 1b, 2a… APW 1a, 1b, 1c…etc.). Results outside of the acceptable extraction recovery range were omitted from data analysis. Calibration curves were calculated by determining the ratio of 10 known quantitation of standard to peak area relative to the known calibration standard concentrations. Limit of detection was determined by the LC-MS instrument detection limit; manually integrated peaks were included in the data report. Method limit of detection is limited to the lowest calibration standard concentration.

2.7.6.2 Glyphosate:

All calibration stock solutions were stored at 4 ℃ until use and working calibration and standard solutions were prepared with each batch run. FMOC derivatization and SPE of glyphosate was verified with laboratory blanks; laboratory replicates, and laboratory sample matrixes were spiked with the enriched surrogate analyte glyphosate-2-13C of known concentration to quantify extraction recovery rate.

LCMS QA/QC was assessed by running initial and continuing calibration standards, laboratory reagent and blanks (18 MΩ·cm H_2O), in addition to the SPE QA/QC blanks, replicates, and standards. Laboratory replicates, initial calibration, and continuing standards fall within ± 25% accuracy; reagent and blanks read < MDL, and recovery fall ± 25% of the average glyphosate-2-13C recovery rate. Calibration curves were calculated by determining the ratio of 10 known quantitation of derivatized and extracted standard to peak area relative to the known derivatized and extracted calibration standard concentrations, detailed. Limit of detection was determined by the LC-MS instrument detection limit; manually integrated peaks were included in the data report. Method limit of detection is limited to the lowest extracted calibration standard concentration.

2.7.6.3 Benzo[a]pyrene:

All calibration stock solutions were stored at 4 °C until use and working calibration and standard solutions were prepared with each batch run. SPE QA/QC was assessed with laboratory blanks of 50/50 dichloromethane and ethyl acetate; laboratory sample matrixes were spiked with non-deuterated standards of known concentrations for checking extraction efficiency and to determine recovery rate; and laboratory replicates. GC-FID QA/QC was assessed by running initial and continuing calibration standards, laboratory reagent and blanks (50/50 dichloromethane and ethyl acetate, and methanol), in addition to the SPE QA/QC blanks, replicates, and standards. Laboratory replicates, initial calibration standards, and continuing standards fall within ± 25% accuracy; reagent and blanks read < MDL, and recovery fall ± 25% of the known benzo[a]pyrene matrix spike concentration. The Labsolutions (v.5.85) software was used to develop the

calibration curves for each standard. Minimum R^2 value for linear calibration fit was > 0.9990 at 95% confidence level. Calibration standards were prepared fresh with each batch run. Five benzo[a]pyrene calibration standards were analyzed per run, and MDL was determined by the concentration of the lowest standard: 25 µg/L.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 CONSTRUCTED WETLAND (OCWD 1A – 1B)

3.1.1 Prado Constructed Wetlands (PCW)

The area of study, Prado Constructed Wetlands, is situated above the Prado Dam between Riverside County and San Bernardino County in Southern California. Considered to be the largest constructed wetland in western U.S., the 450-acre system is composed of 45 open-air shallow ponds. The depth of the constructed ponds ranges from 0.45m to 2.5 m deep with an average retention time of 5 to 7 days, surface flow rate of >35.0 MGD, and hydraulic loading rate of 10 cm/d. The main source of water comes from the SAR whereby half of the base flow, roughly 1.8 to 2.4 m^3/s, is diverted through the wetlands. In addition to the SAR, the Prado Basin also receives water from Chino Creek, Cucamonga Creek/Mill Creek and Temescal Creek. The wetlands are operated by OCWD as part of the district's potable-reuse management infrastructure. During the dry season, from May to October, the bulk of SAR's flow are comprised of wastewater-effluent discharges from WWTPs upstream. As the river continues downstream, it percolates into the groundwater recharge and is subsequently used as the source water for the local DWTPs. PCW enhances aquifer recharge performance by improving river water quality prior to recharge infiltration.

Pathogens and microbes, nutrients, and inorganic contaminants are usually retained when stormwater or wastewater effluent has been processed through a wetland buffer. The different physical, chemical, and biological characteristics of PCW allow for efficient removal of certain aquatic contaminants, thus functioning as kidneys of the potable reuse system[14]. The effectiveness of wetlands is corroborated by the data

collected from OCWD's Prado Constructed Wetlands, where attenuation of nutrients occurred, particularly nitrogen and phosphorous.

Sample locations in PCW are designated as (OCWD 1) and (OCWD 2), as shown in Figures 2 and 3. The wetland influent samples, (OCWD 1), were collected from the SAR right before it was diverted to enter the constructed wetlands. The effluent samples were collected approximately halfway through PCW (the staff had limited accessibility to the wetland outlet as it was submerged underwater during the rainy season). Six total samples were collected at Prado: three samples were collected during the dry season and three during the rainy season (Table 1 lists specific time and dates). The data for the dry and wet seasons were analyzed together in order to provide a comprehensive assessment that considered environmental fluctuations due to temporal and weather differences. Nevertheless, the data from wet and dry seasons were evaluated separately as well, but only significant findings were reported.

3.1.2 Metals

Copper and iron were attenuated in PCW as shown in Figures 9 and 10 [n]. Heavy metals entering a wetland system can be attenuated through several different mechanisms. A wetland copper tracer study was conducted by Babscányi *et al*. (2014) with similar hydro-chemical conditions to this study. Their findings suggested the following retention pathways, (1) adsorption onto mineral phases and complexation with organic ligands, (2) precipitation with other minerals, (3) biological uptake by

[n] Contaminants with 100% removal, shown in figures, are under the assumption that the final/effluent measurement is zero if the concentration is below the instrument or method limit of detection (LOD) level. The percent removal reported in the discussion section accounts for the LOD and factors the lowest possible concentration detectable into the percent calculation. For example, if the LOD is 5 mg/L, the influent measures 100 mg/L and the effluent is BDL, the removal is considered to be >95% rather than 100%. Instrument limit of detection = 1.0 ppb.

macrophyte and microcytic organisms, and (4) sedimentation of precipitated species.[45] According to the speciation modeling for wetlands, most of the copper species were found as organocomplexes.[45, 46] Additionally, in constructed wetlands, copper and iron tends to accumulate in the floc and organic layers of the soil column and proportionally increase or decrease depending on the percent organic matter available in those layers.[47, 48] Therefore, the copper was most likely retained through bio-uptake or bio-adsorption.

Naturally occurring manganese deposits near the constructed wetlands could have contributed to the 47% increase of manganese measured in this study. Additionally, manganese complexation with ligands may have facilitated its mobility and transport through the wetlands.[49] The recontamination with manganese in PCW was not statistically significant. The wetland influent and effluent concentrations exceeded the EPA maximum contaminant level (MCL)[o] for manganese of 0.05 mg/L.

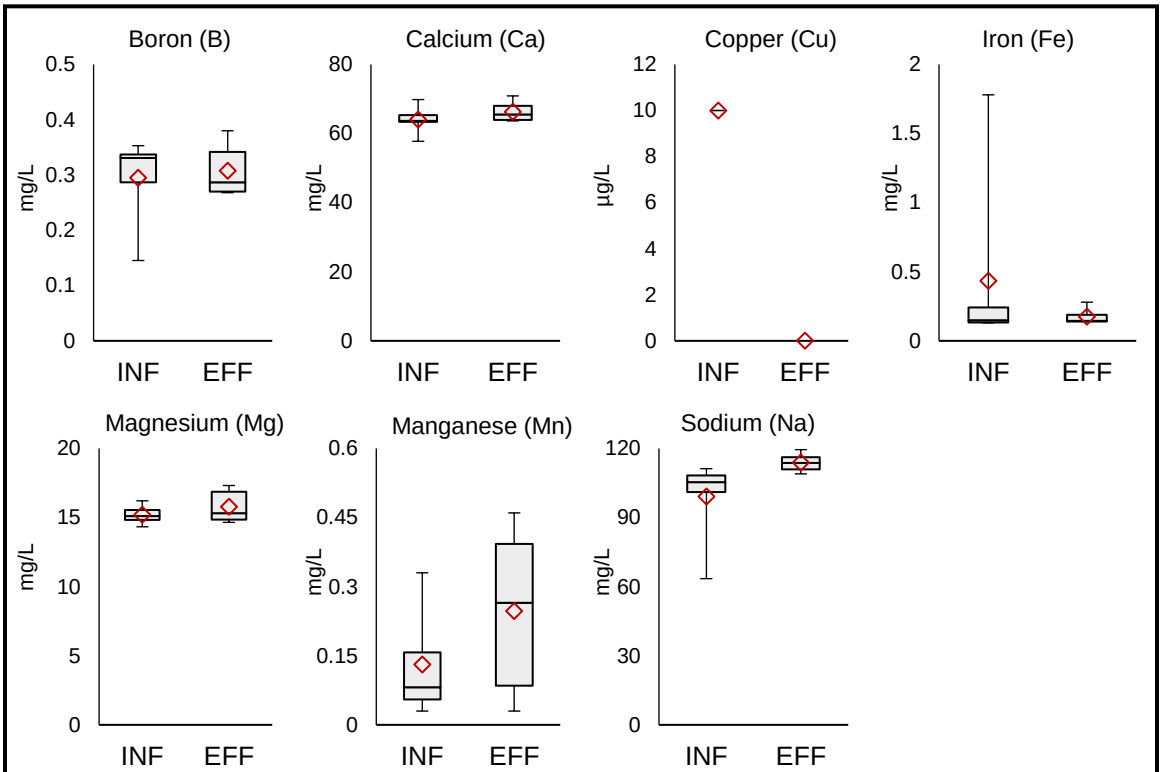
Except for copper and magnesium, the attenuation/recontamination of metals during wet and dry seasons did not differ significantly, as expected, since wetlands functionally imitate equalization tanks which buffer sudden fluctuations from hydraulic and contaminant inflow. Both copper and magnesium measured higher during the high flow conditions of rainy seasons in both the influent and effluent. This may be indicative of non-point source pollution as stormwater runoff can increase soil leaching.

All metals with the exception of iron and manganese were well below the drinking water maximum contaminant level goal (MCLG)[p]. The concentration of iron

[o] The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to MCLGs as feasible using the best available treatment technology and taking cost into consideration. MCLs are enforceable standards (EPA, 2019).

[p] MCLG: The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety and are non-enforceable public health goals (EPA, 2019).

was initially above the MCL standard of 0.3 mg/L with an average of 0.44 mg/L at the intake but was effectively reduced to below the MCL at 0.18 mg/L. Therefore, the wetland was able to provide additional cost savings to the downstream DWTPs in this instance. However, manganese concentration remained above MCL and MCLG levels in both the intake and post PCW purification. Previous tracer studies that utilized copper showed that removal is consistently proportionate to the amount that is introduced; thus, in terms of metal contaminant reduction, it may be cost efficient for wetlands to receive industrial wastewater that contain high concentrations of metal contaminants.[45]



*cadmium (Cd), mercury (Hg), and lead (Pb) = below detection limits.

FIGURE 9. Comparison of metal concentrations for wetland influent and effluent.

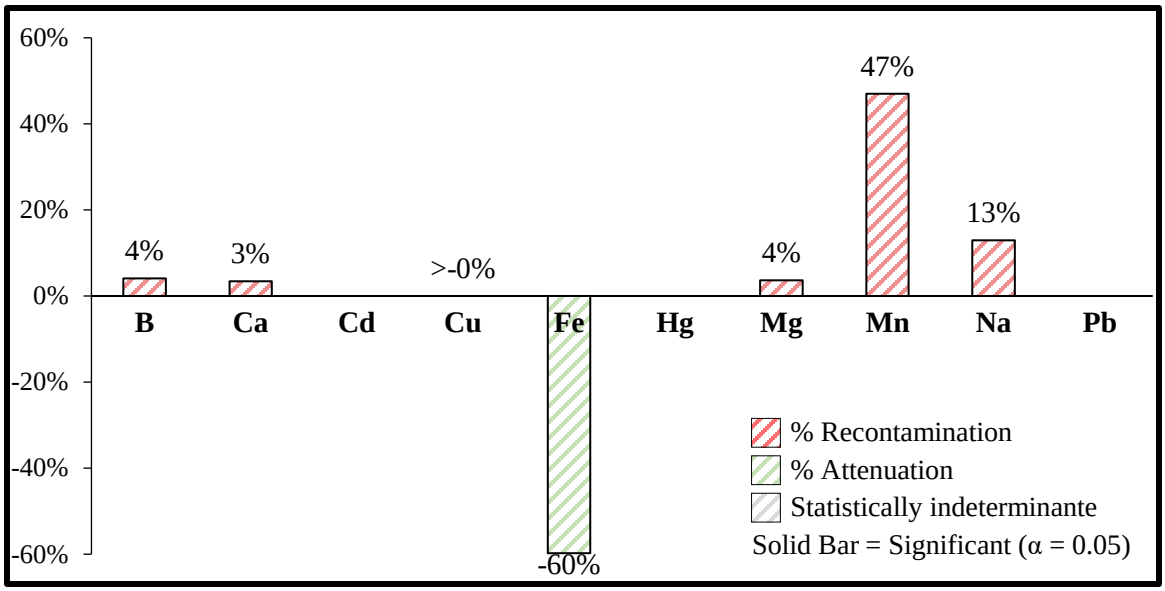


FIGURE 10. Average percent decrease or increase of metals in Prado Constructed Wetlands.

3.1.3 Nutrients and Anions

The location of PCW is considered to be in the major zone of marine influence.[50] Located roughly 50.0 km inland from the Pacific Ocean, the increase in iodide concentration, as shown in Figures 11 and 12, may have been influenced by its proximity to the ion enriched coastal soils and aerosol deposition from the seawater. PCW are also susceptible to the rise and fall of sea level, where the salinity gradients of the wetland may change depending on the ocean water table.[51] This increase was not observed in the other ions measured in this study, this is likely due to the chemical properties of iodide compounds and plant uptake mechanisms. Unlike other macronutrients, the soil and aquatic uptake capacity of iodide through the root system are generally limited as plants prefer to metabolize atmospheric iodide or obtain it through plant surface deposits.[50] This explains why a similar trend was not observed for the other ions. Another potential iodide contributor could be groundwater recharge downstream. The wetland outflows are

directed towards the aquifer recharge downstream of Prado Dam. Although, hydraulically, the wetland are upstream of the recharge, over time, the iodide may saturate the surrounding soil and diffuse into the wetland.[52]

The increased level of iodide can be potentially beneficial. According to the United States Institute of Medicine, the recommended daily allowance of iodide for adults is 150 µg per day.[53] The constructed wetland effluent contains an average of 50 µg/L of iodide; removal may not be necessary as it may be beneficial to consumers who do not receive adequate amounts of the trace element from their diets. Iodine is often used in dairy operations; as a nutrient supplement, sanitizers, and veterinary medications.[54] One study conducted by United States Geographical Survey (USGS) determined that dairy farms are potential contributors to the high concentrations of iodine in found surface waters.[55] The study results corresponded to the levels measured in Prado where it is impacted by multiple dairy farms upstream. The source of iodide can be of concern as well in areas located near nuclear legacy sites or underground nuclear waste disposals. Fortunately, radioactivity is monitored by OCWD, mainly for naturally occurring radioactive contaminants from oil and gas production or mining activities. Another concern for the increase in iodide is the formation of iodinated disinfection byproducts (DBP) such as iodo- trihalomethanes, acids, amides, phenols, and acetaldehydes.[56] The toxicity of iodo-DBP compounds exceed that of chlorinated or brominated disinfection byproducts. In the presence of natural organic materials, these compounds occur during the disinfection stage of drinking and wastewater treatment, particularly from chloramine-based processes.[57, 58] Iodide levels as low as 50 µg/L were observed to have DBP formation potential,[59] and this study measured an average of 150

µg/L in PCW. The presence of those DBP compounds post-treatment is observed to be higher with source waters that contain higher concentrations of iodide and organic matter, which is plentiful in wetlands. To reduce the formation of DBPs, drinking water treatment facilities will need to consider either the removal of organic matter prior to disinfection or the removal of iodide all together. The process to remove organic matter is done via coagulation, flocculation, and sedimentation: common processes that are already in place in conventional drinking water treatment facilities. To increase organic matter removal, higher doses of coagulants may be required. But with the advancement of coagulation research, optimization of coagulation may actually reduce the cost while enhancing organic removal. If facilities are opting for the removal of iodide instead, it may be costlier as it requires the installation of advanced treatment processes such as RO or ion exchange. However, recent research has shown that pre-chlorination can transforms iodide into a more absorbable organic form that can be removed by activated carbon if utilized during a drinking water treatment process.[60]

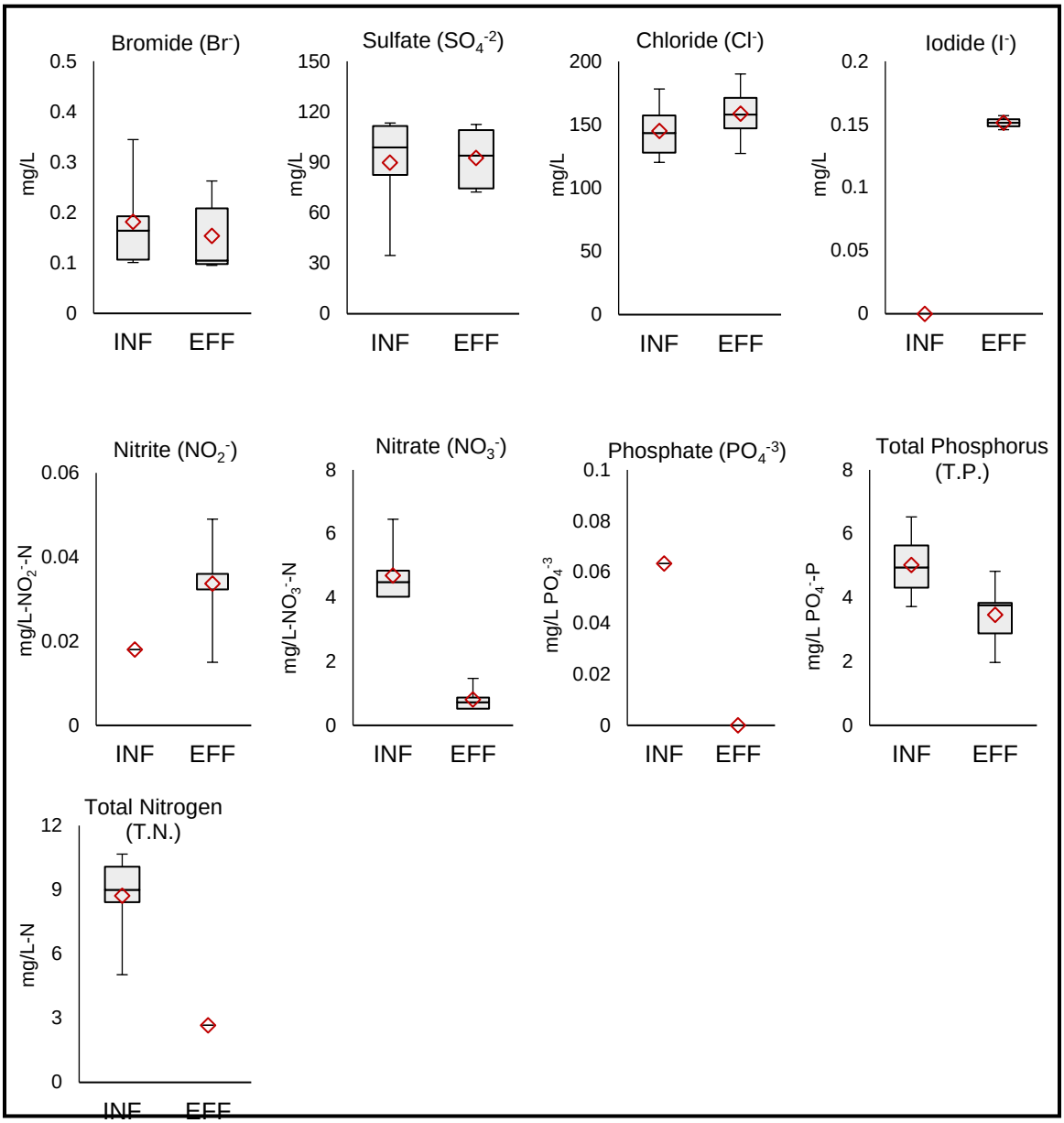


FIGURE 11. Comparison of nutrient and anion concentrations for wetland influent and effluent.

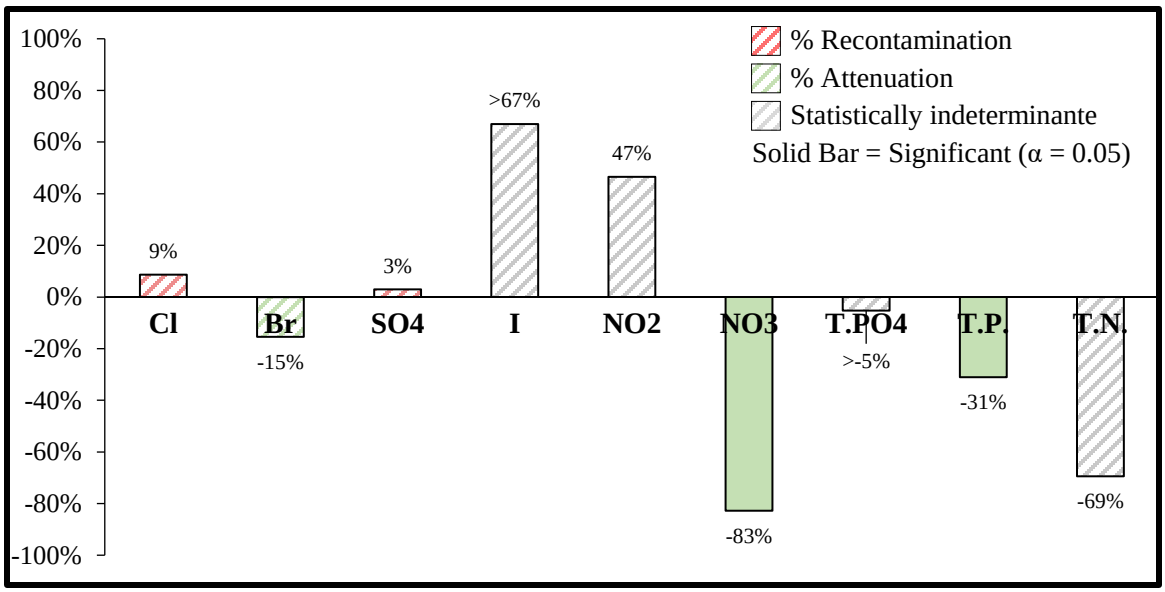


FIGURE 12. Average percent attenuation and recontamination of nutrients and anions; Prado Wetland.

On the other hand, as expected, a significant reduction of nitrogen and phosphorous compounds occurred, which are primary constituents found in most naturally occurring wetlands. The attenuation of those pollutants is most likely due to plant and microbial uptake, and in some cases through photolysis and biotransformation particularly in open-air wetlands with minimal plant cover.[61] [51]

The presence of organic phosphorus is suggested by the large empirical difference between the total phosphorous and total phosphate (inorganic phosphorous) concentrations measured in this study. Livestock manure usually contains high levels of nitrogen and phosphorous. According to literature, 45% to 90% of the phosphorous in livestock manure are composed of inorganic phosphorous.[62] Especially livestock that are fed with a high phosphorus diets exceeding their metabolic capacity, which are excreted in excess of what the livestock can normally process.[63] For example, if daily cattle is fed in excess of 25%–40% of their phosphorus requirements will actually excrete up to 80% of the total phosphorus consumed.[64]

Most aquatic systems receive the majority of their nutrient input from secondary or tertiary streams.[65] Heavy cattle farming around the tributaries of the river that drains into the constructed wetlands contributes greatly to the nutrient load in the wetland influent.[66] The wastewater effluent-dominant SAR is also heavily impacted by increased urbanization, lack of canopy coverage, and cattle farming. Leading to higher total phosphorous load in the SAR surface flow, which are subsequently carried into the constructed wetlands.[67]

In an effort to explain the possible sources of the organic phosphorous in the influent, nutrient balance in freshwater systems was evaluated. Stoichiometrically, similar to mixed liquor volatile suspended solids (MLVSS) in biological wastewater treatments, the level of phosphorous in aquatic biomass may be estimated based on biomass fraction to organic phosphorus content, assuming all volatile suspended solids (VSS) in the sample are composed mainly of bacterial cells. Considering the VSS molecular formula of $C_5H_7O_2NP_{0.1}$, developed by Rittman and McCarty, we can estimate that 2.6% of biomass is phosphorous.[68] Typically, VSS/total suspended solids (TSS) has a ratio of approximately 0.85. PCW inflow contains an average of 62.8 mg/L of TSS, or estimated 53.4 mg/L of biomass dry weight.[69] Therefore, 1.38 mg/L of total phosphorous is organic phosphorous contributed by the wetland biota. The remaining organic phosphorus may be pesticides or agricultural runoffs that leached into sections SAR from several residential neighborhoods, retail areas, as well as the agricultural land.

However, it is very difficult to accurately estimate the nutrient composition using ratio stoichiometry for wetland water columns as it differs drastically depending on the wetland location, input, temporal influences and vegetation.[70-73] C:P:N ratio can vary

widely between different wetland cells even though they exist within the same water system.[74]

The nitrogen compounds in this study were well below the MCL and MCLG for drinking water[q], therefore their removal in wetland treatment does not significantly impact the cost of downstream drinking water treatment. Similarly, the phosphorus levels were also very low. However, the reduction of those compounds demonstrated the capability of wetlands to reduce nutrient levels in influents exceeding the MCL.

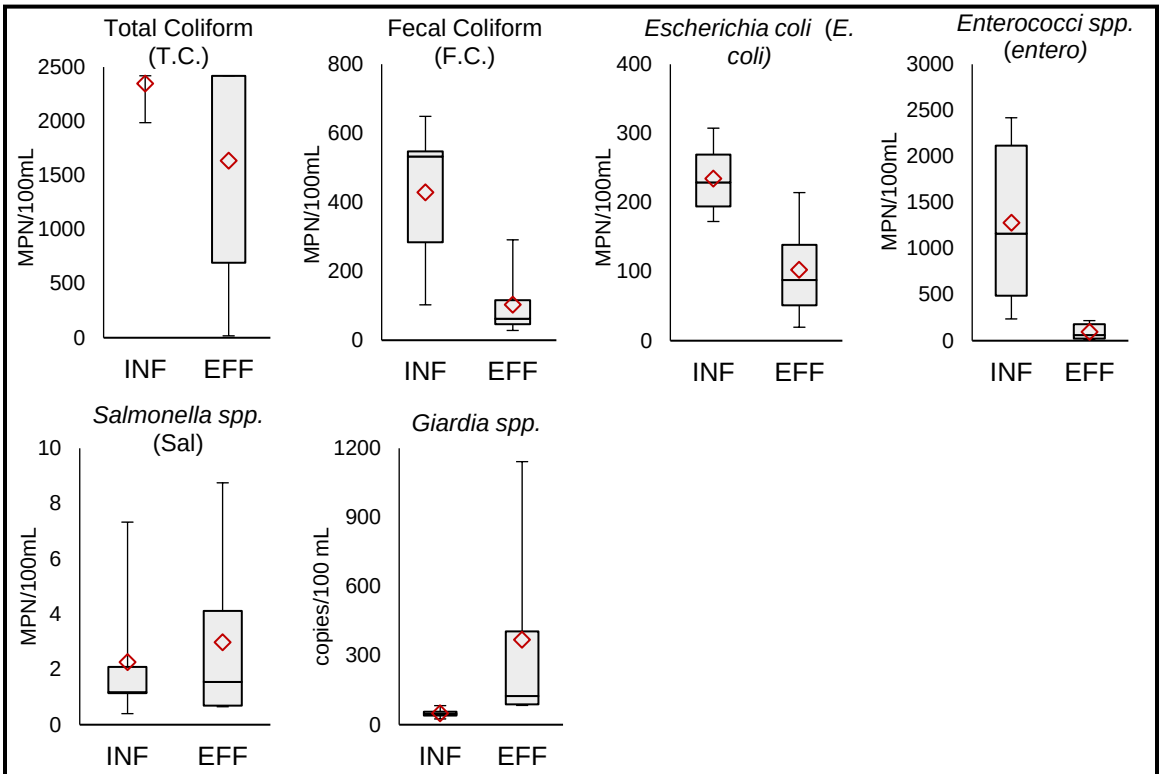
Hydraulic flow had a significant impact on chloride concentrations for both the influent and effluent. Sulfate in wetland effluent also differed significantly between high and low flow events. Both occurred at lower concentrations at during high flow, which can be attributed to dilution occurring with high water volumes in the system.

3.1.4 Microorganisms

Significant removal of *E. coli*, fecal coliforms, and *spp.* was observed as seen in Figures 13 and 14.[75] However, an increase of 86.5% was observed for *Giardia spp.* in PCW, although not statistically significant. Figure 13 shows concentration of microbial contaminants in PCW. The removal of microbes in wetlands is supported by a study conducted by the University of California-Agriculture and Natural Resources (UC-ANR), which saw similar results. The UC-ANR study looked into wetland buffer removal of microbial pollutant removal from agriculture impacted water. The UC-ANR wetland buffer field studies were located in Sacramento Valley and the San Joaquin Valley, California, with discharges into the Sacramento River and San Joaquin River

[q] MCL and MCLG: nitrate (10 mg/L-NO_3^-N); nitrite (1.0 mg/L-NO_2^-N); total nitrogen (n/a).

respectively. The study focused on wetlands with open water design with varied retention times.



Maximum concentration for total coliform, fecal coliform, E. coli, and Enterococci spp. is reported up to 2419.6 MPN/100mL.

FIGURE 13. Comparison of microbial concentration for wetland influent and effluent.

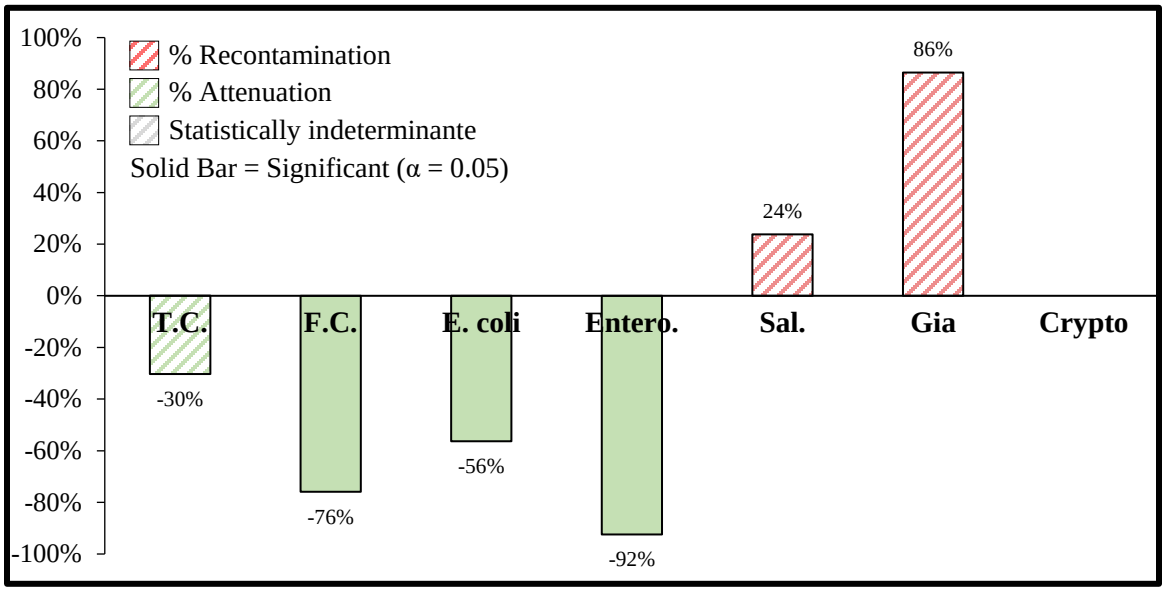


FIGURE 14. Average percent attenuation and recontamination of microbes; Prado Wetland

Several mechanisms can contribute to the attenuation of bacterial contamination in wetlands. Typically, a one-log reduction in fecal coliforms can be expected in free water surface wetlands like Prado.[76] The primary pathway is through sedimentation whereby the suspended particles settle with the decrease in hydraulic velocity and turbulence.[77] Open wetlands such as Prado are more exposed to germicidal ultraviolet (UV) radiation and high temperature fluctuations. Open wetlands are therefore more favorable than canopy covered wetlands, which buffer against the germicidal effects of UV radiation and temperature increase, thus also reducing photodegradation capacity.[78] [79] There are also studies that suggests positive correlations of *Salmonella spp.* culture to wetland coverage, particularly from agricultural areas.[78] Other mechanisms of attenuation include adsorption onto organic or charged particulates, filtration through sediment, and predation from aquatic invertebrates.[80]

On the other hand, wetlands can also harbor wildlife that are zoonotic pathogen vectors such as rodents, waterfowl, amphibians, and wild game. Wetland's

antipathogenic properties may be ineffective against some pathogens depending on pathogen susceptibility and path of transmission.[81] Factors such as fertilizers, ideal soil conditions, algae growth, amphibian vectors, and macrophyte transmissions all increases the likelihood of pathogenic proliferation, especially amongst *salmonella spp.*[82]

Interestingly, the increased presence of *Giardia spp.* in PCW effluent runs counter to what is typically observed of wetlands' effects on aquatic microorganisms. In 2006, a study was conducted on the removal of pathogenic and indicator microorganism via constructed wetlands receiving raw wastewater effluent. The studied area is comparable to PCW in that both are free water surface flow types with rooted plants and visible water. The study observed an average of 95.3% ± 2.4 removal of *Giardia* cysts in the wetland effluent.[83] The same study also saw an average of 3-log *Giardia* removal in the wetland effluent with a subsurface flow (SSF) configuration, meaning water flow occurs subsurface level through the sediment media. In another instance that also practiced potable water reuse, an average of 87.8% reduction was measured in SSF wetlands receiving raw wastewater. No *Giardia* recontamination was detected, BDL = <1 cysts/100L, when it was fed with chlorinated groundwater with no detectable amounts of *Giardia* cysts.[84]

In most cases, the removal of *Giardia* and *Cryptosporidium* is generally greater in SSF type wetlands. When using free surface flow wetland configurations, cells with vegetation saw higher removal than non-planted bare bottomed cells in previous studies. This indicates a higher likelihood that the cysts were removed via sedimentation or adsorption, especially in planted wetlands as vegetation can hinder and slow the water flow.[84, 85] Moreover, *Giardia* cysts concentration was found to be 1 to 3 order of

magnitude higher in the sediments of free water surface constructed wetlands than in the water column.[86] Because of that, *Giardia* removal is often correlated to turbidity removal since the primary removal mechanisms for both is via settling. In addition to the simultaneous settling factor, the positive correlation between cysts and turbidity occurrence can be attributed to the cysts' tendency to adsorb onto organic matter, enhancing the cyst removal at higher turbidity, or TSS, sedimentation rates. However, in this study, the increased occurrence of *Giardia* is inversely proportional to the removal of TSS at 94% average removal. Furthermore, an average increase of 86.5% of *Giardia* cysts was measured in the wetland effluent, which runs counterintuitive to what is commonly observed in constructed wetlands. Although, SAR supplies the main bulk of PWC's influent, but it is not likely the main contributor, evident in the concentrations measured at PCW's intake. The probable source of contamination may have come from the abundant wildlife wetlands can harbor as well as the animal husbandries upstream to the smaller rivers and tributaries that also flow into PCW. This is because in addition to humans, many other mammalian and avian species can play host to the parasite, such as ducks, cattle, and sheep.[87] *Giardia* cysts were found to be still infectious after 28 days in flowing as well as stagnant freshwater systems, [88] thus it may be possible that the *giardia* cysts observed in PCW remained active as it traveled through the wetland with an average of 5 -7 days of retention time. Although similar to other constructed wetlands, the functions of PCW are not exactly comparable. The purpose of PCW is primarily for nitrate and trace organics removal thus not expressly designed for pathogen or microbial removal. [89, 90] This, in addition to potential microbial influx from upstream agricultural

activities, may have resulted in the proliferation of *Giardia* to surpass the wetland's ability to attenuate it.

In conventional drinking water treatment processes, aquatic pathogens can be removed, usually up to 1 – 2 log removal after coagulation, flocculation, and sedimentation.[91] Up to 4 log removal, or 99.99% removal, can be achieved with chlorine disinfectants, UV radiation, or AOP.[76] Effective buffer treatments can minimize the costs and the formation of disinfection by-products associated with drinking water disinfection.[92]

No significant differences were observed between the rain and dry events.

3.1.5 Aggregate Water Quality Assessment

As expected, the low velocity movement aided the removal of suspended particles, in this case, a reduction in TSS at 94% was observed, seen in Figures 15 and 16.[76] No statistical differences were observed between the high and low flow conditions. Neutral to acidic water is characteristic of constructed and naturally occurring wetlands in that the presence of organic matter can lower pH that would otherwise remain relatively neutral; therefore, the reduction in pH from an alkaline measurement in the influent to a more neutral measurement is in line with wetland buffers. The significantly lowered pH value can aid in the charge neutralizing coagulation process of downstream drinking water treatment.

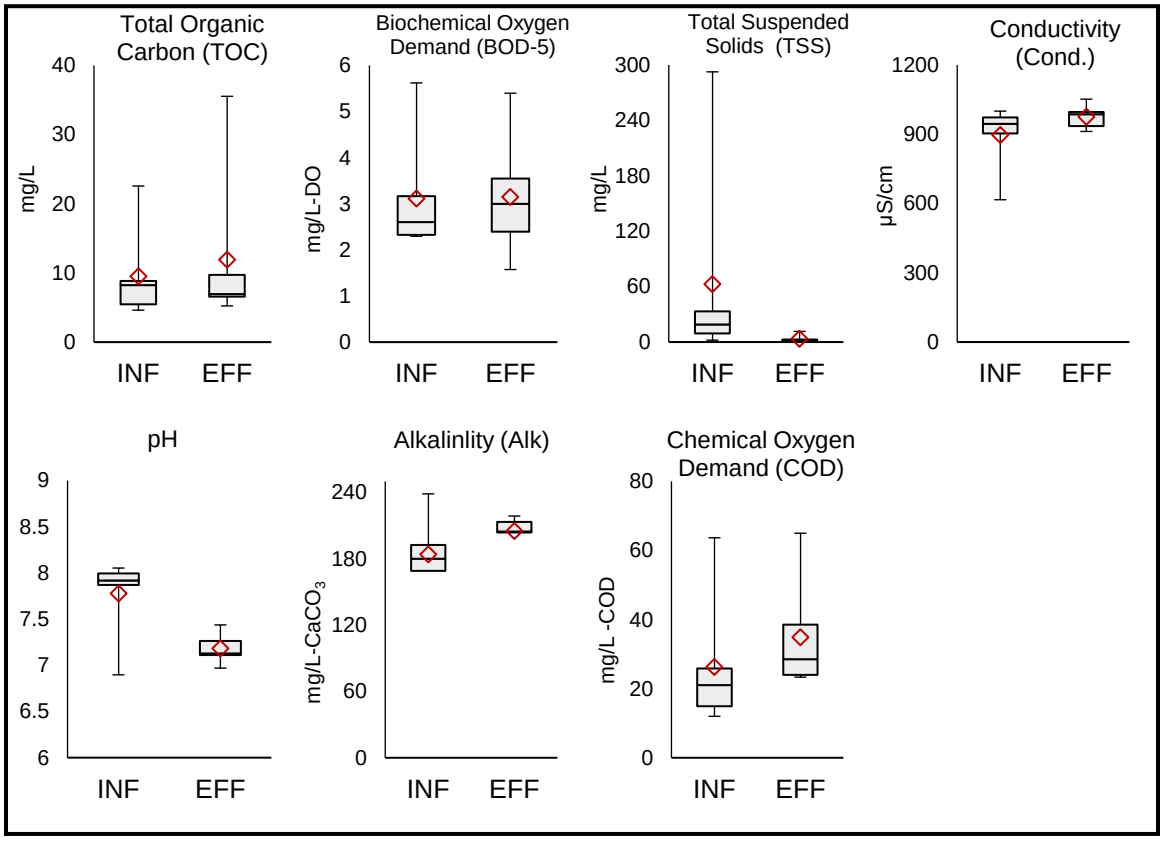


FIGURE 15. Water quality assessment for wetland influent and effluent.

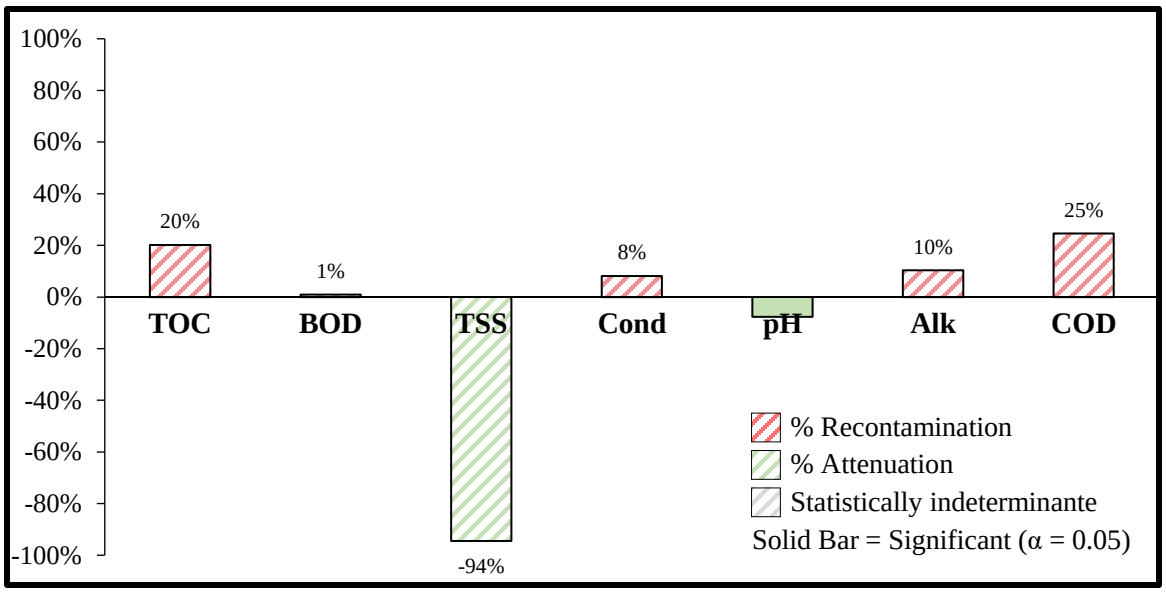


FIGURE 16. Average percent increase or decrease of aggregate water quality parameters; Prado Wetland.

3.1.6 Emerging Contaminants of Concern

Clindamycin was significantly increased at 50% in PCW as shown in Figures 17 and 18. Although not statistically significant, doxycycline notably saw an 82% average increase, sulfamethoxazole at 58%, ibuprofen at 56%, and sucralose at 52%. Ciprofloxacin and penicillin V saw the least change at less than 0.5% decrease and 3% increase, respectively, and atrazine was below detection limits.

According to the Food and Drug Administration (FDA), the most prevalent antibiotics used in the livestock husbandry are tetracyclines, up to 41% by volume compared to other antibiotics. Doxycycline is amongst the most heavily prescribed tetracyclines, because it is cheaper and more effective against a broad spectrum of bacteria.[93] Ionophores account for the second most common veterinary antibiotic, than, to a lesser extent, penicillins.[94] Ionophores are illegal for human consumption in the U.S. and therefore were not examined in this study. On the other hand, penicillin is the most

prescribed antibiotic for humans and goats in the U.S., up to 44% by volume. The southwestern region of California where the samples sites were located is heavily impacted by livestock agriculture, with more than 5 major cattle and dairy farms and 1 goat farm located within 15 miles to the wetland itself. The tributary streams that feed into the wetland are even closer in proximity to the agriculture sites. Doxycycline and penicillin V levels measured in the wetland effluent are found to be higher than the average concentrations measured in wastewater effluent impacted surface waters as shown in Table 24. These findings are not out of the ordinary considering that these antibiotics are three of the most widely used ones. Additionally, as a result of extensive use and minimal degradation in soil and manure, tetracyclines are extremely persistent and stable in the environment, making it difficult to degrade unless certain attenuation pathways are present.[95]

Clindamycin increased significantly post wetland treatment. Although the levels are still within the expected environmental range, the source could also have been introduced from the surrounding agricultural activities as clindamycin is also recognized as an important part of veterinary antibacterial treatments.[96]

Although not statistically significant, attenuation was still observed for amoxicillin, cephalexin, and ciprofloxacin. As part of the beta-lactam (β-lactam) group, amoxicillin and cephalexin can be extremely unstable in aquatic environments as they undergo rapid hydrolysis in ambient aquatic conditions, and are not very susceptible to adsorption.[97-99] Additionally, cephalexin is also sensitive to photodegradation, which may explain its lack of presence in the environment.[100] Although extensive laboratory research has been conducted on the extreme photosensitivity of ciprofloxacin, those

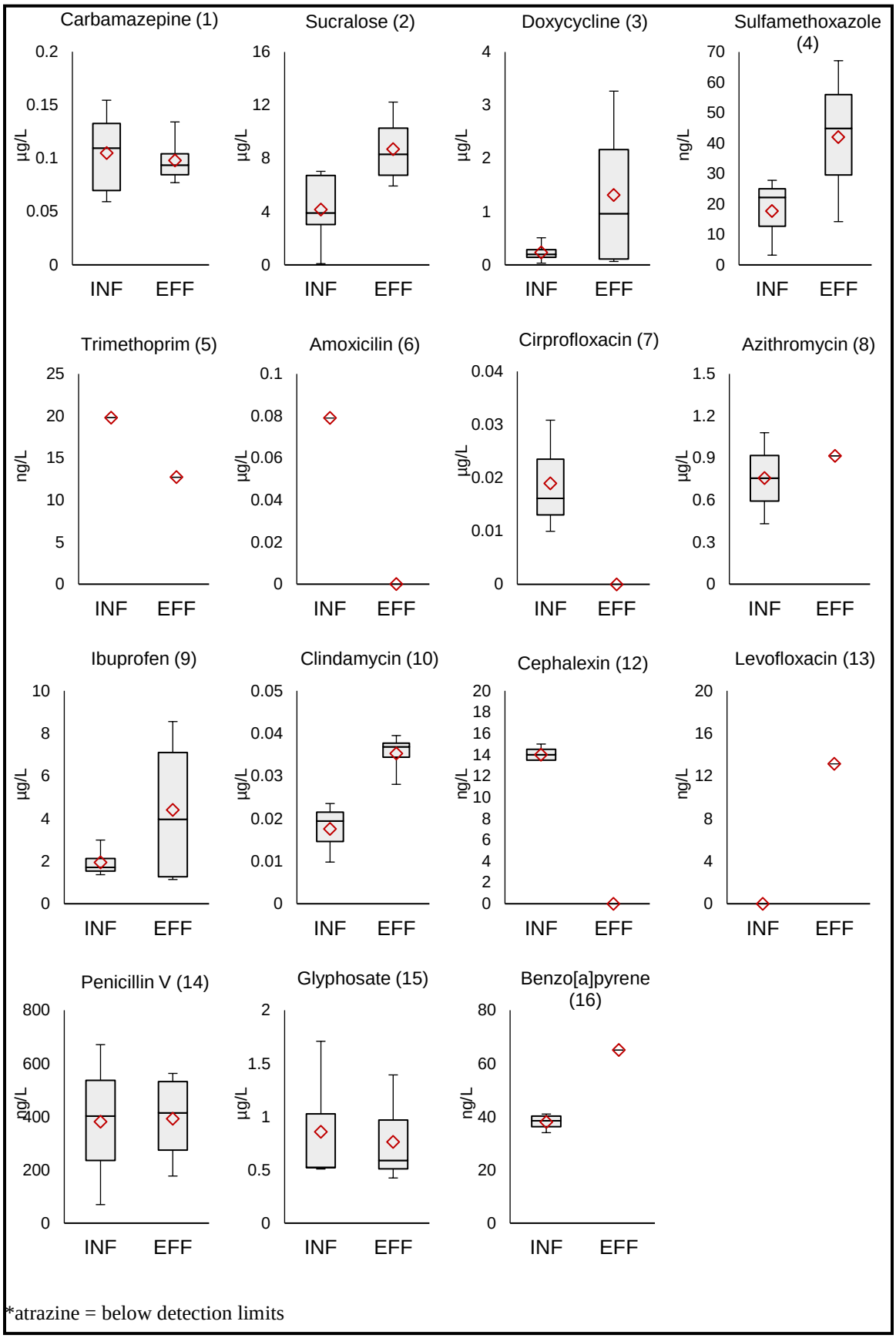
results are not necessarily accurate when used to describe the fate of ciprofloxacin in the environment.[101, 102] However, the effectiveness of photolysis is affected by the level of pH, phosphate, and organics in the water.[103] Due to this, the constant fluctuation of those environmental parameters may change the mechanism of which ciprofloxacin is retained. Other important environmental fates include adsorption onto organic matter and biotransformation. [104, 105] It is difficult to assess which is the dominant pathway. The high levels of organics measured in the wetland alludes to the possibility that adsorption transpired in conjunction with photolysis, just at an unknown degree of effectiveness.[106]

Numerous studies have shown the ubiquitous and persistent nature of PAHs in the environment. Most PAH emission sources are from anthropogenic activities such as pyrolytic processes resulting in the incomplete combustion of carbon fuels.[107] Benzo[a]pyrene (BaP) is one of the PAHs that has been well characterized as it is considered to be highly carcinogenic and mutagenic both human and wildlife.[108] Therefore, BaP is often used as an indicator compound to assess the overall level of PAH contamination in an area of interest.[109] Although the effects of BaP have been well studied, the development of effective remediation strategies is still in its infancy.

The contaminants are often removed from aquatic environments via adsorption onto soil particles or metabolized by soil/aquatic microbials. In wetlands, PAHs are often removed in the soil column via biodegradation, as the low hydraulic flow allows non-polar contaminants to settle onto the soil layer. Positive correlations have been observed between the abundance of bacterial communities such as *Actinobacteria*, *Firmicutes*, and *Proteobacteria* and the rate of PAH degradation in soil.[107] However, the challenge is the breakdown of higher molecular weight PAHs with more than four rings.[110, 111] The five

benzene ring structure contributes to BaP's molecular stability and hydrophobicity, which reduces its bioavailability for plant and microbial uptake. Because of that, BaP can pose a higher ecological risk to the wetland environment when compared to most other smaller PAHs.[112] Recently, studies have found soil microbial communities that rely exclusively on PAHs for their source of carbon and capable of degrading high molecular weight PAHs such as benzo(a)pyrene.[111]

Although BaP was not attenuated in this study, the most likely fate, if it were to be, would likely be via adsorption onto the benthic soil layer. It is also probable that BaP can be removed through uptake of microbial communities with specific BaP-target enzymes. Suggestion for future BaP environmental fate studies will include the sampling and sequencing of known BaP degrading bacterial DNA, and the analysis of soil column samples.



*atrazine = below detection limits

FIGURE 17. Comparison of emerging contaminant concentrations for wetland influent and effluent.

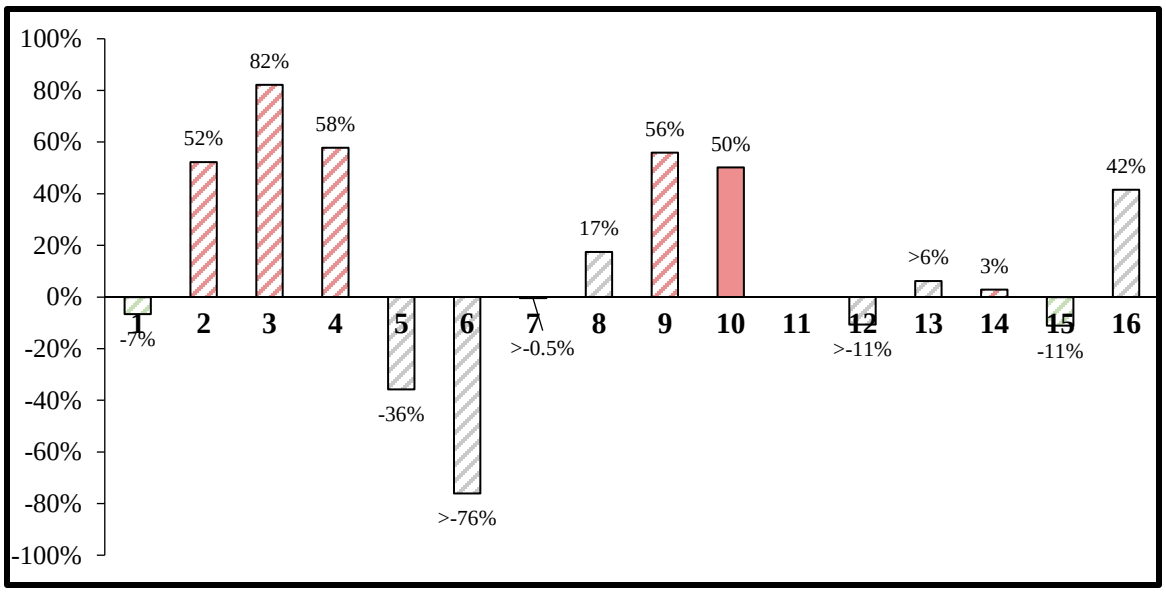


FIGURE 18. Average percent attenuation or recontamination of emerging contaminants; Prado Wetland.

(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

TABLE 24. The average global occurrence* of pharmaceuticals in surface water receiving wastewater effluent discharges, compared to Prado Wetland influent and effluent. [113-120]

Emerging Contaminants	**Typical Surface Water (µg/L)**	**Santa Ana River, Prado Influent (µg/L)**	**Prado Wetland Effluent (µg/L)**
Carbamazepine	<0.001 – 7.1	0.059 - 0.155	0.077 - 0.134
Sucralose	0.12 – 15.0	0.094 - 7.031	5.931 - 12.235
Doxycycline	BDL – 0.08	0.033 - 0.51	0.064 - 3.265
Sulfamethoxazole	BDL – 1.9	0.003 - 0.028	0.014 - 0.067
Trimethoprim	BDL – 0.71	0.02 - 0.02	0.013 - 0.013
Amoxicillin	0.025 – 2.2	0.079 - 0.079	BDL
Ciprofloxacin	BDL – 0.03	0.01 - 0.031	BDL
Azithromycin	BDL – 1.62	0.431 - 1.08	0.915 - 0.915
Ibuprofen	0.0002 – 5.044	1.364 - 2.99	1.137 - 8.556
Clindamycin	BDL – 0.085	0.01 - 0.024	0.028 - 0.039
Atrazine	BDL – 0.058, 201.1**	BDL	BDL
Cephalexin	BDL – 0.1	0.013 - 0.015	BDL
Levofloxacin	0.0062 – 0.0593	BDL	0.013 - 0.013
Penicillin V	BDL	0.07 - 0.671	0.177 - 0.563
Glyphosate	BDL – 1.90	0.508 - 1.708	0.424 - 1.392
Benzo[a]pyrene	BDL – 0.026	0.034 - 0.041	0.065 - 0.065

Significant Attenuation

Significant Recontamination

SAR receives wastewater effluent from multiple municipal sewage treatment facilities upstream to the PCW.

*Australia, Canada, China, Czech Republic, Germany, Italy, Sweden, United States, United Kingdom, Brazil, Norway, and Spain. **Heavy agriculture impacted areas BDL = Below Detection Limit

Most of the contaminants were not affected by hydraulic flow. However, in the SAR influent, carbamazepine and clindamycin had significantly lower concentrations during rain events. In the effluent sample, ibuprofen was significantly lower during rain events than dry events.

3.1.7 Antibiotic Resistance Genes (ARG)

Several of the ARGs showed an increase in abundance during wetlands treatment as shown in Figures 19 and 20. A statistically significant increase was observed for qnrB gene that encodes for the resistance to quinolone antibiotics, such as ciprofloxacin. Another gene responsible for the resistance to quinolones, qnrA, also increased considerably but not statistically significantly. Ciprofloxacin was one of the antibiotics significantly attenuated during wetlands treatment. It is not clear, whether it is coincidental or if this is indicative that there are abundant organisms in this wetland system that can break down fluoroquinolones.

Other ARG that noticeably increased, although not in a statistically significant way, was ereA, a gene encoding for the resistance to macrolide antibiotics. Macrolide azithromycin was not removed in the wetland treatment.

One ARG showed a decrease, tetW responsible for resistance to tetracycline class antibiotics. Another tetracyclines resistance gene remained unchanged in abundance.

Overall, concentration of total microbial community increased in wetlands compared to Santa Ana River, as indicated by a higher abundance of 16S rRNA.

No trends related to wet and dry weather were observed in the data.

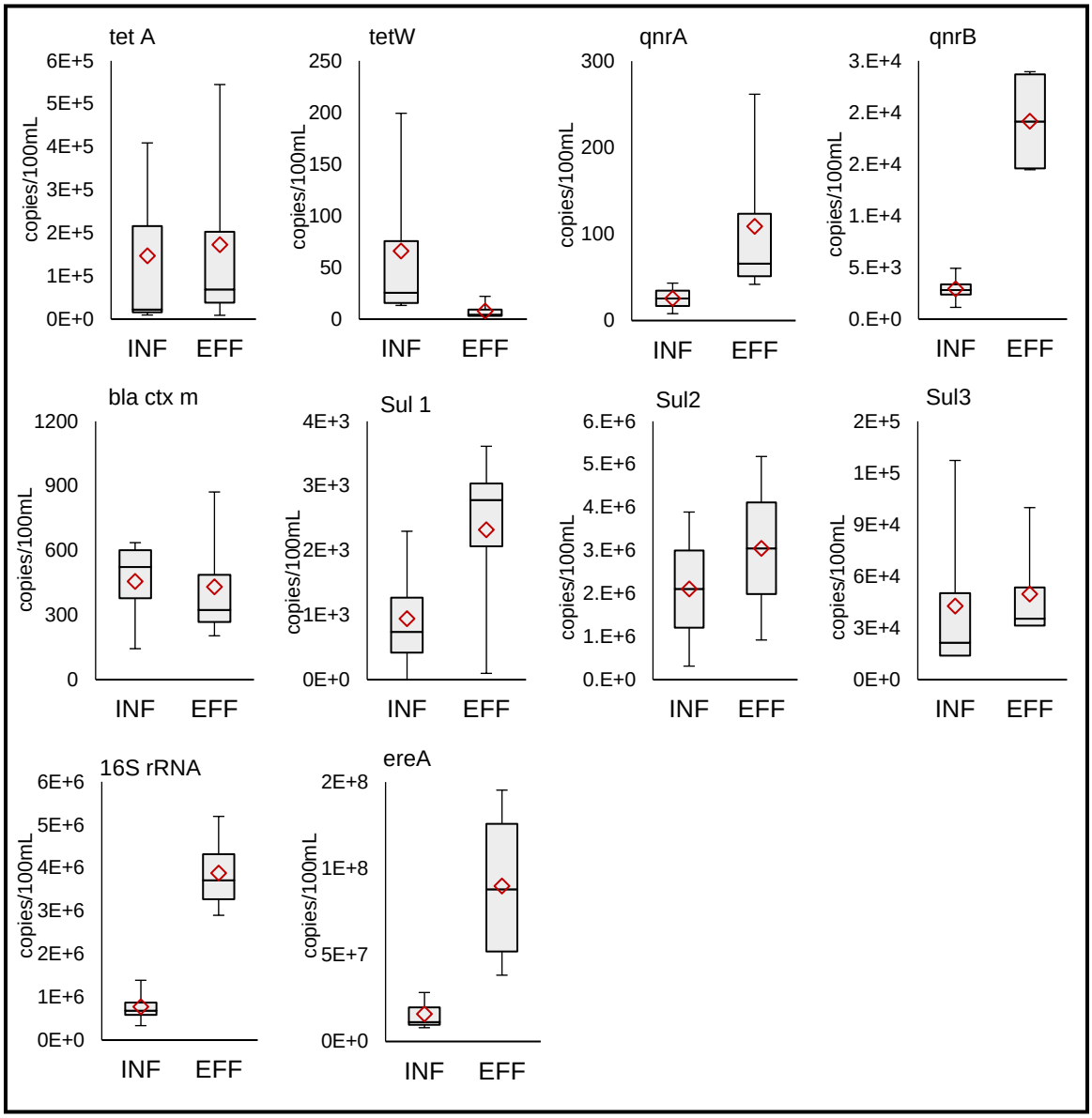


FIGURE 19. Comparison of ARGs for wetland influent and effluent.

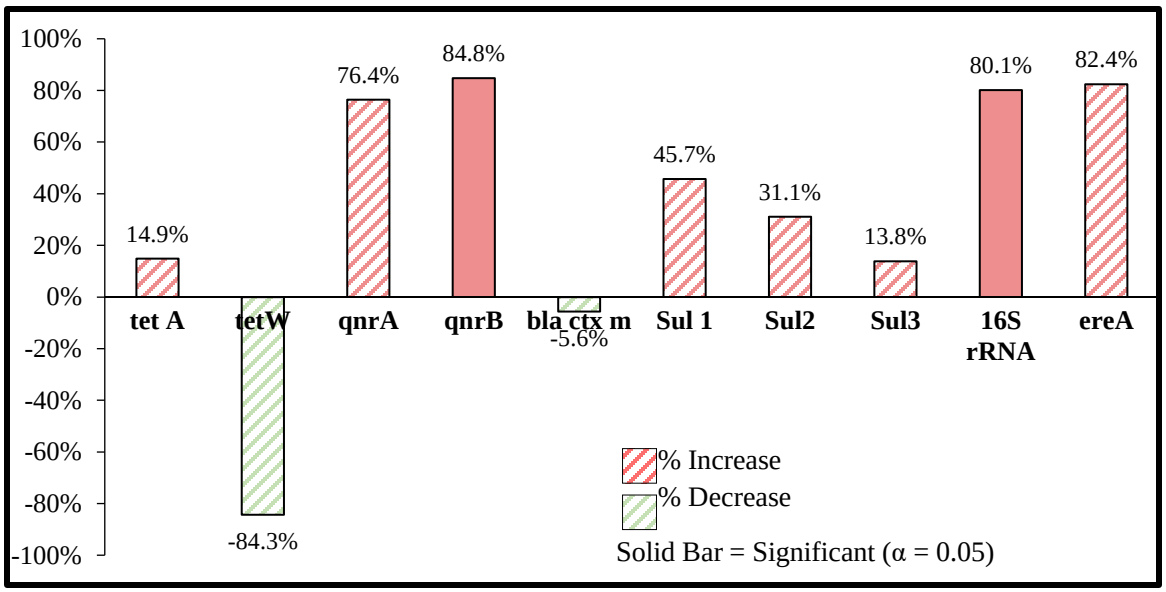


FIGURE 20. Average percent attenuation or ARGs; Prado Wetland

3.1.8 Cost Analysis

In potable reuse, it is common practice for a DWTP to use source water containing wastewater effluent from an upstream WWTP. As the body of water traverses through urbanized areas, the source water can accumulate additional contaminants via non-point source run-offs. One of the main cost-reducing benefits of using environmental buffers in IPR is the buffer's ability to naturally attenuate aquatic contaminants without excessive maintenance. For example, a river sample measured to contain 2.0 mg/L of iron 5.0 miles upstream to and at the drinking water treatment intake. The EPA MCL and for iron is 0.3 mg/L, therefore, the DWTP will need to remove the excess iron prior to final distribution. Alternatively, if the river was treated by a buffer 5.0 miles upstream and now measured 0.1 mg/L of iron at the intake instead, the iron contaminant, as with this case, is now below the MCL and does not need to be addressed during drinking water treatment. Hence, the cost saving effect of the "hands-off" approach to contaminant mitigation using environmental buffers.

However, the average manganese concentration measured in the wetland effluent exceeded the EPA MGL level, which would need to be removed by the downstream DWTPs. Elevated levels of manganese can impair SDWS characteristics like color, odor, or taste of the water. This is especially a concern for making beverages containing tannins, common in coffee and tea, which react with manganese to produce a black sludge like substance. In the distribution system, excess manganese deposits can accumulate in the pipelines, water heaters, and storage tanks, reducing the flow and pressure of the water supply. Over time, the mineral deposits can lead to an increase in energy cost for the transportation of water through constricted pipelines. Also, manganese in water can promote unwanted bacterial growth. Although not a major health threat, these bacteria can form black-brown slime-like biofilms in toilets and sinks and emit an unpleasant musty smell. Dissolved manganese in water can be removed by: (1) installing an ion exchange unit, not commonly recommended for municipal scale treatment because of associated expenses; (2) adsorption using an oxidizing filter such as manganese greensand or zeolite or activated carbon; and (3) filtration of insoluble manganic particulates after oxidation, which is the most cost-effective method. Oxidation can be carried out chemically using potassium permanganate, chlorine, or through aeration.[121]

The City of Orange Water Division consumer confidence report in 2017, reported in Table 25, provided a list of contaminants and their MCL and MCLG. All regulated contaminant concentrations are in compliance with the mandatory health standards published by the United States Environmental Protection Agency (USEPA).

TABLE 25. EPA vs Prado Wetlands [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (SAR)	Effluent (PCW)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.01 ± 0.00	BDL
Iron (Fe) [1]	ppm	0.3	NS	**0.44 ± 0.60**	0.18 ± 0.05
Boron (B) [1]	ppm	NS	NS	0.30 ± 0.07	0.31 ± 0.05
Calcium (Ca) [1]	ppm	NS	NS	64.0 ± 3.58	66.3 ± 2.68
Magnesium (Mg) [1]	ppm	NS	NS	15.2 ± 0.61	15.8 ± 1.21
Manganese (Mn)	ppm	0.05	NS	**0.13 ± 0.12**	**0.25 ± 0.17**
Sodium (Na) [1]	ppm	NS	NS	99.1 ± 16.3	113.8 ± 3.641
Cadmium (Cd)[2]	ppb	2	0.005	BDL	BDL
Mercury (Hg) [2]	ppb	2	0.002	BDL	BDL
Lead (Pb) [1]	ppb	15	0.2	BDL	BDL
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	145.0 ± 20.58	158.8 ± 20.42
Bromide (Br)	ppm	NS	NS	0.18 ± 0.09	0.15 ± 0.07
Sulfate (SO_4^{2-}) [1]	ppm	500	NS	89.8 ± 27.6	92.5 ± 17.8
Iodine (I)	ppm	NS	NS	BDL	0.15 ± 0.01
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.02 ± 0.00	0.03 ± 0.01
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	4.69 ± 0.87	0.81 ± 0.36
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.06 ± 0.00	QAQC
Total Phosphorous	mg/L PO_4^--P	NS	NS	5.02 ± 0.95	3.46 ± 0.93
Total Nitrogen	mg/L-N	NS	NS	8.73 ± 1.85	2.67 ± 0.00
Microorganisms					
Total Coliform[2]	MPN/100 mL	MCL[3]	0	**2347 ± 177**	**2151 ± 1215**
Fecal Coliform[2]	MPN/100 mL	MCL[3]	0	**428.3 ± 219.2**	**103.23 ± 98.6**
Escherichia coli[2]	MPN/100 mL	MCL[3]	0	**234.45 ± 59.9**	**102.38 ± 84.0**
Enterococci[2]	MPN/100 mL	NS	NS	**1278 ± 972**	**96.75 ± 93.9**
Salmonella spp. [2]	MPN/100 mL	NS	NS	**2.27 ± 2.56**	**2.98 ± 3.2**
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	**49.92 ± 24.10**	**294.96 ± 476.89**
Aggregate Water Quality					
Total Dissolved Solids[1]	ppm	500	NS	62.78 ± 113.2	3.46 ± 4.0[r]
pH[1]		6.5 – 8.5	NS	7.78 ± 0.40	7.18 ± 0.15
Total Organic Carbon	mg/L	NS	NS	9.55 ± 6.07	12.0 ± 10.7
Biochemical Oxygen Demand	mg/L-DO	NS	NS	3.12 ± 1.17	3.15 ± 1.21
Conductivity	µS	NS	NS	896.5 ± 129.8	975.9 ± 47.81
Alkalinity	mg/L-$CaCO_3$	NS	NS	184.0 ± 29.3	205.1 ± 11.4
Chemical Oxygen Demand	mg/L -COD	NS	NS	26.3 ± 17.5	34.9 ± 14.7
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	0.858 ± 0.468	0.762 ± 0.352
Benzo[a]pyrene[2]	ppt	200	0.0	38.0 ± 3.0	65.0 ± 0.0

NS: no standard **Red: above MCL**

Green Cells: Significant Decrease Red Cells: Significant Increase

[1] City of Orange Water Division Consumer Confidence Report – 2017

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation." - *National Primary Drinking Water Regulations, USEPA* – 2018.

[r] Reported values are ppm of total suspended solids (TSS)

In urban household settings, the source of metal contamination usually comes from corroded plumbing or other household fixtures that comes into contact with water at low pH. This is usually addressed by facilities by increasing the pH or by the addition of orthophosphate prior to distribution. Other sources of metals include naturally occurring forms found in the environment such as sediments, groundwater, or soil leaching. In those instances, more advanced purification methods will be required, such as distillation or RO. The reduction of heavy metals in wetlands will benefit drinking water facilities, as it will not be necessary to install new treatment processes to remove copper from the raw drinking water influent.

Phosphorus and nitrogen-based nutrients were reduced significantly at PCW. OCWD estimates nitrate removal at cost of $0.85 per pound using PCW as compared to $15 per pound for conventional treatments.[15]

The significant reduction of aquatic microbes and suspended solids can be cost effective to drinking water facilities for disinfection either by reducing the chlorine contact time and dosage, or by reducing the UV dose required. Not only are there less pathogens to address, the reduction of TSS also enhances the effectiveness of both chlorine and UV disinfection. The removal of TSS reduces the amount of chlorine quenching organic compounds therefore requiring less dosage to be effective. Additionally, the removal of TSS is critical to UV disinfection. Collimated beam studies indicate a nearly full log (factor of 10) improvement in bacterial inactivation was observed for the samples with lower TSS.[122] The low TSS value also reduces treatment costs by lowering the effective coagulant dose, reducing filter backwash rate, and the formation of DBPs. Conversely, the increase in iodide may increase the formation of

iodo-DBPs even at low TSS concentrations.[56] Therefore, as a precaution, more stringent TSS removal may be necessary. However, as discussed in previous sections, the increase in iodide may just be an isolated occurrence based on the geographical location of PCW near the ocean. Thus, the issue of increasing iodide levels does not pertain to all drinking water treatment facilities that may utilize wetlands as an environmental buffer in water reuse. However, bear in mind, while iodo-DBPs are a concern from toxicity perspective, they are presently unregulated.

3.2 NATURAL RIVER SYSTEMS (APW 1A, 1B, 1C – 2A)

3.2.1 South Platte River (SPR)

3.2.1.1 Littleton/Englewood Wastewater Treatment Plant (LEWWTP)s

The Littleton/Englewood Wastewater Treatment plant is the third largest treatment works that is publicly owned in the state of Colorado.[1] The plant mainly services the cities of Littleton and Englewood, but also receives sewage from 21 other connector districts around the city as well. An average 23 MGD of wastewater is processed at the plant daily, but the facility is designed to handle up to 50 MGD.[2] The wastewater influent first processed through bar screens for large debris removal; the primary clarifiers and dissolved air flotation tanks for solids removal; then trickling filters as well as aeration basins for biological nutrient removal; and additional solids removal, after the biological treatments, with secondary clarifiers. Beyond conventional treatments, LEWWTP also implemented advanced treatment processes including nitrifying tricking filters and denitrification filters for the removal of ammonia and nitrate.[2] The disinfected effluent is then discharged into the South Platte River (SPR).

3.2.1.2 Metro Wastewater Reclamation District (MWRD)

The Metro Wastewater Reclamation District covers an area of roughly 715 mi^2, and services about 2-million people from the surrounding cities of Denver, Arvada, Aurora, Brighton, Lakewood, Thornton, and Westminster.[3] MWRD has 2 operating treatment plants, the original southern treatment plant that was built in 1966. The Robert

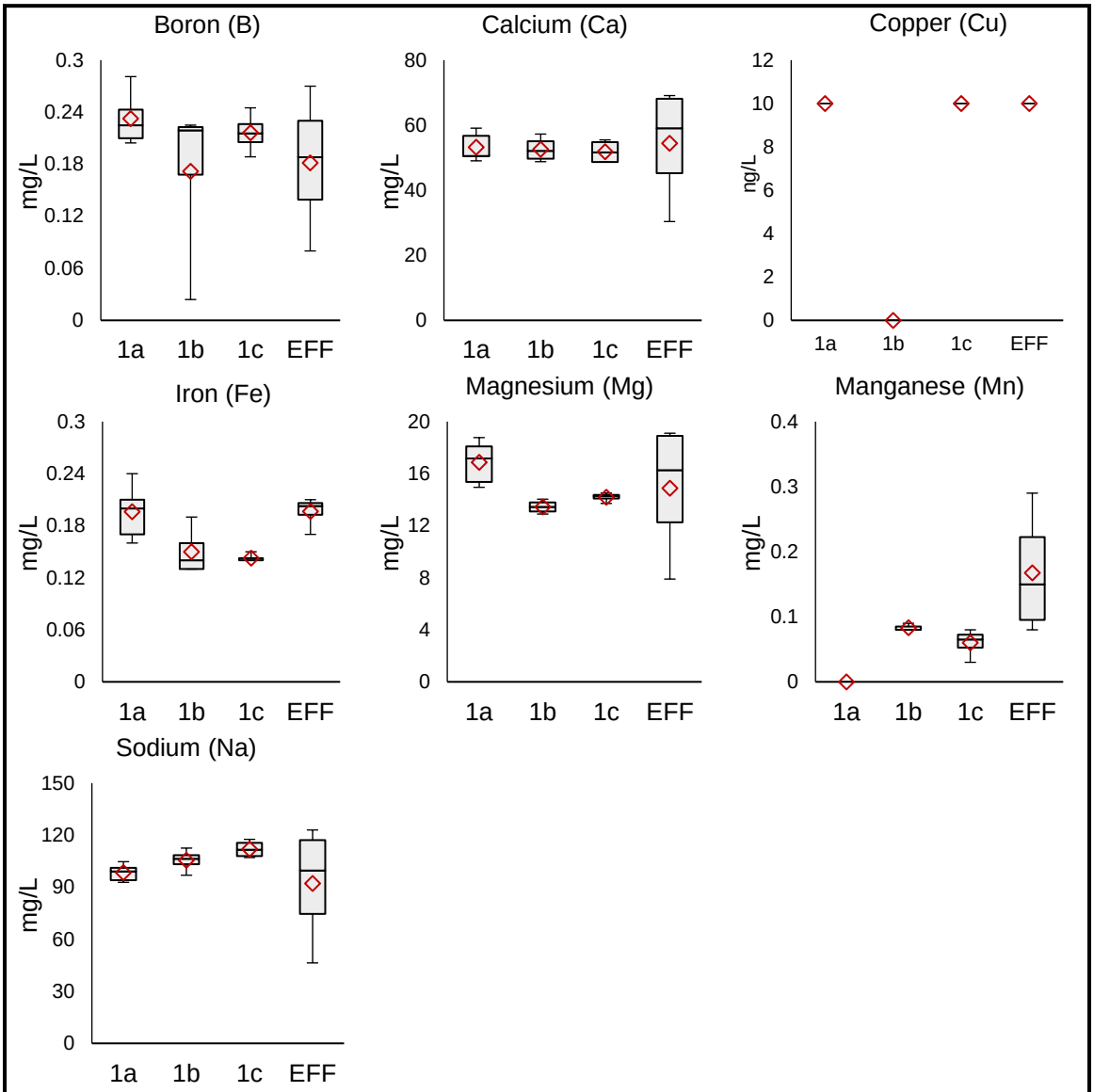
[s] As of April 2018, the facility formerly known as the Littleton/Englewood Wastewater Treatment Plant has been renamed the South Platte Water Renewal Partners. In this report, the facility will be referred to its previous name prior to the rebranding since the samples were collected before April 2018.

W. Hite Treatment Facility treats an annual average of 140 MGD of wastewater and covers 615 mi^2 with roughly 1.6-million residents. Similar to other conventional treatment works, the sewage is first screened to remove large objects, then moves onto primary clarifiers and aeration basins for the removal of solid wastes and organic wastes, the water is then settled again in the secondary clarifier and disinfected before discharging into SPR via two outfalls.[4] All three treatment plants were sampled for this study.

3.2.2 Metals

Concentrations of detected metals/cations were approximately the same in all three WWTPs and in the river at APW intake location (20-13 mi from the WWTPs) as shown in Figures 21 and 22. Calcium and iron saw an average increase of 3.3% and 16% respectively between the three WWTP and downstream of SPR, with a noticeable increased in manganese at 58% as well though none of which were statistically significant. Despite that, the three aforementioned substances are often associated with soil erosion runoffs, which may have contributed to increased concentrations in the river. Iron was below secondary drinking water standard of 0.3 mg/L in all samples. Manganese, however, exceeded the EPA secondary standard of 0.05 mg/L in many of the RWHTF effluent samples (BDL in Littleton/Englewood effluent) and was considerably above that threshold (up to six times) in all samples downstream in the river at an average of 0.17 mg/L. Contamination by manganese to concentrations above the secondary drinking water standard was also observed in the lake system evaluated in this study and described in Section 3.4. The consistency of this result underscores the susceptibility of surface water environmental buffers to contamination by runoff.

Depending on the degree of recontamination and the ability of the treatment processes to remove these compounds, a DWTP may have to consider additional treatment processes for the removal of manganese.



*cadmium (Cd), mercury (Hg), and lead (Pb) = below detection limits.

FIGURE 21. **Comparison of metal concentrations for three WWTP effluents (1a,b, and c) and a downstream river location (EFF).**

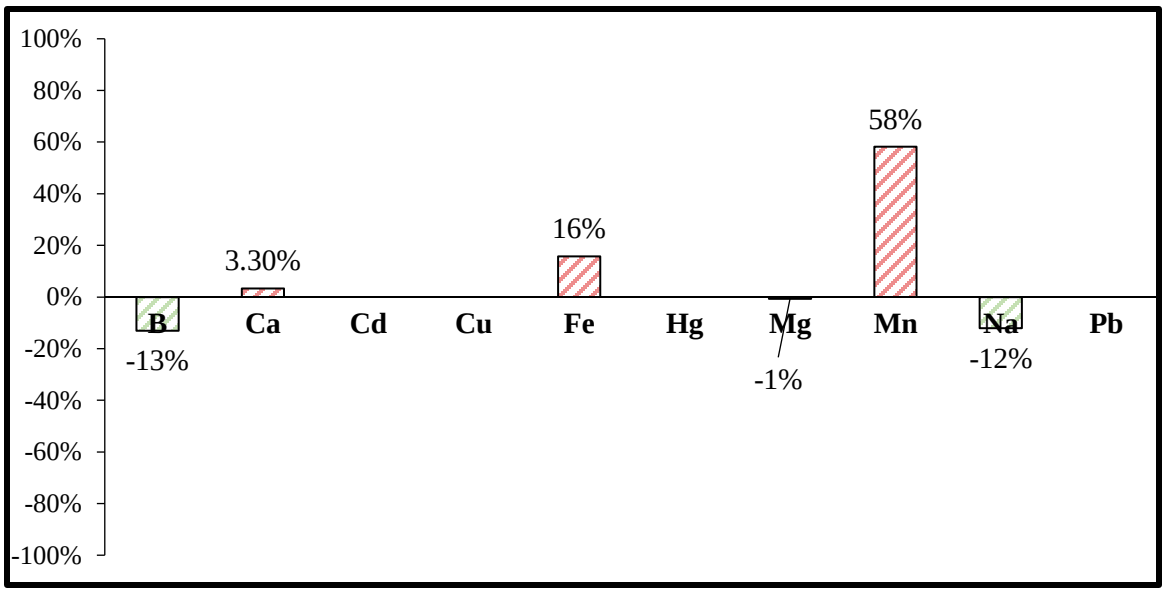


FIGURE 22. Percent decrease or increase of metal concentration between the average of the three WWTP effluents and a downstream river location.

3.2.3 Nutrients and Anions

Iodide was present in treated wastewater from the three plants at relatively steady concentrations of <50 µg/L (MDL) to as high as 446 µg/L (with the average of 200-300 µg/L in the samples above detection limit) as shown in Figures 23 and 24. Iodide in wastewater effluent is not a widely researched topic. One study in Hong Kong reported high concentrations of iodide in domestic wastewater in the range close to the results of our study.[123] Despite the importance of iodide for disinfection byproduct formation during water chlorination,[124] little information is available on the presence of inorganic iodide in treated wastewater. Most of the studies on sources of iodinated DBPs focus on organic iodine from iodinated X-ray contrast media as a source. Iodide was BDL in all of the downstream river samples. However, concentrations well below the iodide detection limit in our study can contribute to formation of iodinated DBPs.[124] Dilution is unlikely to contribute to the attenuation of iodide in South Platte River, as the samples were mostly

taken during the dry portion of the year when the flow from the sampled WWTPs constitutes 90% of the river flow. Most likely the attenuation was via change in speciation of iodide to iodate and methylation by aerobic microorganisms, which can lead to volatilization.[125,126] It appears that release of the effluent into an environmental buffer capable of providing iodide attenuation may improve the compliance with DBP standards for downstream DWTPs. However, in direct potable reuse (DPR) scenario, iodide would be removed in the RO step, as was seen in our field study of AWPS described in Section 3.5. Overall, inorganic iodide in municipal wastewater warrants further research.

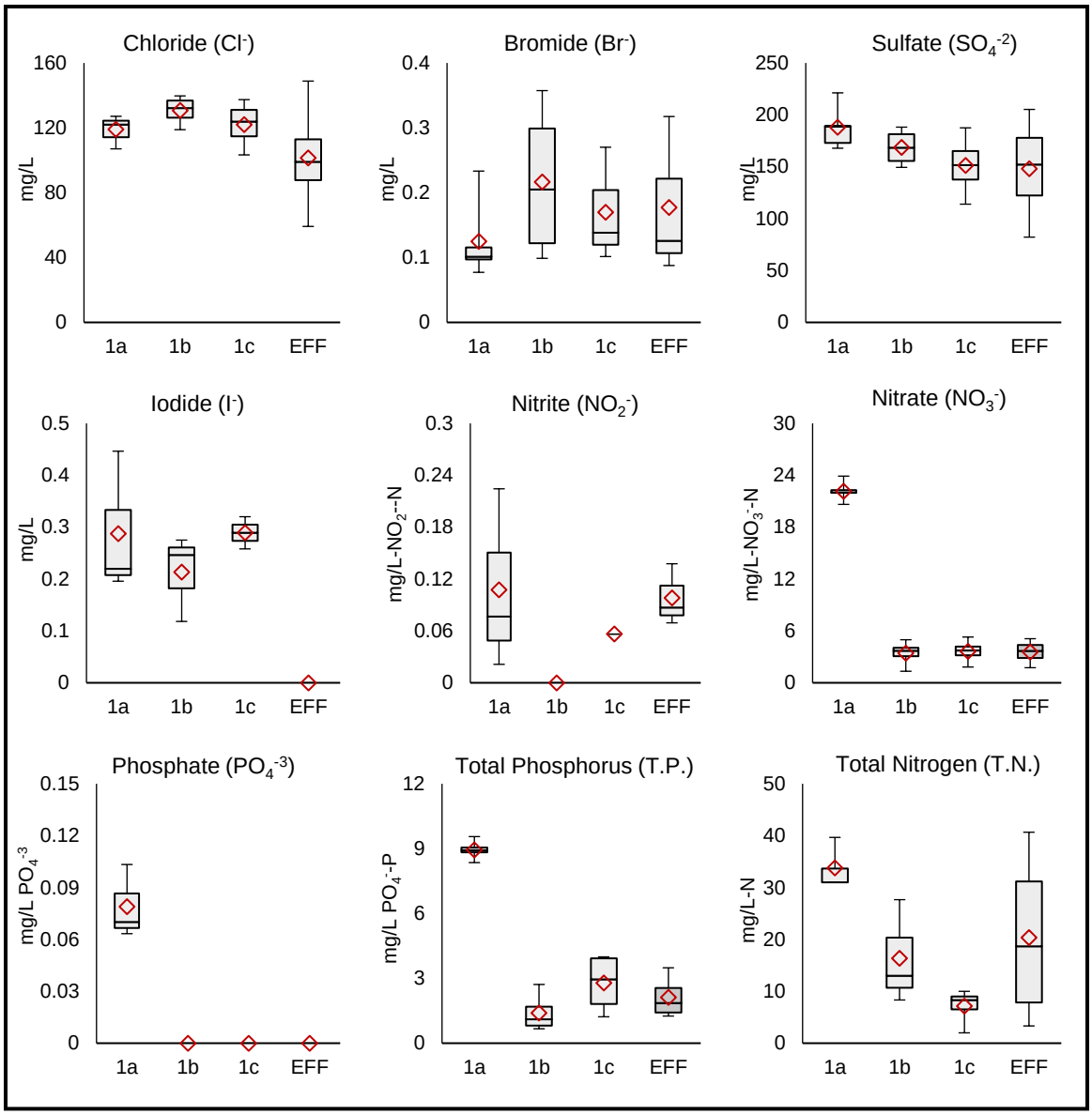


FIGURE 23. Comparison of nutrient and anion concentrations for three WWTP effluents and a downstream river location.

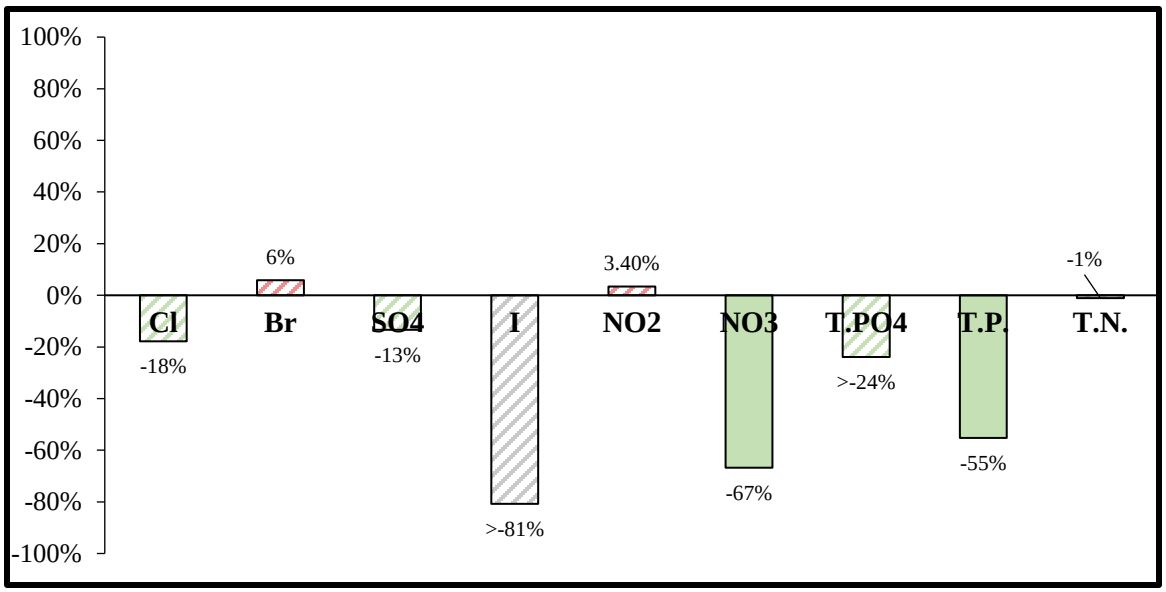


FIGURE 24. Percent decrease or increase of nutrients and anions between the average concentration in the three WWTP effluents and a downstream river location.

Nitrate and total phosphorus in South Platte River match the concentrations in RWHTF effluent. RWHTF discharge is 130 MGD compared to 22 MGD of Littleton/Englewood. While the latter has much higher nitrate and TP in the effluent, RWHTF has the most influence on the river quality because of the volume of water discharged. Surface water environment can be susceptible to nutrient pollution from runoff as well. It was not the case for the sampled stretch of South Platte River, but can be significant for rivers in other regions. South Platte River in the sampling segment traverses a densely urbanized area, with little agricultural impact from its upstream catchment. Nutrient application for urban landscaping is not as common in Colorado as in some other areas of the country due to the general difficulty of maintaining a lawn in the arid environment combined with watering restrictions. This result does not indicate that river environment in general is not subject to nutrient pollution via runoff.

3.2.4 Microorganisms

E. coli was significantly higher in the SAR with fecal coliform and *Salmonella spp.*'s presence considerably higher in the river water as well compared to the effluent from all three sampled WWTPs as shown in Figures 25 and 26. In some instances, despite a big difference in the values, the results are not statistically significant because of BDL values for the analyte in one of the samples. In general, there is evidence that once treated wastewater is released into the surface water environment, it is contaminated with microorganisms, including pathogens, and would require higher disinfectant dose or contact time compared to DPR.

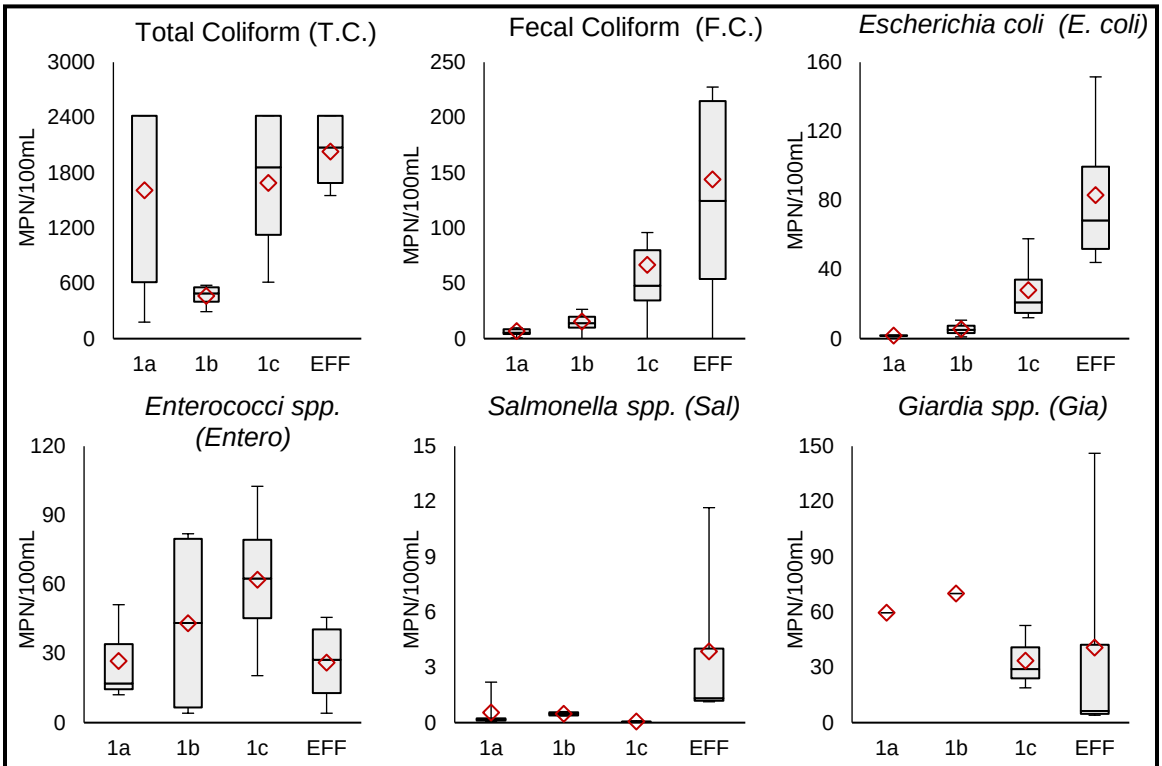


FIGURE 25. Comparison of microbial contaminant concentrations for three WWTP effluents and a downstream river location.

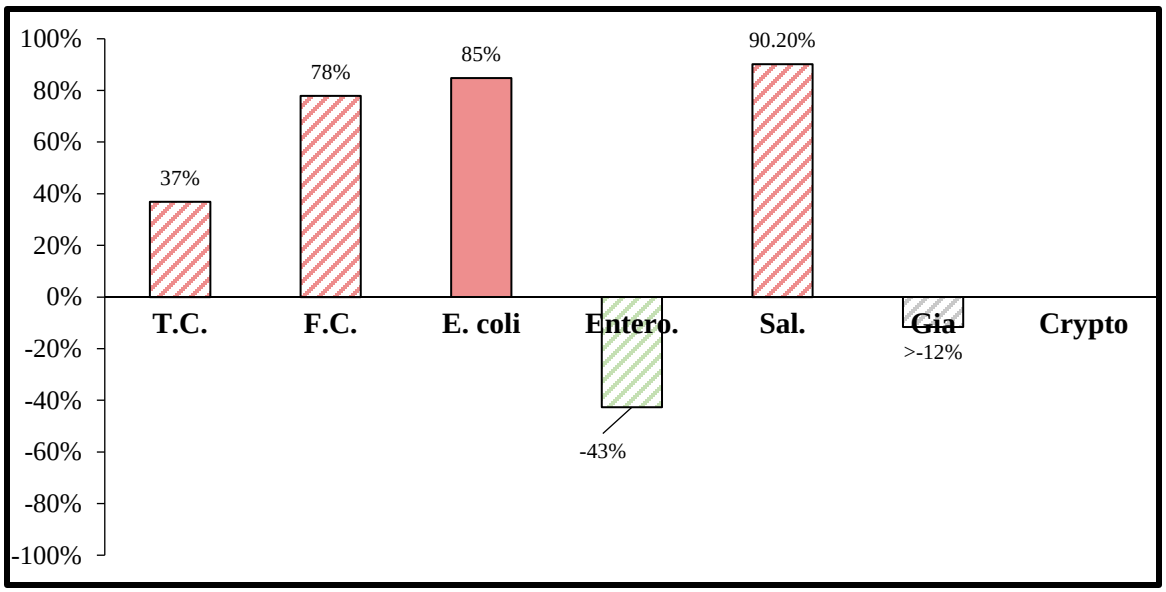


FIGURE 26. Percent decrease or increase of microorganisms between the average concentration in the three WWTP effluents and a downstream river location.

3.2.5 Aggregate Water Quality Assessment

Release of the effluent into the river environment provided some attenuation to BOD_5 and TOC as shown in Figures 27 and 28. Slight, but not statistically significant decrease in COD was also observed. Since the baseflow provides little dilution, the decrease is likely due to biodegradation of residual wastewater organics by environmental microbes. The observed increase in pH is likely the result of biological activity as well. Overall, the attenuation of background organics is beneficial for subsequent drinking water treatment processes. The need for coagulant use and formation of DBPs could be reduced as a result. However, there was a considerable increase in TSS, which negates the benefits achieved by the decrease in TOC and BOD_5. Higher TSS would warrant higher coagulant use and shorter backwash cycles for filters. There was also higher variability in TSS making water quality in the river less predictable than the water quality of effluent and making DWTP process optimization more difficult. Much greater variability was also

observed for conductivity in the river compared to the effluent samples from the WWTPs. The fluctuations in conductivity in the river are likely indicative of runoff impact. The urban and industrial surroundings of the river may input various ionic species to the river water; some of them may be of concern for drinking water quality, even though not analyzed in this study.

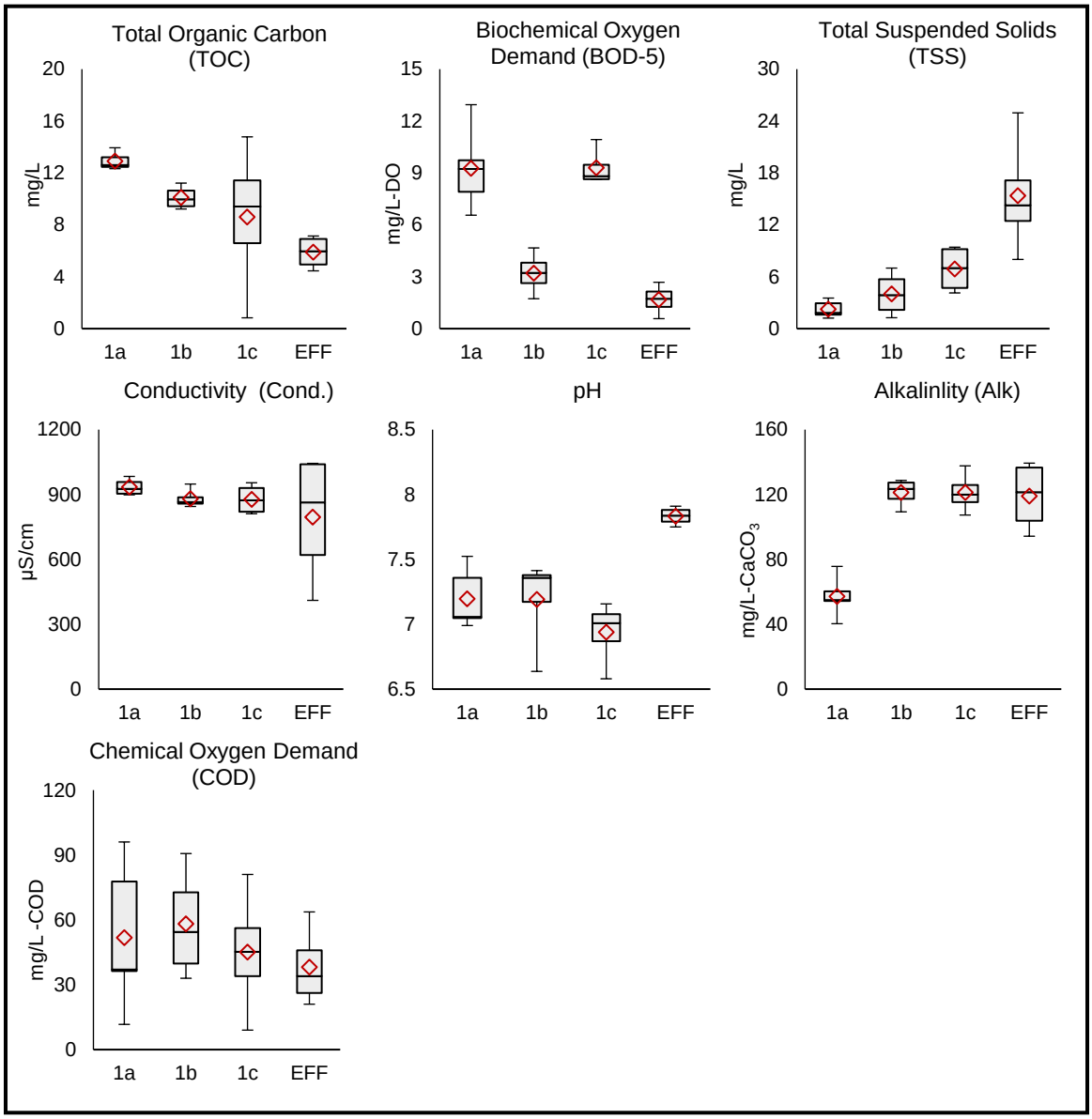


FIGURE 27. Comparison of aggregate water qualities for three WWTP effluents and a downstream river location.

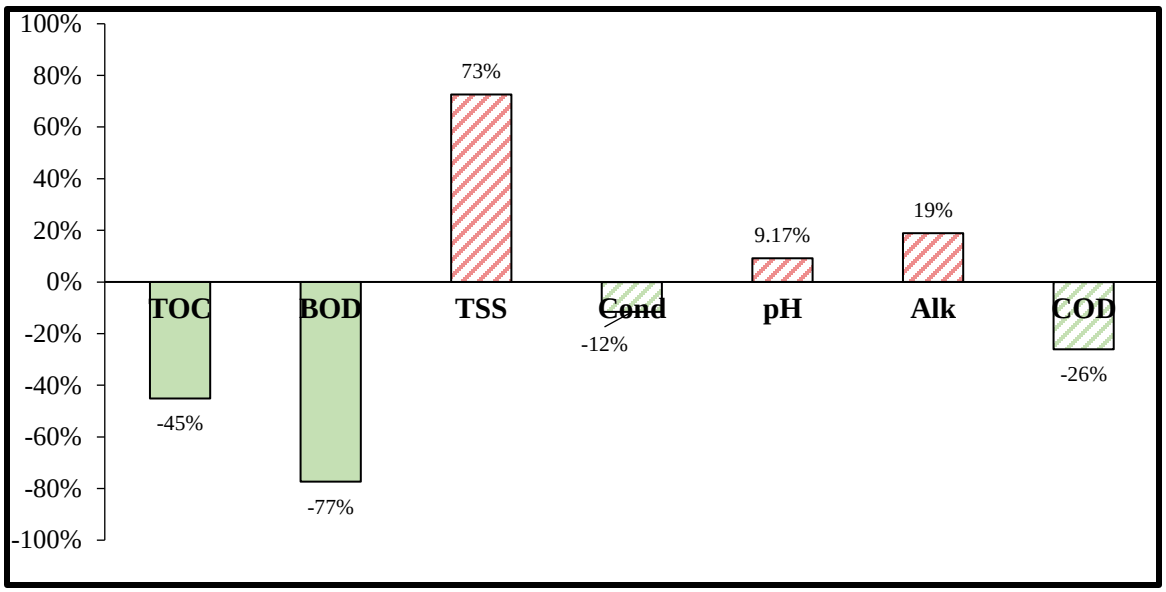
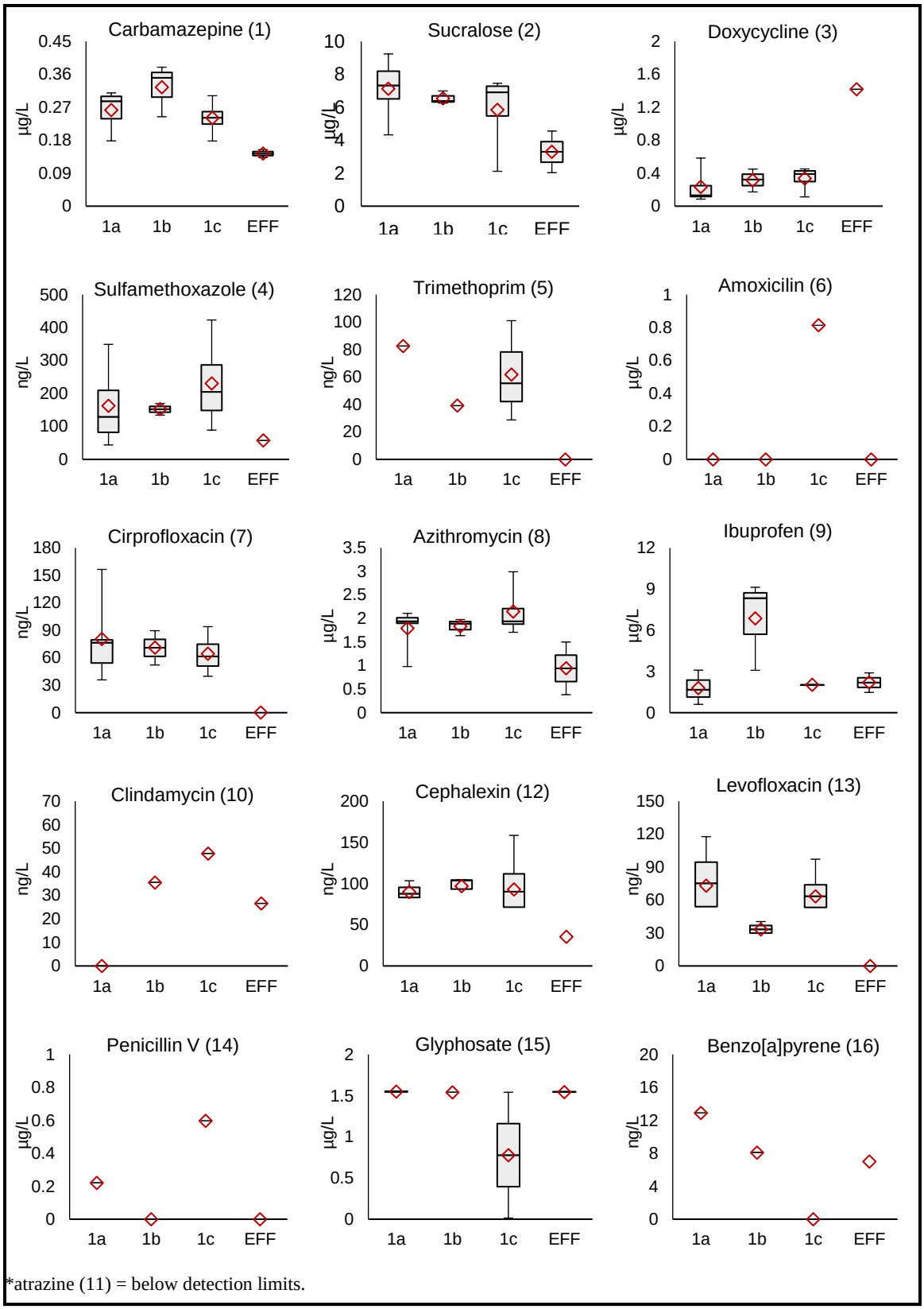


FIGURE 28. Percent change of aggregate water qualities between the average concentration in the three WWTP effluents and a downstream river location.

3.2.6 Contaminants of Emerging Concern

Environmental fate indicators sucralose and carbamazepine both were at approximately half the concentration downstream in the river compared to the concentrations in the effluent as shown in Figures 29 and 30. Sucralose is not susceptible to environmental attenuation, and therefore a decrease would be indicative of dilution. However, the difference was not statistically significant, given the number of samples and the variability in the concentrations between samples from a single location. Other water quality parameters suggest that dilution is not a major factor in this river. The decrease in carbamazepine, while also not statistically significant, may still be indicative of some removal of CECs via adsorption.



*atrazine (11) = below detection limits.

FIGURE 29. Comparison of CEC concentrations for the three WWTP effluents and a downstream river location.

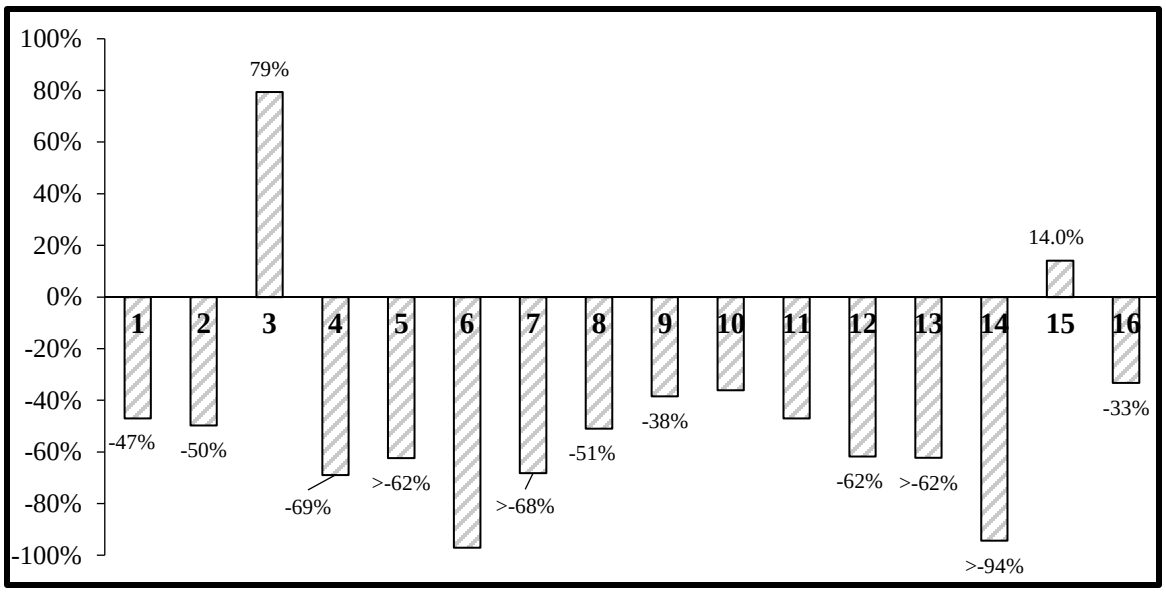


FIGURE 30. Percent change of CEC concentrations between the average concentration in the three WWTP effluents and a downstream river location.
(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

Although the degree of removal is very small. Ibuprofen, a biodegradation indicator compound, remained stable in concentration, suggesting that the residence time in the river is not sufficient to achieve considerable attenuation of biodegradable CECs by the time the river reaches DWTP intake. Sulfamethoxazole and ciprofloxacin both were lower in the downstream location (close to MDL or BDL in many instances) compared to the effluent from the WWTPs where they were consistently at least an order of magnitude above the MDL. Same is true for antibiotic levofloxacin, which is also known to have the rates of solar photolysis similar to those of ciprofloxacin.[127] Decrease in these antibiotics suggests that photolysis plays an important role in a river environment. Unlike in a lake and a wetland environment that were examined in this study, river turbulence provides mixing that allows for better effectiveness of photolysis, as UV radiation from the sun can only reach the upper layers of water in a natural environment. In quiescent water body, the

mixing would rely on a slow process of diffusion to bring the photolabile compound from deeper strata to the surface where it can decompose, while in a turbulent water body mixing is much faster. Cephalexin showed similar results. This antibiotic undergoes rapid hydrolysis in the environment, which is likely its main environmental fate, although it is also susceptible to indirect photolysis with nitrate as a photosensitizer.[128] Because of the impact of WWTPs on South Platte river, there may have been sufficient nitrate to sensitize degradation of pharmaceuticals. Most of the other tested pharmaceuticals were not detected with sufficient consistency to allow conclusions regarding their environmental fate. Table 26 shows the average occurrence of pharmaceuticals in this study vs what is found in surface waters around the world as indicated in literature,

TABLE 26. The average global occurrence* of pharmaceuticals in surface water receiving wastewater effluent discharges, compared to South Platte Rive..[113-120]

Emerging Contaminants	**Surface Water Concentration (µg/L)**	**1a (µg/L)**	**1b (µg/L)**	**1c (µg/L)**	**South Platte River (µg/L)**
Carbamazepine	<0.001 – 7.1	0.178 - 0.309	0.24 - 0.38	0.178 - 0.302	0.13 - 0.15
Sucralose	0.12 – 15.0	4.328 - 9.238	6.26 - 6.99	2.11 - 7.46	2.03 - 4.55
Doxycycline	BDL – 0.08	BDL - 0.58	0.17 - 0.45	0.11 - 0.452	BDL - 1.418
Sulfamethoxazole	BDL – 1.9	BDL - 0.35	0.13 - 0.17	0.089 - 0.423	BDL - 0.057
Trimethoprim	BDL – 0.71	BDL - 0.083	BDL - 0.039	BDL - 0.101	BDL - 0
Amoxicillin	0.025 – 2.2	BDL - 0	BDL - 0	BDL - 0.814	BDL - 0
Ciprofloxacin	BDL – 0.03	0.036 - 0.16	BDL - 0.090	0.0398 - 0.094	BDL - 0
Azithromycin	BDL – 1.62	0.98 - 2.11	1.64 - 1.98	1.708 - 2.993	0.38 - 1.50
Ibuprofen	0.0002 – 5.044	BDL - 3.10	3.09 - 9.13	BDL - 2.046	BDL - 2.90
Clindamycin	BDL – 0.085	BDL	BDL - 0.036	BDL - 0.048	BDL - 0.027
Atrazine	BDL – 0.058, 201.1**	BDL	BDL	BDL	BDL
Cephalexin	BDL – 0.1	BDL - 0.103	0.083 - 0.105	0.033 - 0.159	BDL - 0.036
Levofloxacin	0.0062 – 0.0593	BDL - 0.118	BDL - 0.04	0.030 - 0.097	BDL
Penicillin V	BDL	BDL - 0.22	BDL	BDL - 0.597	BDL
Glyphosate	BDL – 1.90	BDL - 1.56	BDL - 1.54	BDL - 1.54	BDL - 1.548
Benzo[a]pyrene	BDL – 0.026	BDL - 0.013	BDL - 0.0081	BDL	BDL - 0.007

Significant Attenuation
Significant Recontamination
SPR receives wastewater effluent from multiple municipal sewage treatment facilities.
*Australia, Canada, China, Czech Republic, Germany, Italy, Sweden, United States, United Kingdom, Brazil, Norway, and Spain. **Heavy agriculture impacted areas BDL = Below Detection Limit

Atrazine was BDL in all the samples. Glyphosate, however, was consistently on the order of low μg/L in majority of the samples. Glyphosate is used not only in agriculture and landscape management, but also for maintaining weed-free spaces in a variety of settings, e.g. sidewalk expansion joints, right-of-ways, etc.

BaP was present in only a few samples and at low concentrations close to detection limit. This was unexpected considering highly urbanized and industrial areas in the proximity of South Platte River.

3.2.7 Antibiotic Resistance Genes (ARG)

ARGs in the river mirrored closely the ARGs in the effluent from the WWTPs discharging into the river as shown in Figure 31 and Figure 32. Only one of the genes, tetW that encodes for resistance to tetracycline antibiotics, was considerably decreased in the river. This gene was in low abundance (copies per unit volume) compared to other ARGs. Overall, no positive or negative effect on ARGs was determined in the river environment.

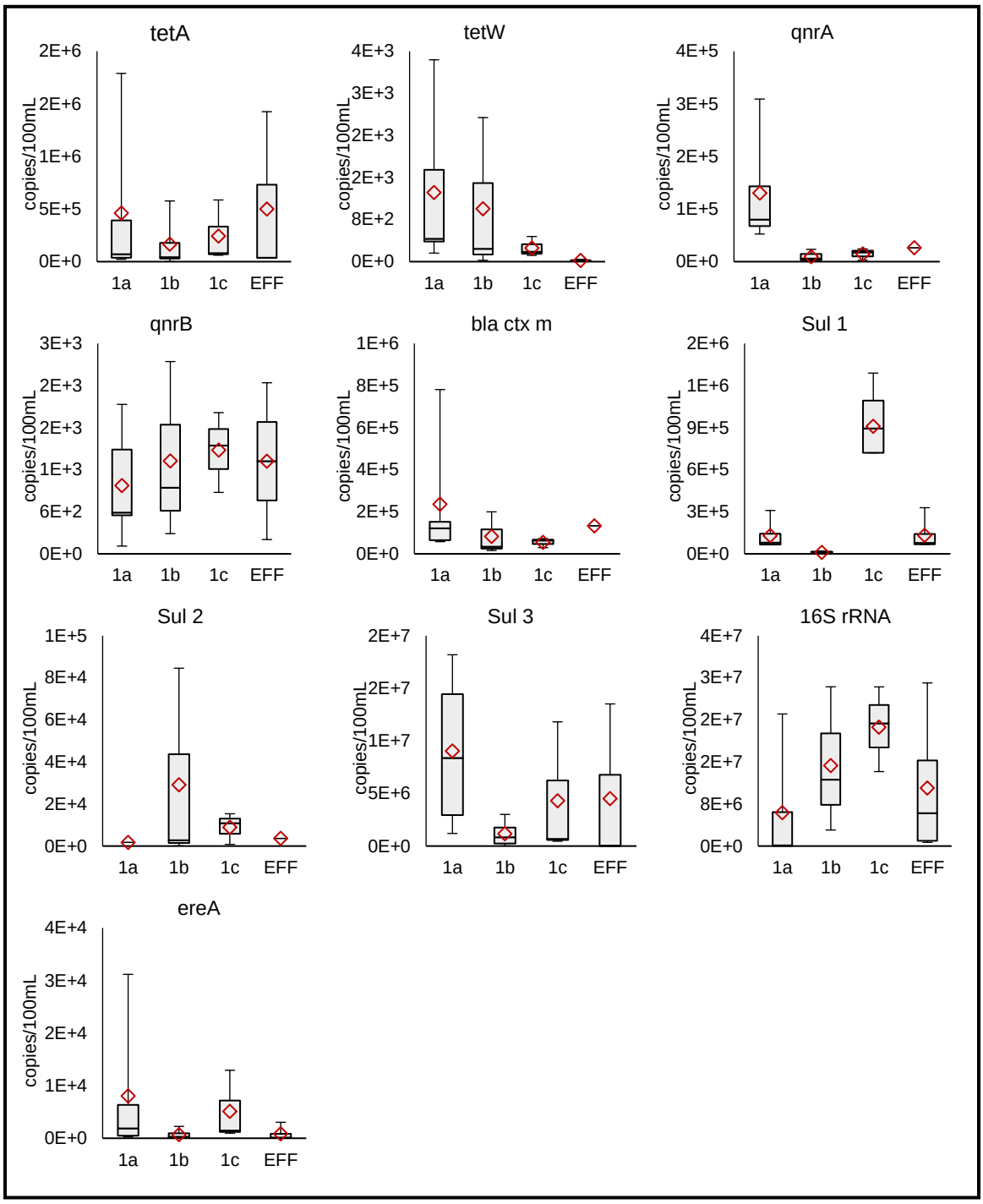


FIGURE 31: Comparison of antibiotic resistance genes concentrations for three WWTP effluents and a downstream river location.

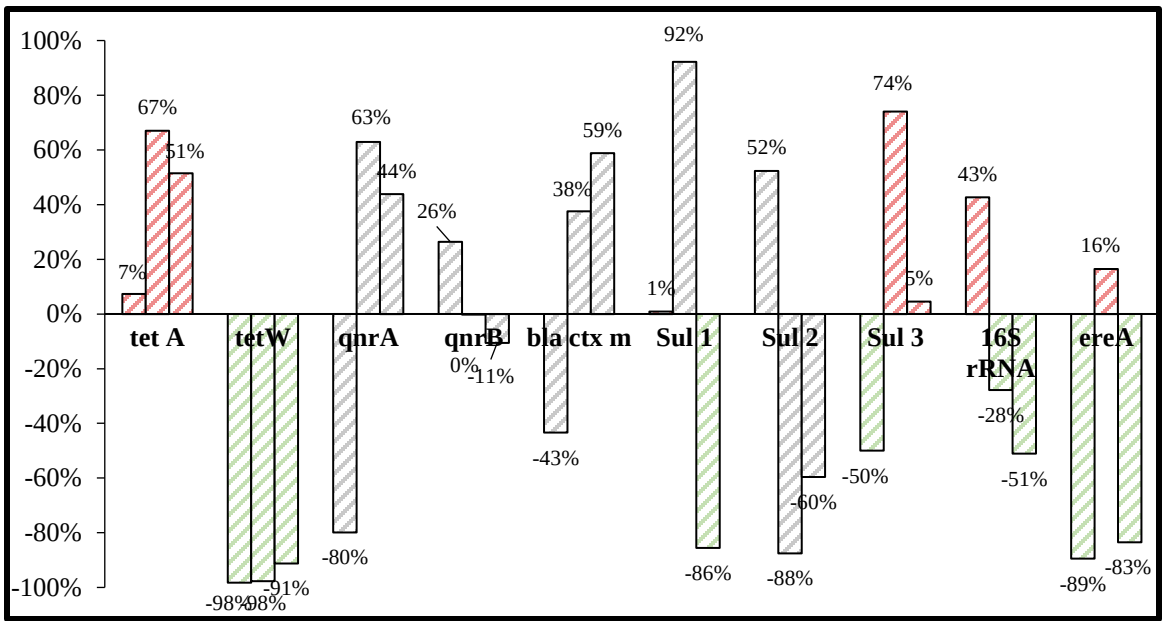


FIGURE 32: Average percent decrease or increase of antibiotic resistance genes between three WWTP effluents and a downstream river location.

3.2.8 Cost Analysis

The most significant positive impacts of effluent release into the river environment were from attenuation of some nutrients, *E. coli*, and apparent photolysis and hydrolysis of CECs that are susceptible to those processes. Additionally, the decrease in iodide may have an effect on formation of iodinated DBPs, however, this is a topic that warrants further investigation, as the decrease may be the result of the change in speciation.

Negative impacts of releasing treated effluent into the river as an environmental buffer between a WWTP and a DWTP is the significant increase in microorganisms, including pathogens, and an increase in TSS and its variability. While the benefits achieved as a result of this environmental buffer are difficult to quantify, the impacts from the increased microorganisms and TSS would have quantifiable monetary impact on a downstream DWTP, compared to DPR, especially if the environmental buffer does not provide considerable dilution. Table 27 shows the average influent between the 3 WWTP and the SPR in terms of meeting EPA MCL guidelines.

TABLE 27. EPA vs South Platte River [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (WWTP 1a – 1c)	Effluent (SPR)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.01 ± 0.0	0.01 ± 0.0
Iron (Fe) [2]	ppm	0.3	NS	0.17 ± 0.03	0.2 ± 0.02
Boron (B) [2]	ppm	NS	NS	0.21 ± 0.06	0.18 ± 0.08
Calcium (Ca) [2]	ppm	NS	NS	52.6 ± 3.6	54.4 ± 18.2
Magnesium (Mg) [2]	ppm	NS	NS	15.0 ± 1.8	14.9 ± 5.28
Manganese (Mn) [2]	ppm	0.05	NS	**0.07 ± 0.02**	**0.17 ± 0.1**
Sodium (Na) [2]	ppm	NS	NS	104.7 ± 7.4	92.1 ± 34.9
Cadmium (Cd)[1]	ppb	2	0.005	0.00 ± 0.0	0.00 ± 0.0
Mercury (Hg) [1]	ppb	2	0.002	0.00 ± 0.0	0.00 ± 0.0
Lead (Pb) [1]	ppb	15	0.2	0.00 ± 0.0	0.00 ± 0.0
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	123.5 ± 10.7	101.5 ± 36.7
Bromide (Br) [2]	ppm	NS	NS	0.17 ± 0.09	0.18 ± 0.12
Sulfate (SO_4^{2-}) [2]	ppm	500	NS	170.7 ± 25.6	147.9 ± 52.3
Iodine (I)	ppm	NS	NS	0.26 ± 0.09	0.00 ± 0.0
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.09 ± 0.08	0.1 ± 0.04
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	**10.7 ± 9.1**	3.56 ± 1.42
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.08 ± 0.02	0.00 ± 0.0
Total Phosphorous	mg/L PO_4^--P	NS	NS	4.7 ± 3.5	2.12 ± 1.01
Total Nitrogen	mg/L-N	NS	NS	20.6 ± 12.7	20.3 ± 17.2
Microorganisms					
Total Coliform [2]	MPN/100 mL	MCL[3]	0	**1281.4 ± 932.8**	**2031 ± 454**
Fecal Coliform [2]	MPN/100 mL	MCL[3]	0	**31.8 ± 41.1**	**143.9 ± 115.7**
Escherichia coli [2]	MPN/100 mL	MCL[3]	0	**12.6 ± 16.1**	**83.1 ± 48.4**
Enterococci [2]	MPN/100 mL	NS	NS	45.5 ± 33.3	26.1 ± 19.46
Salmonella spp. [2]	MPN/100 mL	NS	NS	0.38 ± 0.56	3.87 ± 5.2
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	**46.1 ± 19.1**	**40.7 ± 70.3**
Aggregate Water Quality					
Total Dissolved Solids [2]	ppm	500	NS	4.2 ± 2.7	15.36 ± 7.04
pH[1]		6.5 – 8.5	NS	7.11 ± 0.28	7.83 ± 0.07
Total Organic Carbon [1]	mg/L	NS	NS	10.7 ± 3.4	5.88 ± 1.31
BOD_5	mg/L-DO	NS	NS	7.4 ± 3.2	1.68 ± 0.88
Conductivity	µS	NS	NS	900.1 ± 52.6	795.6 ± 305.5
Alkalinity	mg/L-$CaCO_3$	NS	NS	96.6 ± 32.8	119.1 ± 22.0
COD	mg/L -COD	NS	NS	51.6 ± 27.3	38.17 ± 18.72
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	1.33 ± 0.54	1.54 ± 0.0
Benzo[a]pyrene[2]	ppt	200	0.0	11.0 ± 2.4	10.0 ± 0.0

NS: no standard **Red: above MCL**

Green Cells: Significant Decrease Red Cells: Significant Increase

[1] Denver Water, Water Quality Report - 2018

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation." - *National Primary Drinking Water Regulations, USEPA* – 2018.

3.3 RIVERBANK FILTRATION (RBF) (APW 2A – 2B)

3.3.1 Aurora Prairie Waters Project (APW)

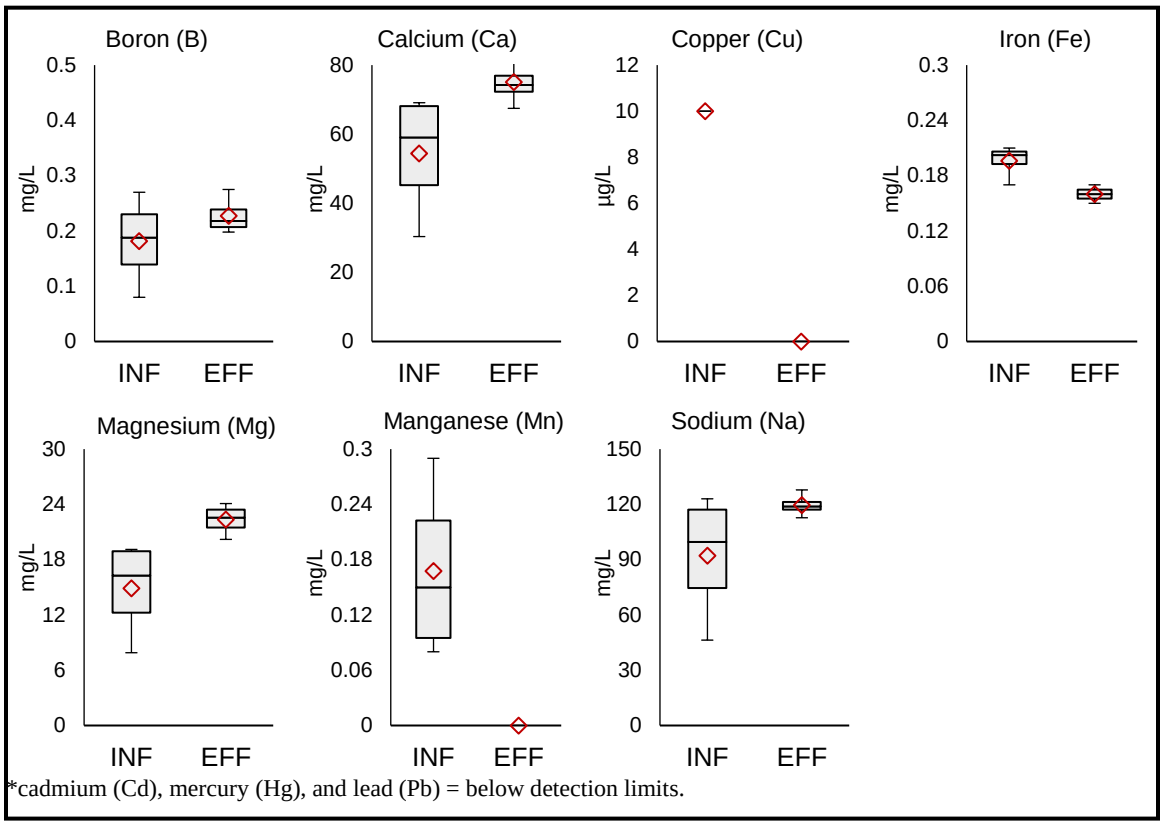
The year 2001 marked the beginning of the longest ever recorded duration of drought to have occurred in the state of Colorado. According to the US Drought Monitor website, the drought conditions lasted more than 7.5 years from October of 2001 to May of 2009. In response, the Prairie Waters Project (PWP) was devised as a preventative measure for any future severe drought conditions. The project combines natural purification processes with state-of-the-art purification technology to provide up to 12 MGD of drinking water to its local residents.[5] To do this, the city of Aurora exercised its ownership rights to the water in the SPR basin, which also includes the Colorado and Arkansas river basins as well, meaning the-right-of-use policy allows the city to use the water native to the river to its entirety.[6]

The overall project is conducted in two separate purification steps. The first step is to process the water using a multi-barrier purification approach. The process utilizes RBF by using 23 alluvial well pumps, installed 300 ft from the river, to pull water from SPR through 100 feet of sand and gravel, with a travel time lasting 7 to 10 days.[7] Then, the filtered water is transferred via 27 extraction well pumps to a 200-acre recharge basin for additional treatment as it percolates through an aquifer recharge for roughly 20 days.[7-8] The groundwater is then transferred via series of 3 pump stations to the Peter D. Binney Water Purification Facility. The facility is equipped to handle up to up to 50 MGD of raw water with advanced processes including advanced ultraviolet oxidation, and activated carbon adsorption.[9-10] The treated water is said to exceed federal and state

drinking water regulations and is distributed directly to residents and business for immediate use.

3.3.2 Metals

Iron and manganese, while introduced via runoff to the river water, were significantly removed by riverbank filtration (RBF). In fact, manganese was not detectable in any of the samples that went through RBF as shown in Figures 33 and 34. Concentrations of calcium and magnesium increased slightly, from 54 to 75 mg/L and from 15 to 22 mg/L on average, respectively. Overall, the hardness in this water is very high even before the RBF, and it increased from the contact with subsurface minerals. Softening would be required for a drinking water treatment plant using South Platte River water, however, the consumable cost of chemicals will be approximately 30% higher to achieve the same final water hardness after RBF.



*cadmium (Cd), mercury (Hg), and lead (Pb) = below detection limits.

FIGURE 33. Comparison of metal concentrations for river water before and after riverbank filtration and aquifer recharge and recovery.

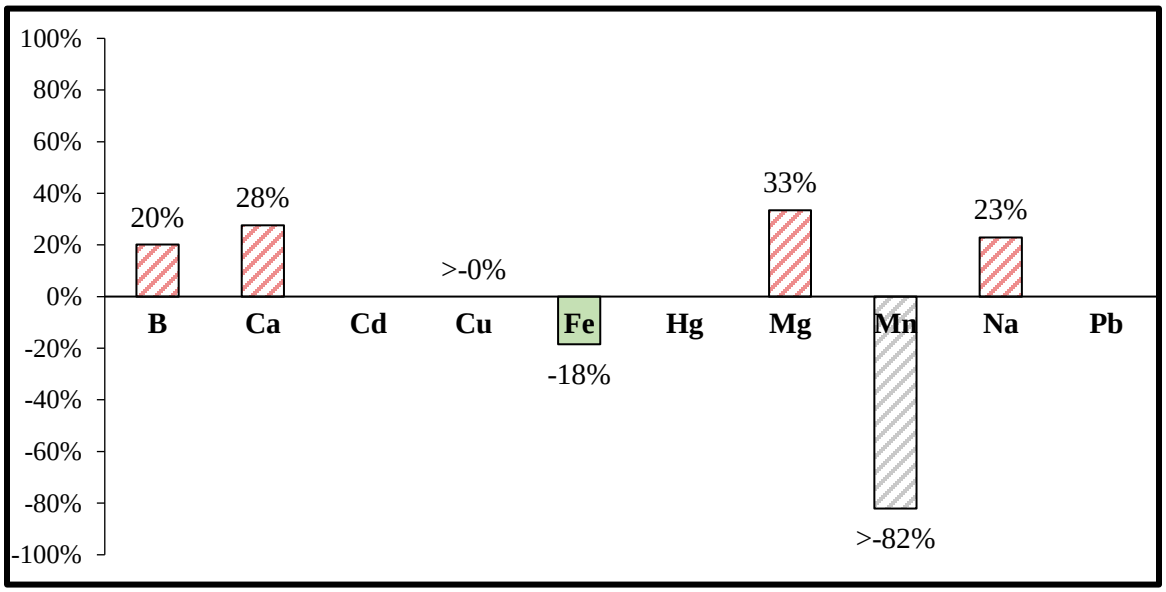
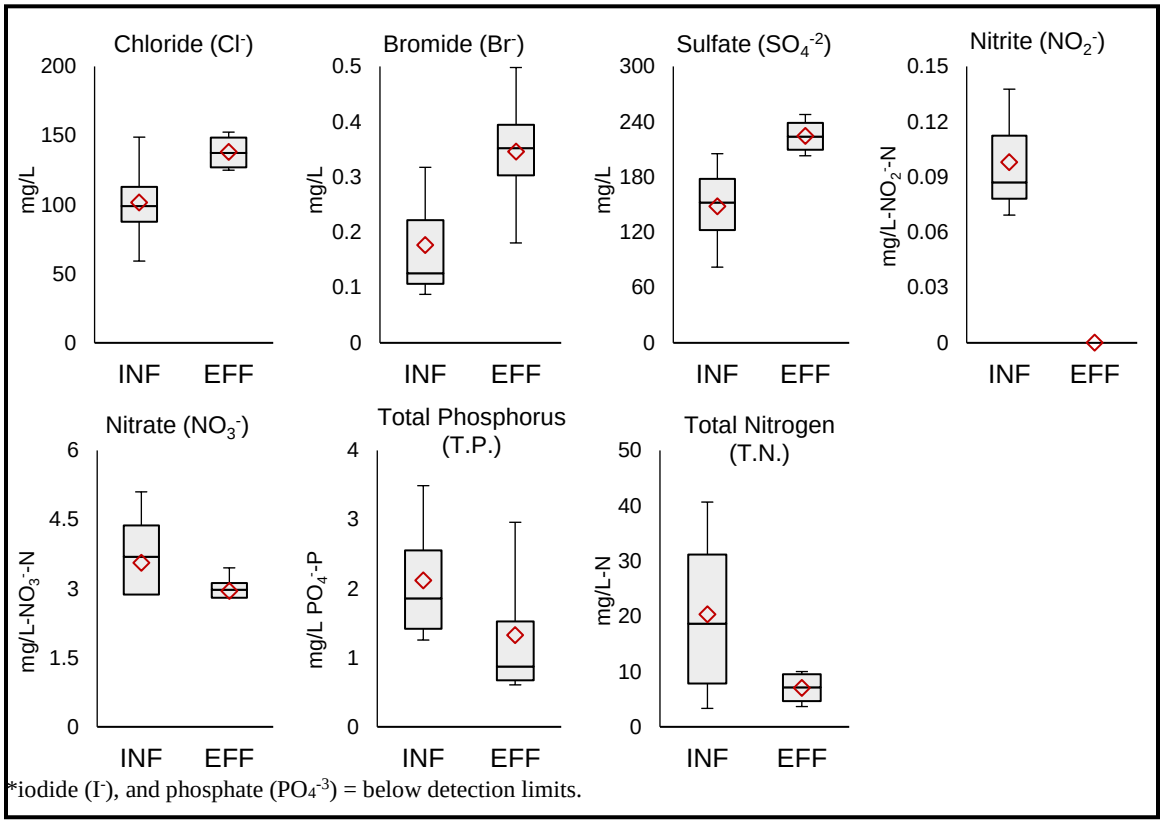


FIGURE 34: Average percent decrease or increase of metals in river water before and after riverbank filtration and aquifer recharge and recovery.

3.3.3 Nutrients and anions

RBF provided little nutrient attenuation, with the most effect observed on TN. Nitrate, however, was not removed, as shown in Figures 35 and 36, therefore, the decrease is most likely due to removal of larger nitrogen-containing organic molecules and microbes via filtration. Concentrations of chloride, bromide and sulfate were slightly higher after RBF, possibly due to mixing with groundwater that has higher ionic content. In general, those concentrations were relatively high in the river water before and after RBF, compared to typical values in freshwater streams and in groundwater. High levels of these ions reflect that South Platte River is heavily affected by human activity and wastewater discharges. Municipal effluent typically has higher ionic loads contributed by water use and treatment. Apart from increase in bromide potentially having an effect on DBP formation, RBF did not impact nutrients and anions in the river water in a notable way.



*iodide (I^-), and phosphate (PO_4^{-3}) = below detection limits.

FIGURE 35. Comparison of nutrient and anion concentrations for river water before and after riverbank filtration and aquifer recharge and recovery.

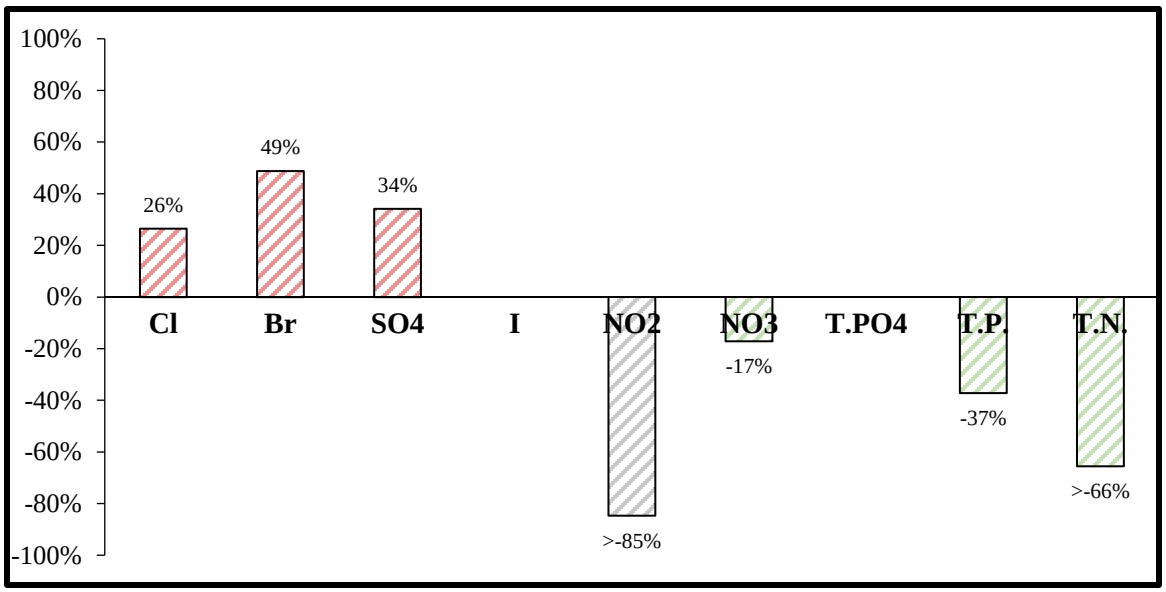
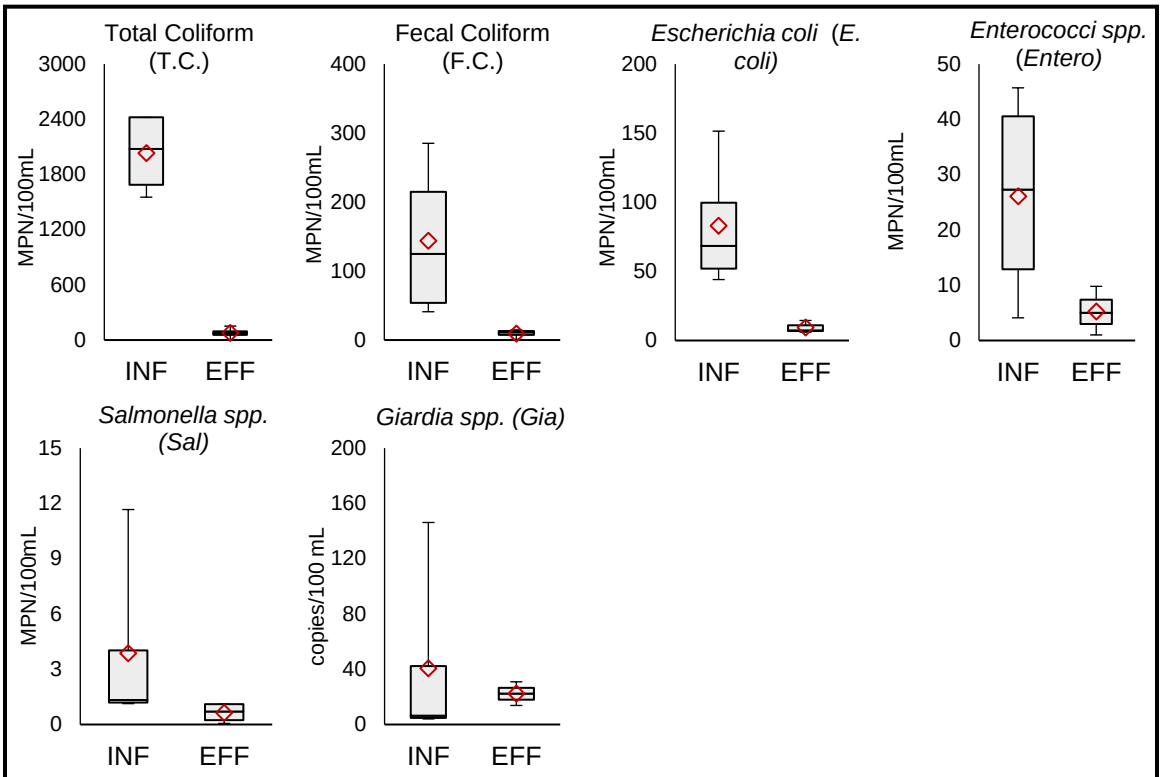


FIGURE 36. Average percent decrease or increase of nutrients and anions in river water before and after riverbank filtration and aquifer recharge and recovery.

3.3.4 Microorganisms

The water was greatly improved in microbial quality after RBF as shown in Figures 37 and 38. The microbial concentrations were lower in all measured categories, many of them showing at least 1-log removal. Any microbial recontamination of treated effluent after its release into the river was mitigated by RBF.



Maximum concentration for total coliform, fecal coliform, E. coli, and Enterococci spp. is reported up to 2419.6 MPN/100mL.

FIGURE 37. Comparison of microbial contaminants for river water before and after riverbank filtration and aquifer recharge and recovery.

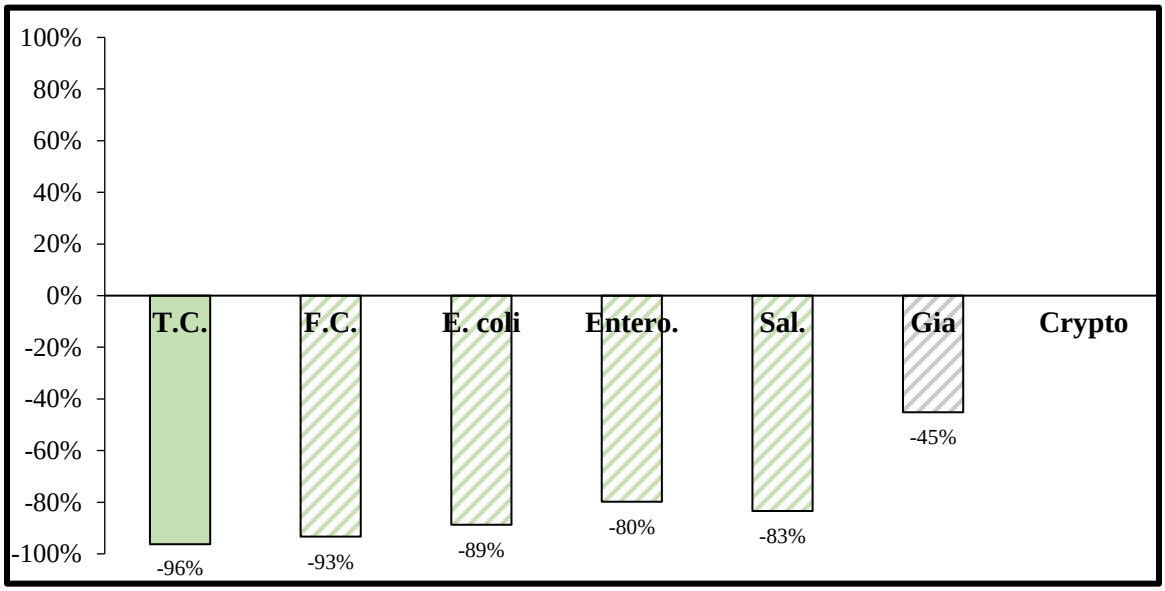


FIGURE 38. Average percent decrease or increase of microbial contaminants in river water before and after riverbank filtration and aquifer recharge and recovery.

3.3.5 Aggregate Water Quality Assessment

Decrease in TOC and BOD_5 was achieved in RBF. The most significant impact was on the removal of TSS (92% on average), again mitigating the increase in TSS that resulted from the release of WWTP effluents into South Platte River as shown in Figures 39 and 40. A slight increase in conductivity (expected considering the increase in most major ions) and a more considerable increase in alkalinity (from 120 to 160 mg/L as $CaCO_3$ on average) were observed, but were not expected to affect downstream water treatment in a significant way. An increase in alkalinity could provide a cost saving for chemicals used in water softening.

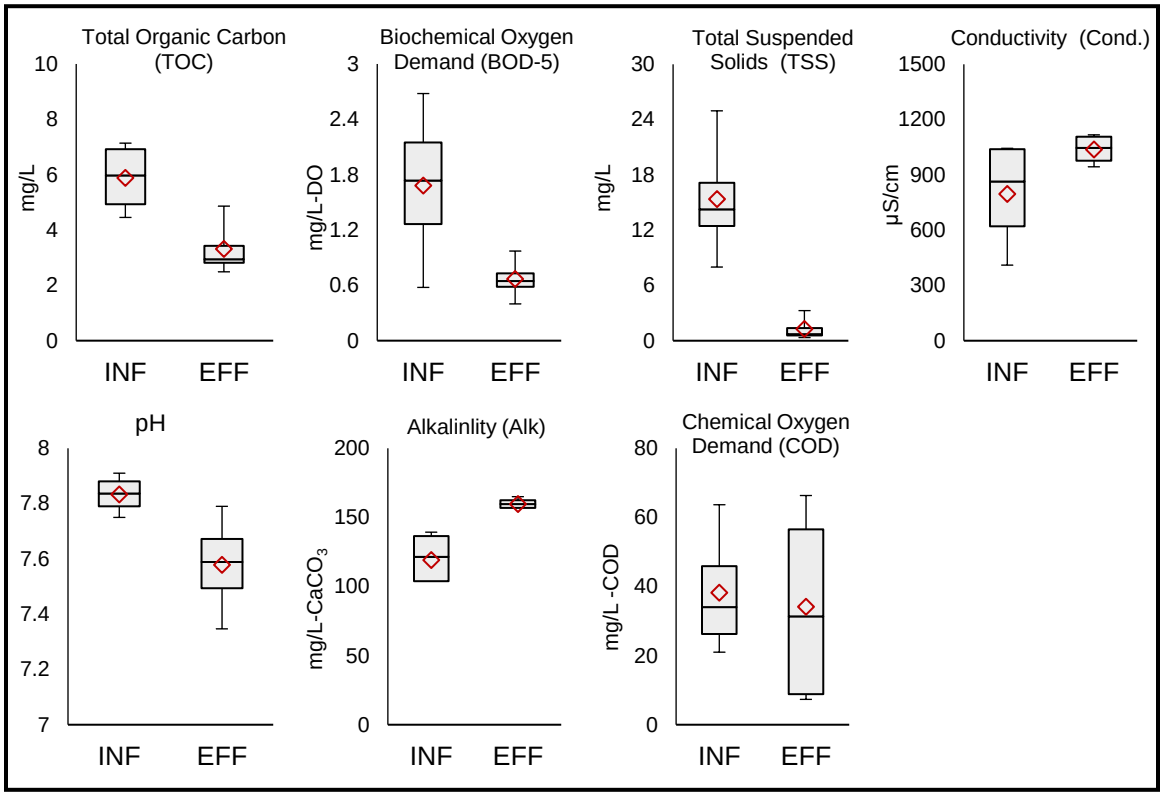


FIGURE 39. Comparison of aggregate water quality parameters for river water before and after riverbank filtration and aquifer recharge and recovery.

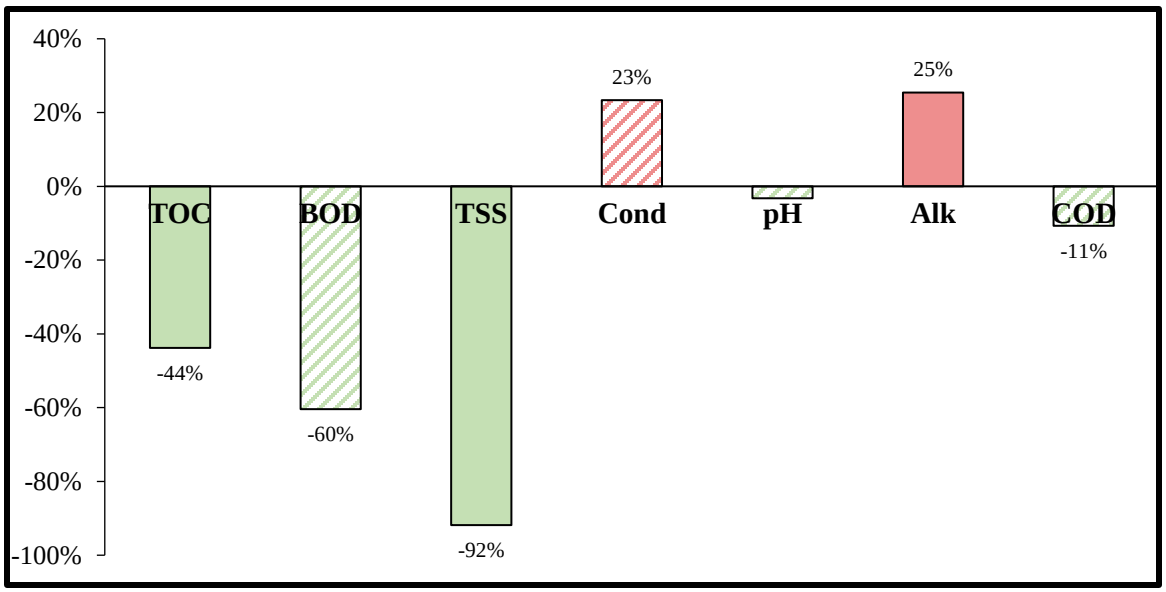


FIGURE 40. Average percent decrease or increase of aggregate water quality parameters in river water before and after riverbank filtration and aquifer recharge and recovery.

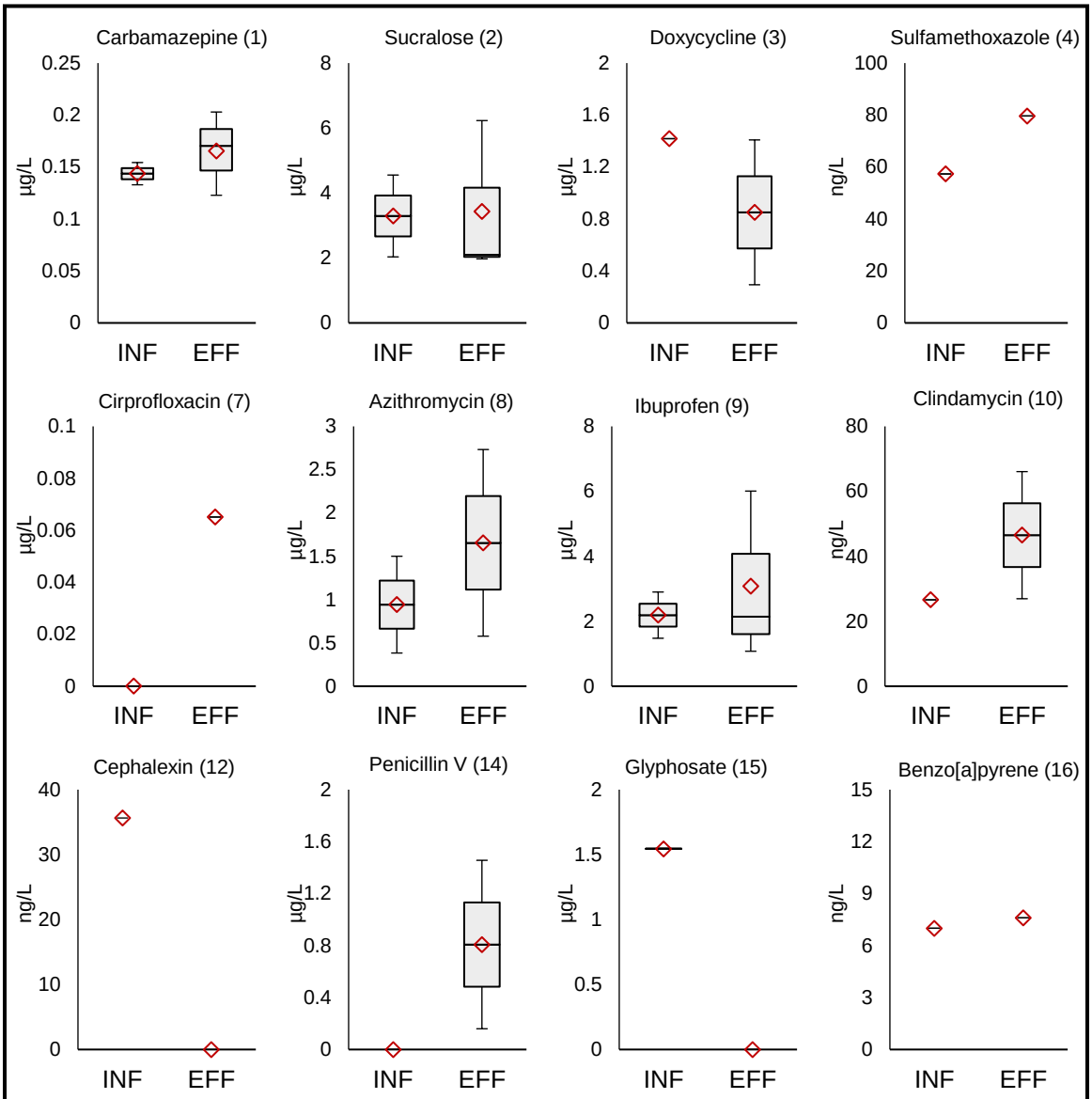
3.3.6 Contaminants of Emerging Concern

The compounds that were consistently detected in river water (carbamazepine, sucralose, azithromycin and ibuprofen) were present at the same levels in the water after RBF as shown in Figures 41 and 42. Two of these compounds were used as environmental fate indicator of adsorption (carbamazepine) and biodegradation (ibuprofen). Their stable concentrations indicate that these two environmental attenuation pathways are not relevant to this RBF system. These pathways may still be of relevance in instances of higher organic soil content or higher abundance of subsurface microbial life. This outcome would also be affected by the average travel time of water through the riverbank.

Penicillin V, while BDL in all of the river samples, was present well above detection limit (in one instance at 1.5 µg/L) in three of the four samples of RBF effluent. The source of this increase in difficult to explain as the location is not in the proximity to livestock operations, which could have contaminated groundwater. Penicillin V is highly

susceptible to hydrolysis in the environment, and typically does not travel long distances and is only found in the environment in the vicinity of the discharge.

Glyphosate, while present in the river water at a consistent concentration of approximately 1.5 μg/L was not detectable (<2.5 ng/L) in RBF effluent indicating that some removal mechanism for glyphosate is present in the subsurface environment. Glyphosate is a zwitterionic substance at near neutral pH, and is believed to have high affinity for adsorption to mineral surfaces in soil, in particular minerals containing iron and aluminum oxides.[129] The soil adsorption capacity reported in literature is on the order of μg/g.[129] However, groundwater in agricultural areas may have high ambient glyphosate levels. The removal of glyphosate in this RBF system is not necessarily indicative that pesticides cannot increase in water in the subsurface environment.



*trimethoprim (5), amoxicillin (6), atrazine (11), and levofloxacin (13) = below detection limits.

FIGURE 41. Comparison of concentrations of contaminants of emerging concern for river water before and after riverbank filtration and aquifer recharge and recovery.

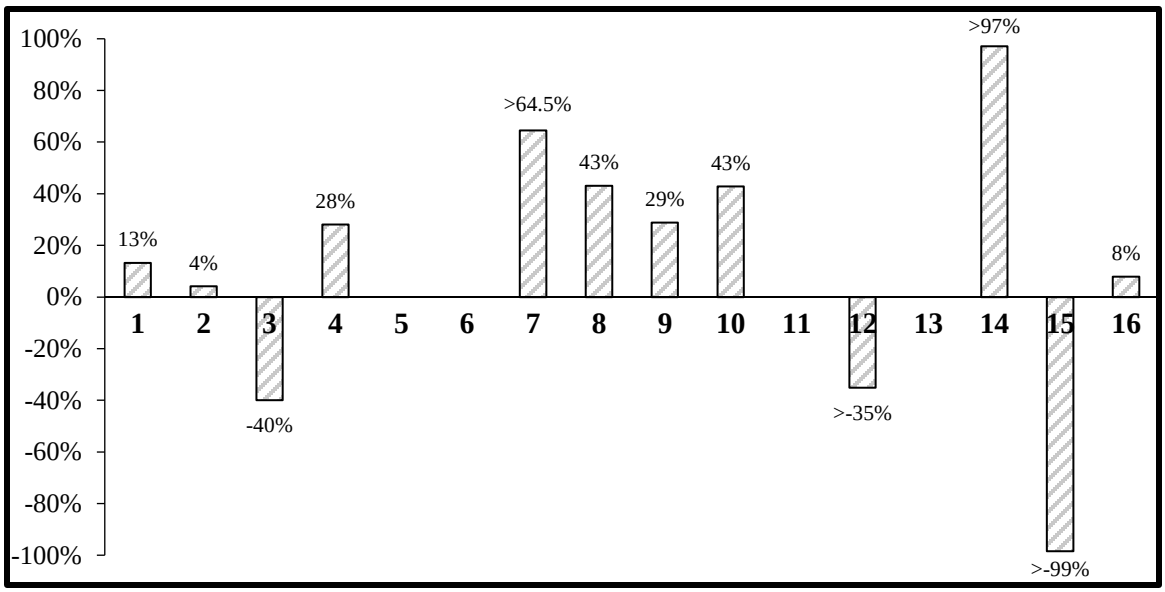


FIGURE 42. Average percent decrease or increase of contaminants of emerging concern in river water before and after riverbank filtration and aquifer recharge and recovery.

Antibiotic Resistance Genes (ARG)

All of the ARGs that were present in the river water decreased considerably (16–97%) during RBF and ARR as shown in Figures 43 and 44. The three sulfonamide resistance genes decreased by >90%. The result is expected as lower overall abundance of microorganisms typically corresponds to lower levels of ARGs.

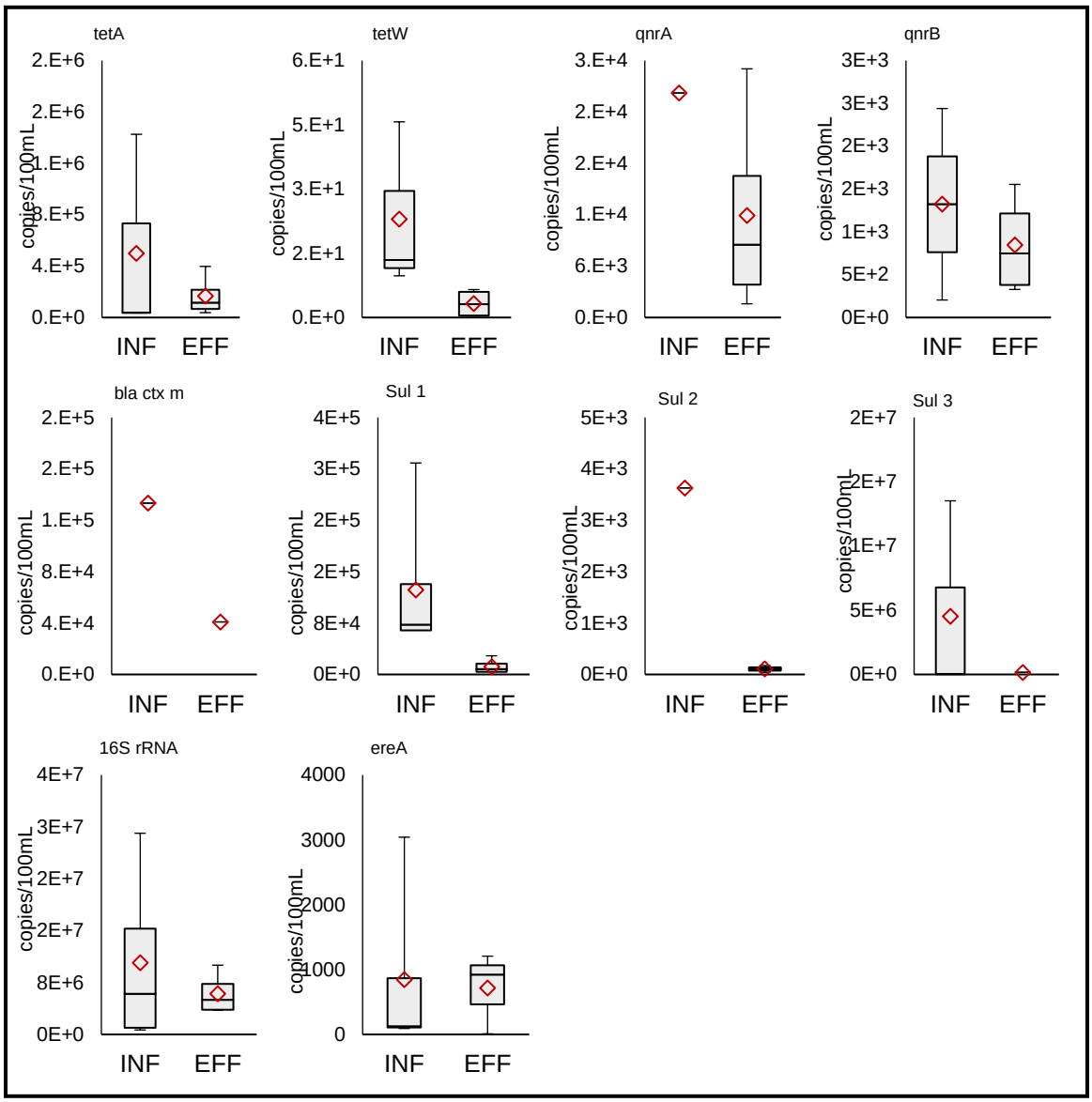


FIGURE 43: Comparison of antibiotic resistance genes concentrations for river water before and after riverbank filtration and aquifer recharge and recovery.

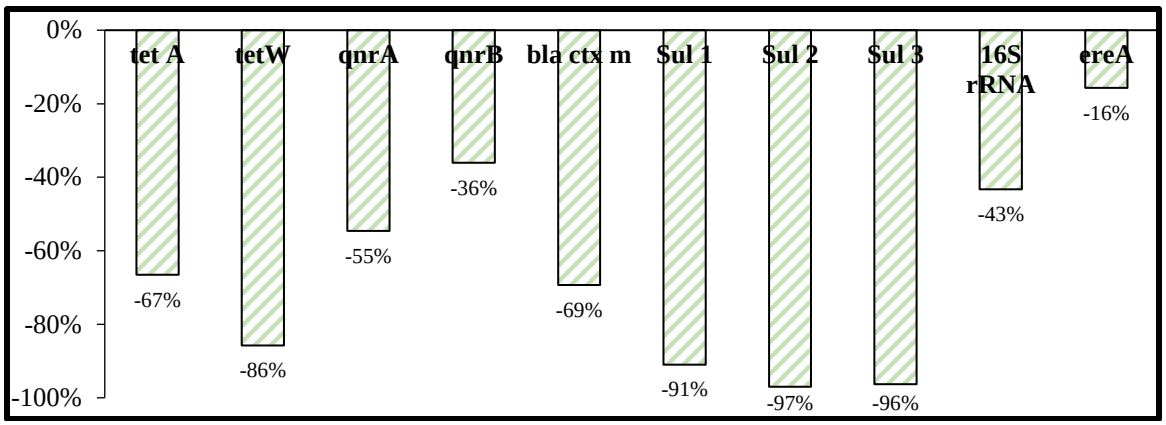


FIGURE 44: Average percent decrease or increase of antibiotic resistance genes in river water before and after riverbank filtration and aquifer recharge and recovery.

3.3.7 Cost Analysis

RBF mitigated the increase microorganisms counts and higher TSS that resulted from the release of the effluent from the WWTPs into the river as shown in Table 28. With the decrease in microorganism concentrations, the abundance of ARGs decreased as well. RBF provided little to no further attenuation to CECs with the exception of glyphosate. There was an increase in overall dissolved ions and hardness. In general, RBF presented little concern for reintroduction of any of the measured contaminants and significantly improved water quality in the categories that would have the highest impact on the cost of drinking water treatment. The cost of added pumping and any maintenance related to RBF needs to be considered as well to determine the net financial benefit of using RBF.

TABLE 28. EPA vs Aquifer Recharge [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (SPR)	Effluent (Aquifer)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.01 ± 0.0	0.00 ± 0.0
Iron (Fe) [1]	ppm	0.3	NS	0.2 ± 0.02	0.16 ± 0.01
Boron (B) [1]	ppm	NS	NS	0.18 ± 0.08	0.23 ± 0.03
Calcium (Ca) [1]	ppm	NS	NS	54.4 ± 18.2	75.1 ± 7.0
Magnesium (Mg) [1]	ppm	NS	NS	14.9 ± 5.3	22.36 ± 1.69
Manganese (Mn)	ppm	0.05	NS	0.17 ± 0.1	0.00 ± 0.0
Sodium (Na) [1]	ppm	NS	NS	92.1 ± 34.9	119.6 ± 6.2
Cadmium (Cd)[2]	ppb	2	0.005	0.00 ± 0.0	0.00 ± 0.0
Mercury (Hg) [2]	ppb	2	0.002	0.00 ± 0.0	0.00 ± 0.0
Lead (Pb) [1]	ppb	15	0.2	0.00 ± 0.0	0.00 ± 0.0
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	101.5 ± 36.7	138.0 ± 13.8
Bromide (Br)	ppm	NS	NS	0.18 ± 0.12	0.35 ± 0.13
Sulfate (SO_4^{2-}) [1]	ppm	500	NS	147.9 ± 52.3	224.6 ± 20.7
Iodine (I)	ppm	NS	NS	0.00 ± 0.0	0.00 ± 0.0
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.1 ± 0.04	0.00 ± 0.0
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	3.56 ± 1.42	2.95 ± 0.44
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.00 ± 0.0	0.00 ± 0.0
Total Phosphorous	mg/L PO_4^--P	NS	NS	2.12 ± 1.01	1.33 ± 1.1
Total Nitrogen	mg/L-N	NS	NS	20.3 ± 17.2	7 ± 3.14
Microorganisms					
Total Coliform[2]	MPN/100 mL	MCL[3]	0	2031.3 ± 454.3	75.7 ± 61.1
Fecal Coliform[2]	MPN/100 mL	MCL[3]	0	143.9 ± 115.7	9.53 ± 6.03
Escherichia coli[2]	MPN/100 mL	MCL[3]	0	83.1 ± 48.4	9.33 ± 4.52
Enterococci[2]	MPN/100 mL	NS	NS	26.1 ± 19.5	5.27 ± 4.41
Salmonella spp. [2]	MPN/100 mL	NS	NS	3.87 ± 5.2	0.64 ± 0.54
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	40.7 ± 70.3	22.3 ± 12.1
Aggregate Water Quality					
Total Dissolved Solids[1]	ppm	500	NS	15.36 ± 7.04	1.25 ± 1.35
pH[1]		6.5 – 8.5	NS	7.83 ± 0.07	7.58 ± 0.19
Total Organic Carbon	mg/L	NS	NS	5.88 ± 1.31	3.31 ± 1.06
Biochemical Oxygen Demand	mg/L-DO	NS	NS	1.68 ± 0.88	0.67 ± 0.24
Conductivity	µS	NS	NS	795.6 ± 305.5	1038.1 ± 85.5
Alkalinity	mg/L-$CaCO_3$	NS	NS	119.1 ± 22.0	159.7 ± 4.7
Chemical Oxygen Demand	mg/L -COD	NS	NS	38.2 ± 18.7	34.1 ± 30.2
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	1.54 ± 0.0	0.00 ± 0.0
Benzo[a]pyrene[2]	ppt	200	0.0	10.0 ± 0.0	10.0 ± 0.0

NS: no standard **Red: above MCL**

Green Cells: Significant Reduction Red Cells: Significant Increase

[1] Denver Water, Water Quality Report – 2018

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation." - *National Primary Drinking Water Regulations, USEPA* – 2018.

3.4 LAKES AND RIVER SYSTEMS (CW 1 – CW 3)

3.4.1 Mountain Island Lake (MIL)

Lake Norman of the Catawba Watershed is the largest artificial lake in NC expanding across 4 counties with an estimated surface area of 130 km^2.[130] Lake Norman was founded and currently owned by Duke Energy and is a major source for local hydroelectric generation as well as a popular area for recreational activities. The lake water is used for cooling purposes by the McGuire Nuclear Station; it receives occasional contamination from the Marshall Steam Station, such as the coal-ash spill incident in 2014; and is currently used as the source of hydroelectric generation for the Cowans Ford Dam.[131] Situated downstream of Lake Norman, MIL is also owned by Duke Energy, serves as the main drinking water supply source for the City of Charlotte and also a source of electricity generation via the Riverbend Steam Station and the Mountain Island Steam Station. At 13 km^2, the surface area of MIL is relatively small compared to its Lake Norman counterpart with an average hydraulic retention time of only 12 days.[42]

McDowell Creek (MDC) WWTP is situated roughly 1.3 miles upstream of MIL next to McDowell Creek, a tributary that feeds into MIL. MDC WWTP services households and businesses located in the northwest Mecklenburg County and the towns of Huntersville and Cornelius.[132] The WW treatment processes at MDC WWTP involve several stages: primary treatment for the removal of large particles and objects via screens, grit chamber, and primary clarifiers; constituent, mainly nutrient, removal via activated sludge in aeration basins; the settling of solids and microorganisms in secondary clarifier basins; additional tertiary treatment with granular filters for the removal of fine particles; lastly, disinfection using UV irradiation.[133] The recent

expansion at the WWTP saw an increased capacity to 12 MGD, and in 2018, MDC WWTP received its 10th consecutive Platinum Award from the National Association of Clean Water Agencies (NACWA) for excellence in permit compliance.[133]

The raw water intake for Franklin Water Treatment Plant (FWTP) is situated 2.5 miles downstream from where McDowell Creek Tributary joins the lake body. The Catawba River Pump Station can pump up to 350 MGD of raw water from MIL to the reservoirs located at FWTP.[134] The plant is capable of processing up to 181 MGD, but more often operates at an average capacity of 80 MGD.[134] Sample locations in MIL are designated as CW 1 and CW 3, as shown in Figures 6 and 7.

MDC WWTP effluent served as influent samples (CW 1) into the MIL, although the overall influent volume is dominated by the Lake Norman outflow upstream. The buffer effluent (CW 3) samples were collected with a peristaltic pump at the intake before it entered the Catawba River Pump Station. Teflon tubing was used to minimize contamination and a small segment of peristaltic pumping tubes with metal fittings was used to accommodate the roller assembly.

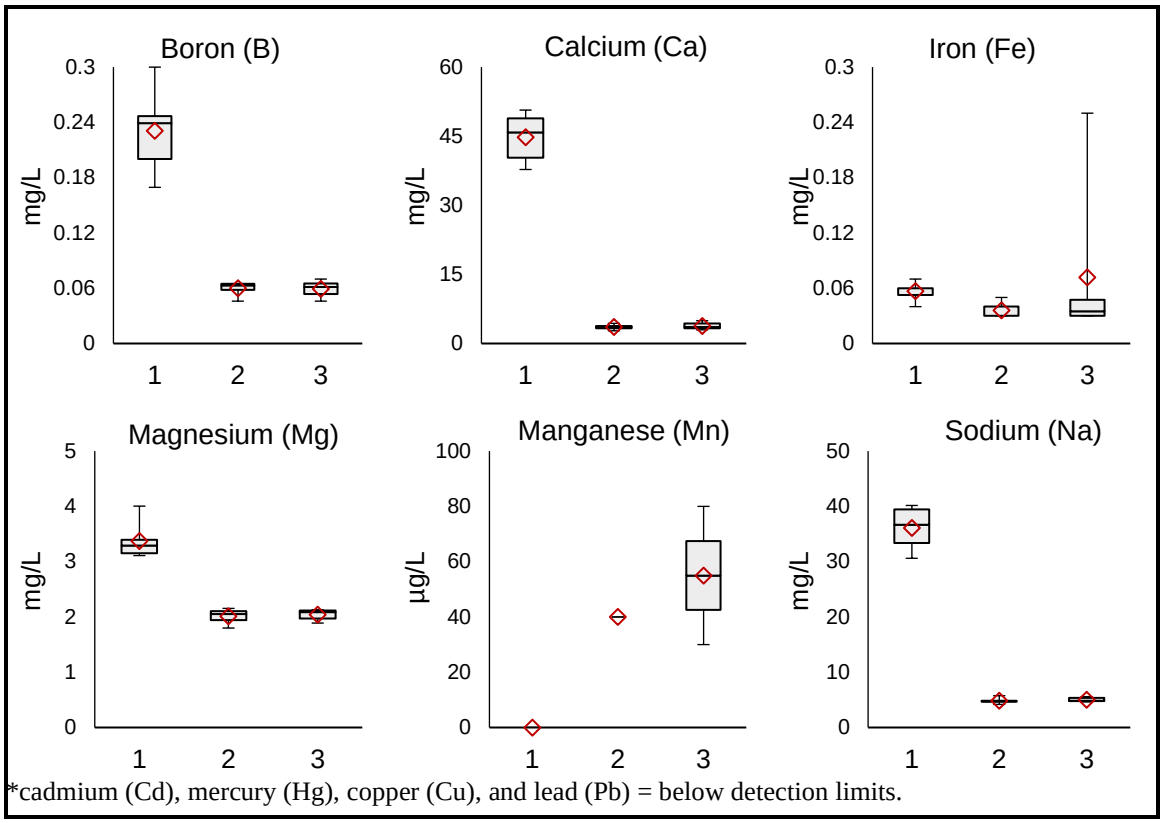
A canoe was used to access CW 2 sampling location in the middle of MIL. This method was used in lieu of a motorized boat to prevent any biasness in the benzo[a]pyrene as a result of incomplete fuel combustion from the aquatic motor vehicle. CW 2 sample locations were kept consistent with GPS coordinates. The primary purpose of CW 2 samples is for the evaluation of environmental degradation pathways beyond dilution. CW 1 samples constitute a very small portion of MIL's influent, < 0.17%, hence, the primary environmental fate of contaminants was due to dilution.[135] However, dilution will not be a main degradation process between CW 2 and CW 3 samples since

the change in discharge rate between the two locations are negligible. Therefore, the occurrence of additional environmental attenuation processes such as photodegradation or biodegradation will be reflected between CW 2 and CW 3 samples. CW1 was compared to CW2 in water quality to evaluate a hypothetical DPR scenario as an alternative to discharge into a lake prior to uptake for drinking water treatment.

Six total samples were collected at MIL: three samples were collected during the dry season and three during the rainy season (Table 2 lists specific time and dates). The data for the dry and wet seasons were analyzed together in order to provide a comprehensive assessment that considered environmental fluctuations due to temporal and weather differences. The wet and dry samples were also evaluated separately to see if rain events had any significant impact on the fate and transport of the contaminants.

3.4.2 Metals

The concentration of boron, calcium, magnesium, and sodium from MDC wastewater effluent discharge was significantly lower after passing through MIL as shown in Figures 45 and 46. Boron concentrations saw an average of 74.3% reduction post lake/river buffer processing. In nature, boron almost never exists in its free elemental state, instead, it is often bound to oxygen to form borate ions. [136]



*cadmium (Cd), mercury (Hg), copper (Cu), and lead (Pb) = below detection limits.

FIGURE 45. Comparison of metal concentrations for MDC WWTP effluent, MIL, and FWTP influent.

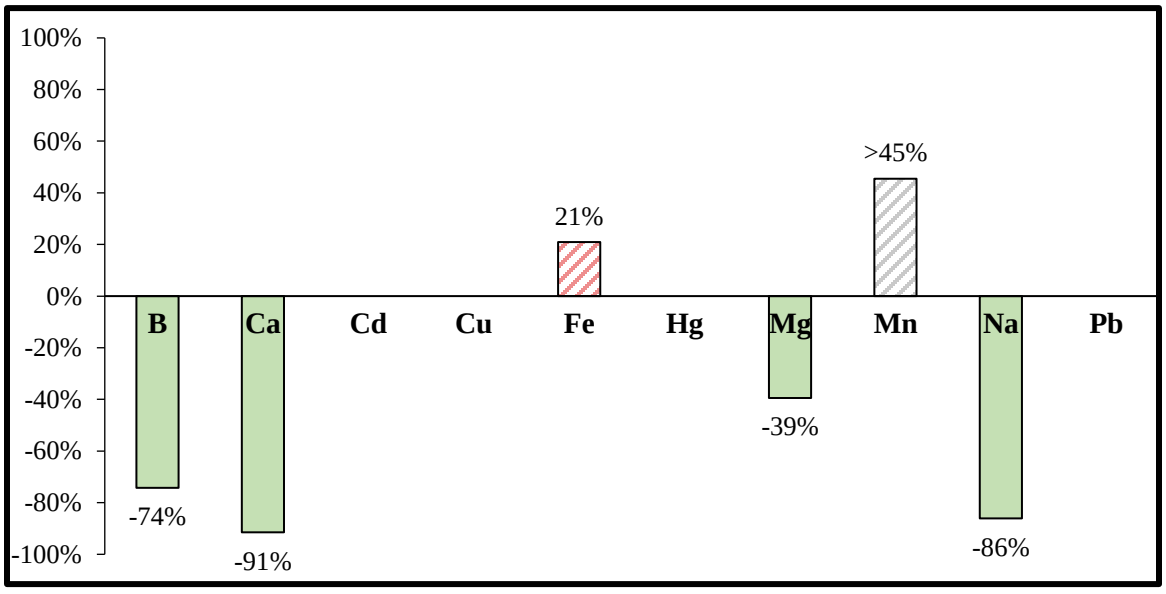


FIGURE 46. Average percent increase and decrease of metals (via dilution); CW 1 and CW 3 and (via environmental processes); CW 2 and CW 3.

In aquatic environments, borate ions are readily hydrolyzed to form boric acid ($B(OH)^3$) and its conjugate base ($B(OH)_4^-$). [137] Borate and its derivatives are stable in water under ambient environmental conditions and are not bioavailable to most aquatic plants and animals. [138] Additionally, because of its chemical stability, boron's ultimate fate is most likely to be adsorbed onto inorganic soil. [139] However, the likelihood for adsorption to occur in lakes and rivers is relatively low. The adsorption of suspended boron onto benthic soil and deposition onto riparian zones is impeded by the high velocity and turbulent nature of large rivers and lakes, which does not allow for adequate settling or contact to occur. Therefore, the primary environmental fate and attenuation of boron in fast moving bodies of water is most likely via dilution. The dilution hypothesis is supported by the negligible difference of 0.7% measured between the mid-buffer (CW 2) and buffer effluent (CW 3) samples. If additional environmental processes had occurred, such as photolysis or biodegradation, the attenuation would have continued throughout the entirety of MIL, and higher reduction percentages would have been measured between samples CW 2 and CW 3. Similarly, the significant attenuation of calcium and sodium in MIL may be attribute to the dilution processes. Although, attenuation via precipitation is plausible, since dissolved calcium cations in wastewater effluent discharges can precipitate with inorganic anions in the environment to form minerals. [140, 141]

Lake Norman is home to numerous Duke Energy electricity generating facilities, including the Marshall Steam Station and the McGuire Nuclear Station. In recent years, the coal ash storage basins located near the Catawba River Basin to the north of Lake Norman have experienced occasional breaches. The contents of the coal ash pond leaked

through the concrete basin barriers resulting in the seepage of coal burning byproducts into local groundwater and surface water supplies. In 2018, Duke Energy also reported the high radioactivity levels from radium in the local groundwater reservoirs, 2.5 times higher to be exact, than the federal drinking water standard.[142] CW 2 and CW 3 samples from MIL and at the drinking water intake measured elevated level of iron and manganese as well, exceeding that of local and federal water quality standards. The results of this study were corroborated by local government monitoring data that also measured exceptionally high levels of toxic heavy metals. [143, 144] The re-contamination measured in this study, therefore, may be caused by the local soil erosion as a result of infrastructural developments in the Charlotte metro area or potentially by the coal ash ponds located alongside Lake Norman and MIL.

No significant increase or decrease in measured parameters were observed between wet weather and dry weather events.

3.4.3 Nutrients and Anions

With the exception of nitrite, which was either below detection limits or did not pass QAQX in all directions, all nutrients and anions were shown to have attenuated in MIL as shown in Figures 47 and 48. For iodide specifically, only one measurement was above detection limits in the downstream sample, therefore, although the measured value itself is higher than the average than the effluent discharge, it can still be considered attenuated in a sense. Significant reduction was measured for chloride, sulfate, nitrate, and total phosphate levels between the wastewater effluent and the drinking water intake, primarily through dilution. Sources of naturally occurring chloride ions or chloride salts typically come from mineral formations in aquifers and brackish waters. Sources of

anthropogenic contamination can originate from sewage and landfill wastes, road salts, fuel additives and solvents, and some industrial processes. As stated previously, the main attenuation pathway for chloride in this study is likely due to dilution since: 1) chloride ions are highly soluble and mobile in aquatic environments and are not susceptible to most aquatic chemical or metabolic degradation processes, commonly used in tracer studies; and 2) significant reduction was only observed for the wastewater effluent after mixing with MIL water, but no significant reduction occurred within the lake/river system (samples CW 2 and CW 3), suggesting dilution to be the primary attenuation mechanism. [145]

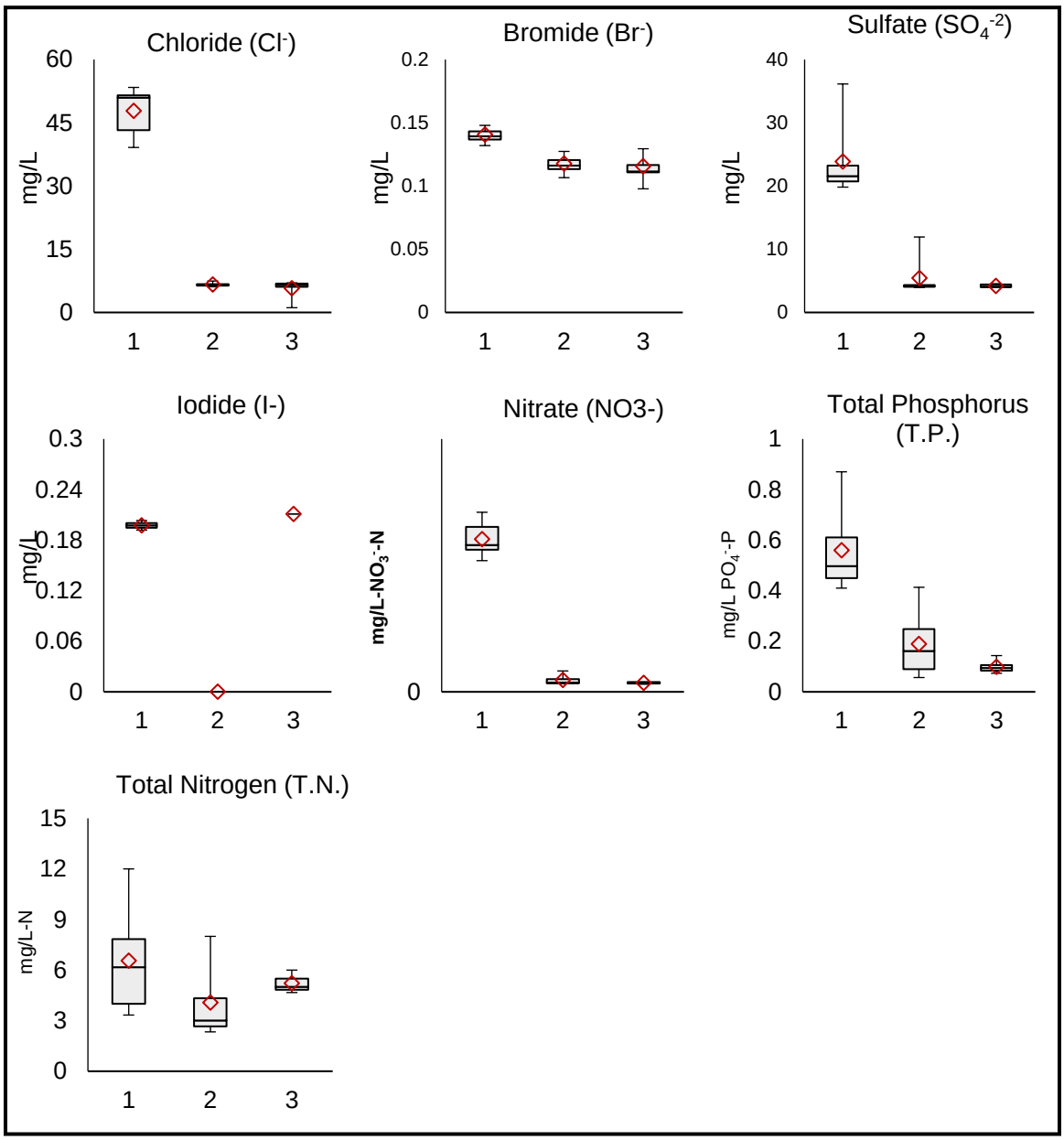


FIGURE 47. Comparison of nutrients and anions for MDC WWTP effluent, MIL, and FWTP influent.

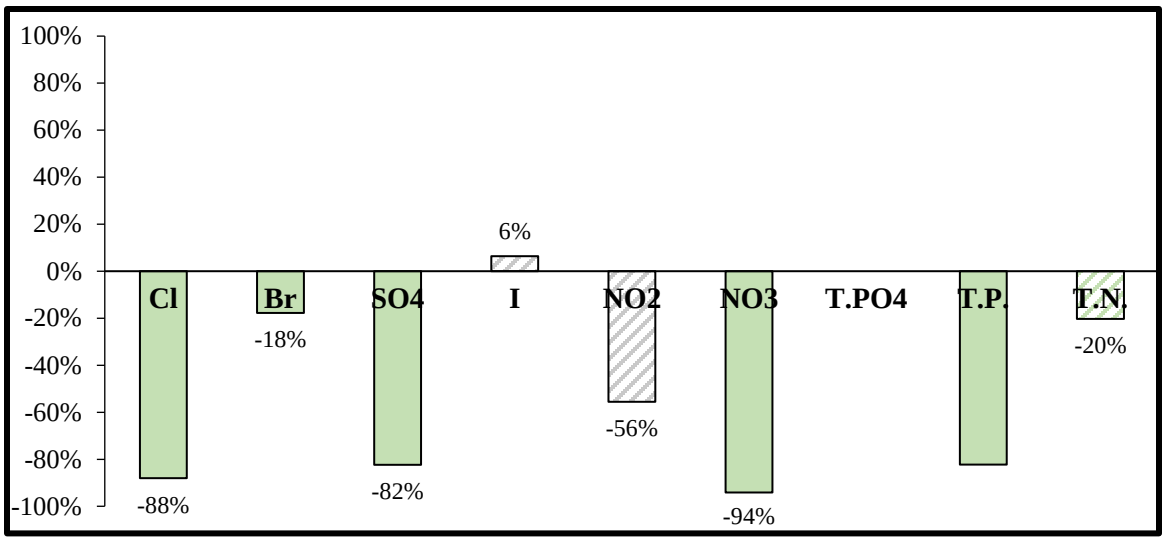


FIGURE 48. Average percent increase and decrease of nutrients and anions (via dilution); CW 1 and CW 3 and (via environmental processes); CW 2 and CW.

Sulfate is one of the most abundant compounds found on earth, naturally occurring sulfate is the result of the oxidation of elemental or organic sulfur, and sulfide minerals. However, the anthropogenic activities are the main contributor to the high occurrence of sulfate in both municipal and industrial wastewater, such as the burning fossil fuels, the use of detergents, tannery chemicals, industrial use in steel mills, pulp mills, textile plants, and the manufacturing of fertilizers.[146-148] Comparable to chloride's fate in aquatic environments, sulfate is very stable in freshwater and is not known to spontaneously reduce under typical environmental conditions. Formation of soluble sulfate salts can occur with low molecular weight alkaline metals such as potassium, sodium, and magnesium.[149, 150] Uncharged sulfate salts such as sodium sulfate and potassium sulfate does not have a strong affinity towards adsorption but are more likely to be transported throughout the environment and are not expected to bioaccumulate. In oceans and brackish waters however, reduction of sulfate does occur from sulfate-reducing obligate anaerobes under saline conditions. [151] In this study, the sulfate levels in

McDowell Creek's wastewater effluent at 24 mg/L measured well below EPA's secondary drinking water standard of 250 mg/L. Furthermore, with an average of 5 mg/L, the sulfate levels in MIL were below the average concentration measured in urban, agricultural, and mixed surface water samples as reported by the EPA as well.[152] Although sulfate salts are often added to distilled water for taste enhancement, optimum concentrations at 270 and 90 mg/L for calcium and magnesium sulfate respectively, elevated levels and anaerobic conditions can produce an unpleasant taste and smell that can be quite offensive to consumers.[153] The taste threshold for sulfate salts in drinking water differs depending on the metal ion; for sodium sulfate, it is between 250–500 mg/L; calcium sulfate at 250–1000 mg/L, and 400–600 mg/L for magnesium sulfate.[154] The presence of sulfate in drinking water does not pose a major health threat to humans, but in excess, it can have a laxative effect. Consequently, sulfate salts are often listed as the active ingredient in over-the-counter laxatives to increase the water content in the intestines via osmotic pressure. Consumers have reported experiencing cathartic effects at concentrations exceeding 600 mg/L, with dehydration as a result of prolonged exposure.[155] Therefore, although not a life-threatening substance, it can still cause some quite unpleasant acute gastrointestinal symptoms. As a part of their air scrubbing process, the power plants upstream on Lake Norman generates flue gas wastewater with a high sulfur content. However, it seems to have little impact on MIL water quality. Therefore, the main attenuation pathway for sulfate in MIL likely due to dilution.

In recent years, Lake Norman has seen outbreaks of non-toxic algae blooms, during which as part of the eutrophication process, the photosynthetic organisms deplete the lake of excess sulfate, nitrate, and phosphate contaminants. Biodegradation aside,

dilution, again is the most likely the key mechanisms in reducing the nutrients and anions in MIL. This conclusion is supported by the wet weather and dry weather analysis in this study, whereby the total phosphate concentration increased during low flow. For bio-uptake, in established waterways, the checks-and-balances sort of relationship between the microbial population and available nutrients results in a delicate homeostatic equilibrium whereby the uptake of nutrients is proportional to their availability, hence, algae blooms occur.[156, 157] Therefore, if biodegradation was the main mechanism of attenuation then the concentration of nutrients should remain relatively stable regardless of water level or flow. Lowered volume of water in MIL during low flow events provided less solvent for the nutrient solutes, resulting in higher concentrations. Furthermore, stormwater run-offs during rain, or wet weather events will typically introduce non-point source pollutants to the river system. However, in this case, the opposite occurred, meaning the source of pollution is likely from specific point-source discharges not affected by environmental factors. An average of 7.0% increase was observed for nitrite between the effluent discharge and drinking water intake, and an average of 7.9% increase was measured between CW 2 and CW 3. Although the concentration increased, it was still lower than the average occurrence of nitrite around the globe in various lake and river systems.[t] However, because the phosphate and nitrite concentration were very low, close to the BDL, the difference seen between the samples may lack some accuracy, inherent from working with such low concentrations.

[t] Average global occurrence of nitrite in lakes and rivers: 0.02 - 0.03 mg/L NO_2 – N, with lake systems measuring >0.50 mg/L NO_2 – N. 158. USEPA *Contaminant occurrence support document for category 2 contaminants for the second six-year review of National Primary Drinking Water Regulations*; United States Environmental Protection Agency, Office of Water Washington D.C., 2009.

A significant reduction of nitrate, an average of 94.6%, occurred between the wastewater effluent samples and at the drinking water intake. A non-significant decrease of nitrate at 16.8% was observed between mid-buffer and the drinking water intake.

In plants and animals, nitrite and nitrates are formed endogenously from metabolic processes. As part of the nitrogen cycle, the oxidation, or nitrification, of ammonium to nitrate is a two step-process whereby toxic nitrogen compounds are removed from the environment via microbial or plant metabolic processes. The effectiveness of nitrification in lakes is highly dependent on environmental factors such as pH, dissolved oxygen, temperature, substrate, and bacterial density. The biggest impact on nitrification is aquatic pH; an imbalance of pH can cause disruptions to bacterial homeostatic equilibrium thus reducing microbial metabolic activity; also, pH affects the acid-base equilibrium of nitrogen species, NO_2^-/HNO_2 and NH_4^-/NH_3, and depending on the species present, there may be less substrate available for bacterial uptake. [159, 160] Although, under typical environmental conditions, nitrite and nitrate exist as deprotonated ionic form with pK_a values of -1.3 and 3.4 respectively and would be available for bio-uptake. [161] Additionally, the optimum temperature for the nitrifiers is between 30 °C and 40 °C, which is consistent with this study's time of sample collection. [162, 163] The 57.1% increase in total suspended solids (TSS) between CW 1 and CW 3 should not have affected nitrifier's metabolic activities, but should have enhanced it equally. [164] Lastly, adsorption is not likely the primary fate of nitrate in MIL since nitrate compounds do not easily adsorb onto soil particles and have high mobility potential within the aquatic system. [165]

Typically, ammonium oxidation is the rate-limiting step in the nitrification portion of the nitrogen cycle. Since ammonium is the "primary producer" nutrient equivalent for the nitrification chain, the amount of available ammonia can limit the population of subsequent species of nitrifying bacteria. However, there are instances where nitrite is the rate-limiting nutrient in the chain of nitrification, and this usually occurs concurrently to the accumulating of nitrite in the environment. This may or may not be applicable to the re-contamination of nitrite in this study as the temperature and pH ranges during sample collection at MIL should not have inhibited any biological nitrite oxidation activity for Nitrosomonas.

The nitrite and nitrate both were attenuated with nitrate showing significant reduction. One study showed that the absence or overabundance of ammonia can inhibit nitrite oxidation activity, that is because the population of *Nitrobacter* was found to be highly dependent on Nitrosomonas population density; 1/3 reduction in *Nitrobacter* activity was observed at $1/10^{th}$ of Nitrosomonas density.[165] Whereas on the other hand, Nitrosomonas activity was not affected the population ratio between the two nitrifiers. There may exist some type of enzymatic commensalism or communication via biochemical-energy-transfer between the two genera, but more research will be necessary for a more confident conclusion. [165] Therefore, the increase in nitrite concentration at MIL can be because of a reduced population of Nitrosomonas present in the lake system, however, if this is accurate, the amount of nitrite oxidation, or nitrate concentration, should have increased as well.

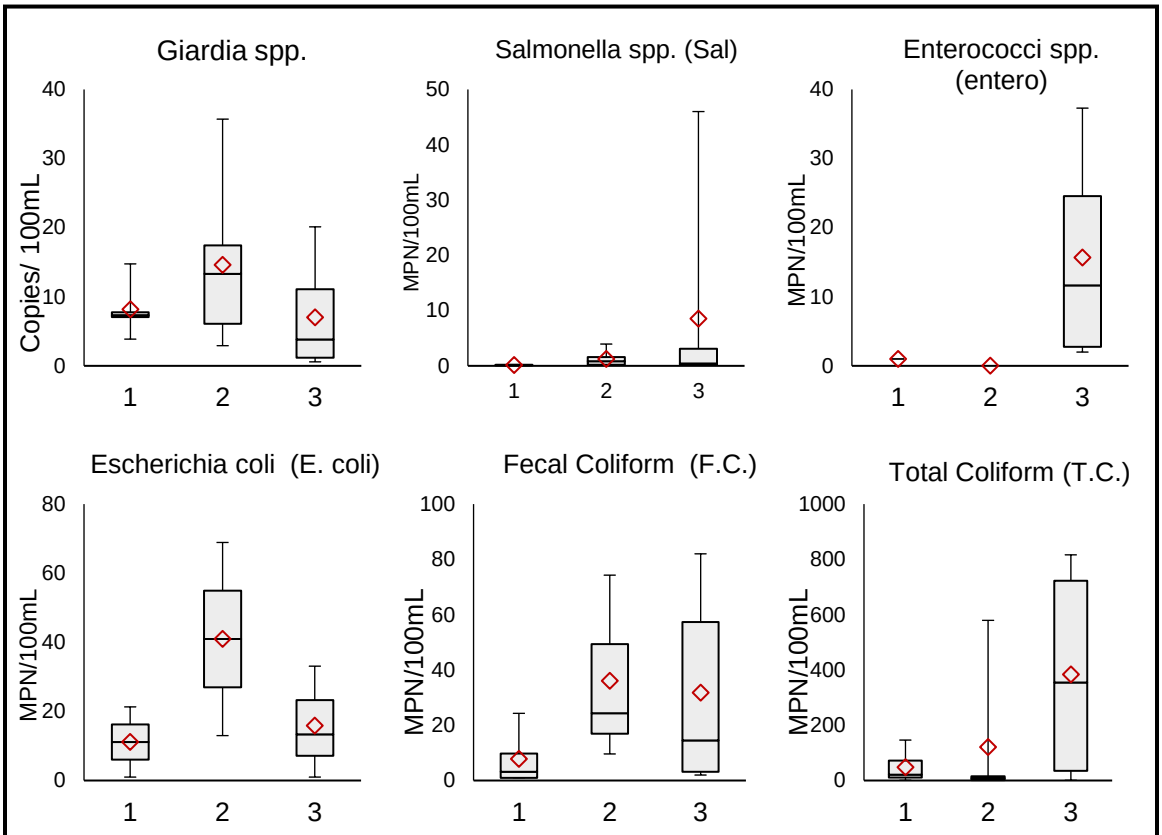
Iodide concentration from MDC WWTP was significantly reduced when compared to the drinking water intake. Interestingly, no iodide was detected from mid-

buffer (CW 2), and no subsequent recontamination occurred as the water migrated towards the drinking water intake (CW 3). Although the formation of iodinated disinfection by-products is a concern for general water treatment and reuse, studies have shown that the extent of iodine substitution in DBPs when compared to other halogens, such as bromide and chloride, of the identical concentrations is substantially lower. That is because iodide is more readily oxidized to in water treatment processes, especially those with ozone pretreatments that selectively oxidizes iodides before disinfection. [166] [167] Although FWTP does not utilize ozone pretreatment, it does however, filters the water through powered activated carbon, reducing the amount of natural organic matter, which also mitigates DBP formation.[134] In spite of iodide levels, the formation of iodinated trihalomethane (THM), haloacetic acids (HAA), and total organic halogens in the presence of chlorine is less of a concern when compared to other halogenated by-products only because it is currently unregulated.

3.4.4 Microorganisms

McDowell Creek WWTP disinfects the effluent with UV prior to discharge. The results in this study showed the reintroduction of aquatic pathogens, >50% recontamination in all microbial categories as shown in Figures 49 and 50. No wastewater treatment facilities lie upstream of MIL and no major animal husbandry farms are located in the surrounding area; with the exception of a wildlife raptor sanctuary located in the Latta Plantation and a few small recreational farms housing a few goats and horses. Although a less likely candidate unless receiving leachate directly, a potential source of pollution to the buffer may be from the Lake Norman Landfill that is located less than 2 miles from the Lake Norman outflow into MIL.[168] *E. coli* and fecal coliform

saw an average of 53.1% and 75.3% respective increase between the CW 1 and CW 3 indicating human and wildlife contamination. However, since the counts are still relatively low, it could also be a result of regrowth of these organisms in the lake once they are introduced into the system from the wastewater effluent. Since MIL is flanked by privately owned non-residential land, the source of contamination points to Lake Norman, located upstream of MIL, which is surrounded by residential areas and is abundant with recreational activities.



Maximum concentration for total coliform, fecal coliform, E. coli, and Enterococci spp. is reported up to 2419.6 MPN/100mL.

FIGURE 49. Comparison of microorganisms for MDC WWTP effluent, MIL, and FWTP influent.

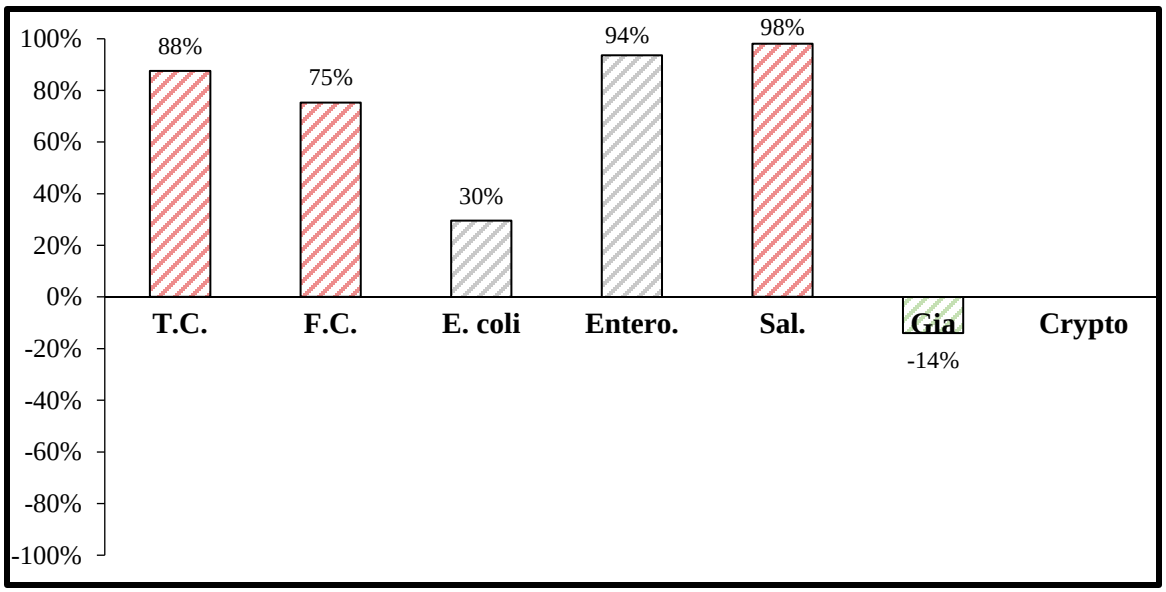


FIGURE 50. Average percent increase and decrease of microbes (via dilution); Mountain Island Lake (CW 1 and CW 3).

The indictor organisms, *Enterococci spp.* and *Salmonella spp*., both saw >98% recontamination between the effluent discharge and drinking water intake. The two pathogens are used as proxies to monitors specific human and wildlife fecal pollutions with *Enterococci spp.* more commonly used in salt water monitoring as it can withstand high salt concentrations. [169] In this instance, the previously mentioned *Enterococci spp.* and *Salmonella spp* indicators saw proportional levels of recontamination at an average of 98.4% and 98.1% respectively, since the average recontamination of other non-specific indicator organisms such as total coliform are lower than that of the human/mammalian specific indicators (*Salmonella spp* and *Enterococci spp.*), it can assumed that most of the aquatic pathogens may have originated from human or wildlife metabolic activity and not from industrial processes.[170, 171] One particular peculiarity was that *Enterococci spp.* measured complete attenuation mid-buffer (CW 2) but was reintroduced en route to the drinking water intake. As stated before, the areas immediately surrounding CW 2 are privately owned and undeveloped land with little to no human impact, whereas the area

downstream of CW 2 encompassed residential areas and also the Historic Latta Plantation with forested areas containing local wildlife, which may have contributed to the increased *Enterococci spp.* and *Salmonella spp.* count. [172]

3.4.5 Aggregate Water Quality Assessment

The significant increase in suspended solids measured in MIL may be a result of the turbulent nature of lakes and rivers which does not allow for settling, unlike wetlands, see Figures 51 and 52 for concentrations. Dilution is probably the primary process for the reduction in conductivity, alkalinity, COD, and total organic carbon. The pattern of contaminant attenuation is similar to what was described in section 3.1.1. whereby CW 1 and CW 3 showed significant attenuation, but CW 2 and CW 3 measured little change.

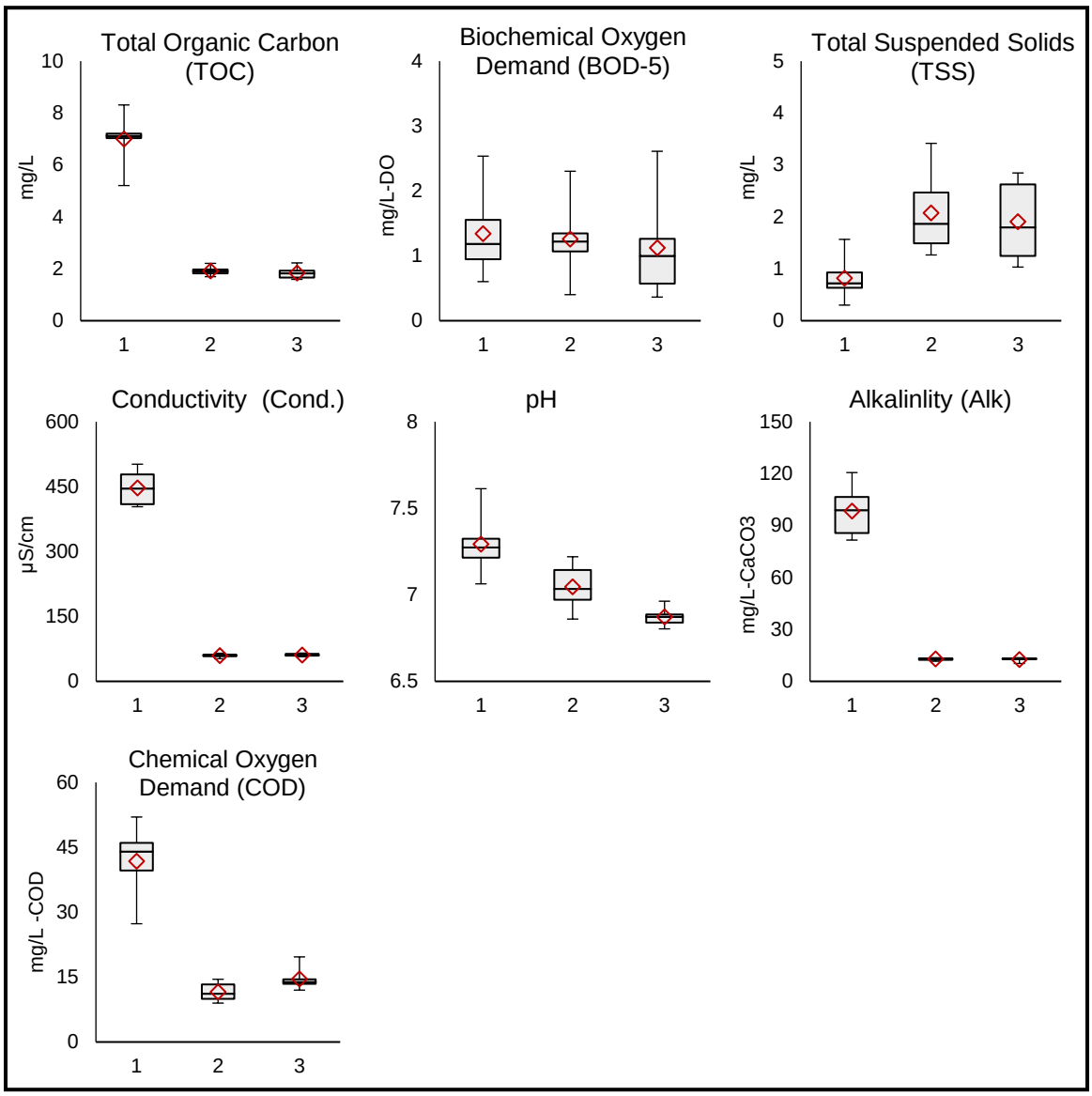


FIGURE 51. Comparison of water quality parameters for MDC WWTP effluent, MIL, and FWTP influent.

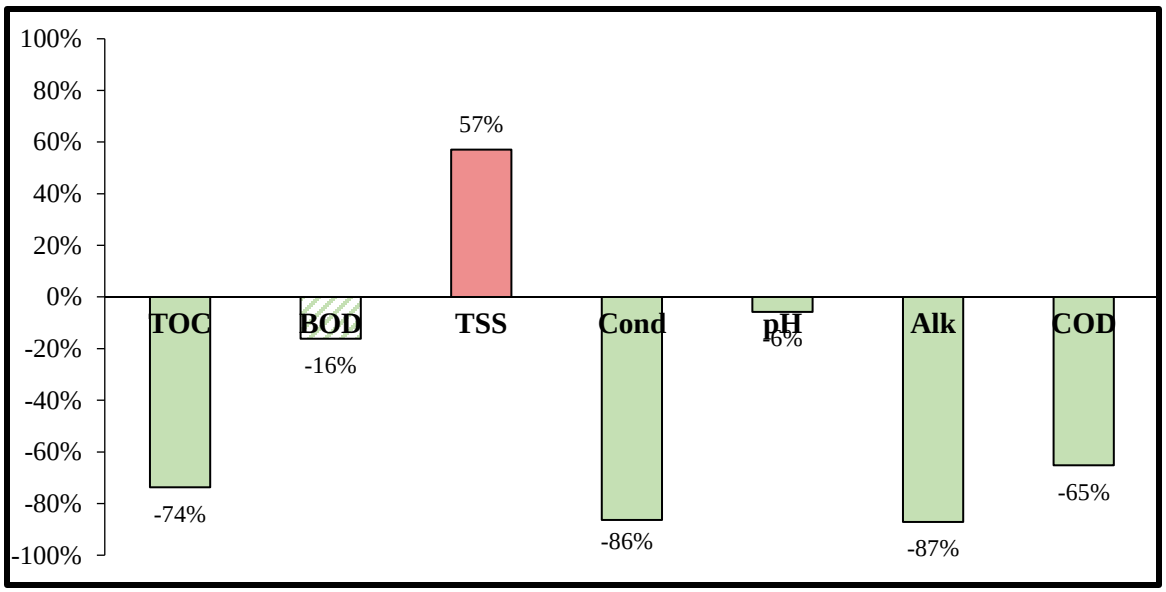


FIGURE 52. Average percent increase and decrease of aggregate water quality parameters (via dilution); Mountain Island Lake (CW 1 and CW 3).

3.4.6 Emerging Contaminants of Concern

Sucralose and azithromycin were significantly attenuated post buffer treatment as shown in Figures 53 and 54. This indicates the occurrence of attenuation via dilution and adsorption processes. However, because the increase of those two compounds was seen between CW 2 and CW 3 and the concentration of these compounds measured at CW 3 were very close to the detection limit, therefore, instrumental error may cause some inaccuracy in the results given.

The reduction of sucralose is representative of dilution in MIL, as the compound is immune to other degradation processes and is often used in environmental tracer studies because it is not readily degradable via physical, chemical, or biological means in moving surface waters.[173, 174] Although not statistically significant, ibuprofen still saw >60% reduction between CW 1 and CW 3, but an slight increase of 22.3% between CW 2 and CW 3 samples. Although in this study dilution is likely the primary attenuation

process for the reduction of emerging contaminants; most pharmaceuticals are removed from lakes and rivers via adsorption onto benthic soil or deposition onto riparian zones.[175, 176] However, those processes are more effective in slow moving waters, such as wetlands, that allows for efficient settling. Compared to the attenuation of stubborn compounds like sucralose with an average decrease of 98%, the occurrence of other degradation pathways is less apparent when compared to dilution. Ciprofloxacin, and atrazine showed recontamination between CW 1 and CW 3, however, due to insufficient sample size from QA/QC procedures, no statistical analysis could be conducted often with only 1 data point in CW 3 for comparison for ciprofloxacin and all values were below detection limits at CW 1 for atrazine. At CW 2, carbamazepine, sulfamethoxazole, amoxicillin, ciprofloxacin, and cephalexin were below detection limits and were re-introduced to the system between CW 2 and CW 3, however, only one measurement of carbamazepine, sulfamethoxazole, and cephalexin were detected in CW 3. No wastewater treatment facilities or animal husbandries lay between CW 2 and CW 3 so the most likely a source of recontamination may be from non-point source pollution. However, this contradicts the results between high and low flow events, which showed no significance, suggesting minimal contamination from stormwater run-offs.

The concentration of carbamazepine was higher at the drinking water intake when compared to the wastewater effluent, however, between mid-buffer and the drinking water intake, carbamazepine saw 97.8% reduction. In river and lake systems, carbamazepine is highly persistent due to its structural and chemical stability in water. Under typical environmental conditions, with a high pK_a of 13.9, carbamazepine exists as

a noncharged compound with only moderate affinity for adsorption, especially in fast moving water columns (log K_{ow} = 2.25). [177]

Doxycycline saw an average of 82% reduction seen between CW 1 and CW 3, lower than that of sucralose, which indicates dilution as the primary attenuation process as sucralose is mostly resistant to environmental degradation. In aquatic environments with other attenuation processes, tetracyclines are sparingly soluble in environmental pH conditions and exist primarily as a cationic, or zwitterion, species. They have been observed to form complexes with chelating ions and adsorbs onto proteins and silanol groups.[178-180]

The concentration of sulfamethoxazole and trimethoprim in aquatic environments should, theoretically, be proportional to each other as are commonly prescribed together and are resistant to most environmental degradation processes. However, from CW 1 to CW 3, sulfamethoxazole saw an average decreased of 2% while trimethoprim increase was below detection limits. If trimethoprim was detected, it would likely be at higher concentration than sulfamethoxazole as trimethoprim is rather resilient in the environment. Studies have found that trimethoprim is highly resistant to hydrolysis, photodegradation, and biodegradation in natural aquatic environments.[181-183] On the other hand, a study found that sulfamethoxazole can be affected by photodegradation in river systems, but only if the process transpires simultaneously with biodegradation.[184]

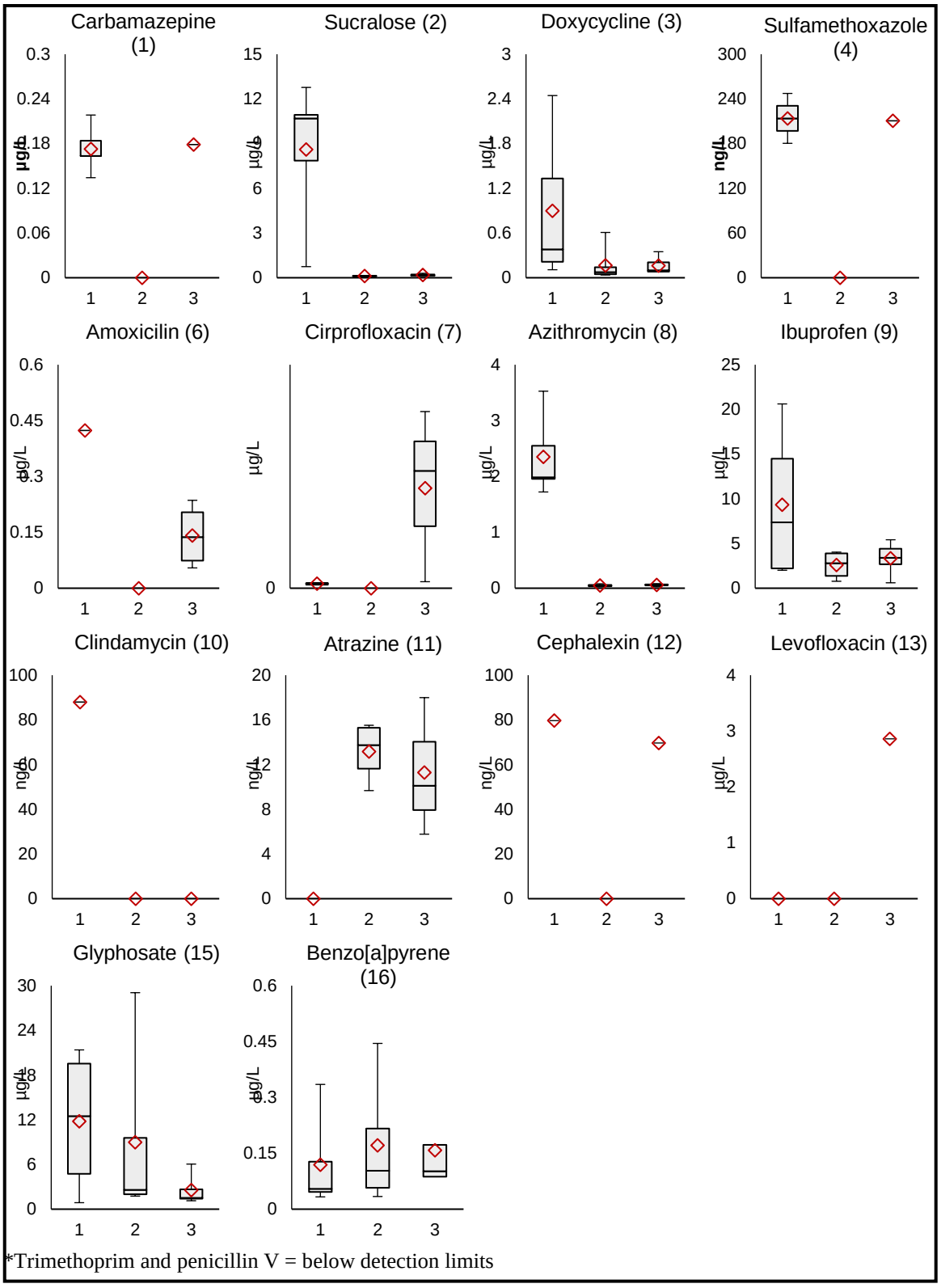


FIGURE 53. Comparison of CEC concentrations for MDC WWTP effluent and FWTP influent (CW 1 and CW 3).

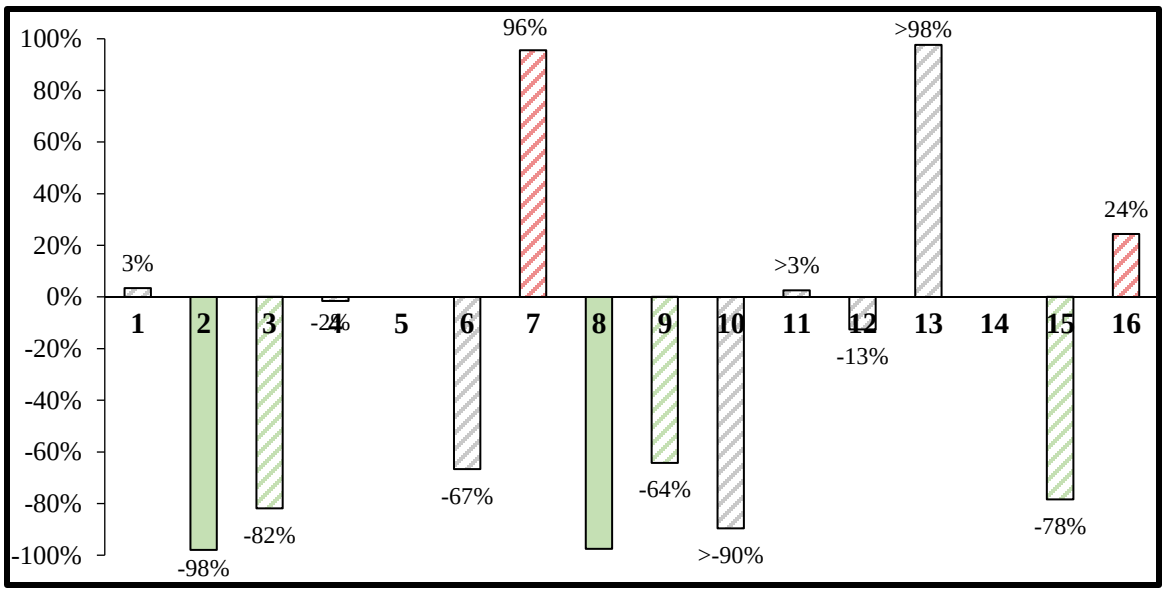


FIGURE 54. Average percent increase and decrease of CECs (via dilution); Mountain Island Lake (CW 1 and CW 3).
(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

Unlike the aforementioned pharmaceuticals, β-lactams (amoxicillin, penicillin, and cephalexin) is the only group susceptible to environmental processes beyond just dilution. The 4-member cycloalkane ring structure is a hallmark of β-lactams. The unstable bond strains are easily hydrolyzed in aquatic conditions.[185]

Although not statistically significant, glyphosate increased an average of 24.4% as the water traversed from the WWTP to the lake (CW 1 and CW 2), but decreased from MIL, CW2, to the drinking water intake. The source of glyphosate contamination may have originated from local developments near CW 2. The west bank of MIL (CW 2) is flanked by land devoted to Duke energy's power transmission systems and are exclusively for power lines. According to the McGuire Nuclear Station Environmental Report, Duke has a right-of-way vegetation management program that integrates mechanical and chemical vegetation clearance methods to minimize the growth of trees

and other vegetation around transmission lines. The McGuire report also states that low-lying marshy areas near the transmission systems are regularly treated with Accord®, the active ingredient being glyphosate, to prevent vegetative obstruction.[186] This study found glyphosate concentration to be the highest in MIL, CW 2, the very same location Duke runs its powerlines through. Therefore, the use of herbicides for vegetation control may be the main contributor to the increased glyphosate concentration measured in this study.

Benzo[a]pyrene (BaP) is a byproduct of incomplete combustion of carbon materials. Lake Norman is a popular recreational area such as boating and fishing, most of which uses gasoline powered aquatic vehicles. Also, the coal burning steam stations in Lake Norman also probably contributed to the contamination, especially with coal ash leakage. [187, 188] All of which likely contributed to the 24% increase in BaP measured in MIL.

Although BaP is quite persistent in the environment, attenuation may result from bioaccumulation in addition to dilution. In water, BaP less polar and much more lipophilic than most other anthropogenic contaminants due to its polyaromatic hydrocarbon (PAH) structure. The compound has a tendency to bioaccumulate in rivers and streams with abundant aquatic wildlife that may not be present in wetlands or groundwater. In one study, researchers measured the amount of BaP parent compounds in various species of macroinvertebrates: mosquito larva were found to bioaccumulate 46% of the total parent compound and daphnia at 90%. Since macroinvertebrates are a major source of food for other aquatic insects and animals, BaP can eventually bioaccumulates up the food chain; with biomagnification factors fish at 930; algae, 5258;

mosquito, 11,536; snail, 82,231; and daphnia, 134,248.[u] [189] Through the uptake process, BaP is then degraded via in-vivo hydroxylation and conjugation producing polar BaP derivatives. [190, 191] As a bonus, the polar metabolic byproducts can be removed via adsorption or deposition onto soil columns.[191] With respect to dilution, the elevated BaP concentration from Lake Norman may have be mediated the less trafficked MIL. Since both lakes are constantly stocked for fishing purposes, the abundance of macroinvertebrates and their predators in the less trafficked MIL could have contributed to the attenuation of BaP discharged by Lake Norman upstream once it reached the drinking water intake. Despite that, as Table 29 shows, BaP levels are still well below what could occur in average surface water levels found around the world.

TABLE 29. The average global occurrence* of pharmaceuticals in surface water receiving effluent and lakes compared to Mountain Island Lake. [192 113-120]

Emerging Contaminants	**Surface Water Concentration (µg/L)**	**Lakes (µg/L)**	**MIL, CW 1 (µg/L)**	**MIL, CW 3 (µg/L)**
Carbamazepine	<0.001 – 7.1,		BDL-0.218	BDL-0.179
Sucralose	0.12 – 15.0		BDL-12.775	BDL-0.491
Doxycycline	BDL – 0.08	BDL – 0.947	BDL-2.446	0.079-0.626
Sulfamethoxazole	BDL – 1.9	BDL – 1.2	BDL-0.155	BDL-0.175
Trimethoprim	BDL – 0.71	BDL – 0.135	BDL-0.004	BDL-0.005
Amoxicillin	0.025 – 2.2	BDL – 0.004	BDL-0.423	BDL-0.194
Ciprofloxacin	BDL – 0.03	BDL – 0.822	BDL-0.124	BDL-0.146
Azithromycin	BDL – 1.62		BDL-3.526	0.026-0.086
Ibuprofen	0.0002 – 5.044		BDL-20.616	0.606-5.421
Clindamycin	BDL – 0.085	BDL – 0.503	BDL-0.056	BDL-0.051
Atrazine	BDL – 0.058, 201.1**		BDL-0.009	0.003-0.018
Cephalexin	BDL – 0.1		BDL-0.080	BDL-0.062
Levofloxacin	0.0062 – 0.0593		BDL	BDL
Penicillin V	BDL		BDL	BDL
Glyphosate	BDL – 1.90		BDL-21.381	1.137-6.051
Benzo[a]pyrene	BDL – 26.0		BDL-0.335	BDL-0.340

Significant Decrease
Significant Increase

*Australia, Canada, China, Czech Republic, Germany, Italy, Sweden, United States, United Kingdom, Brazil, Norway, and Spain. **Heavy agriculture impacted areas BDL = Below Detection Limit

[u] biomagnification factor = C_B / C_A
C_B = [contaminant] in an organism
C_A = [total contaminant] in the organism's diet

3.4.7 Antibiotic Resistance Genes (ARG)

There was significant decrease of some of the ARGs upon discharge of MDC effluent into the lake water as shown in Figure 55 and 56. In general, microbial 16S rRNA was much lower in the lake water indicating lower abundance of microbial life in general. On the other hand, some of the ARGs remained relatively the same or increased. It is impossible to conclusively assess the value of the lake environment for attenuation of ARGs from the results of this case study. No significant or consistent decrease or proliferation of ARGs was found between points CW2 and CW3.

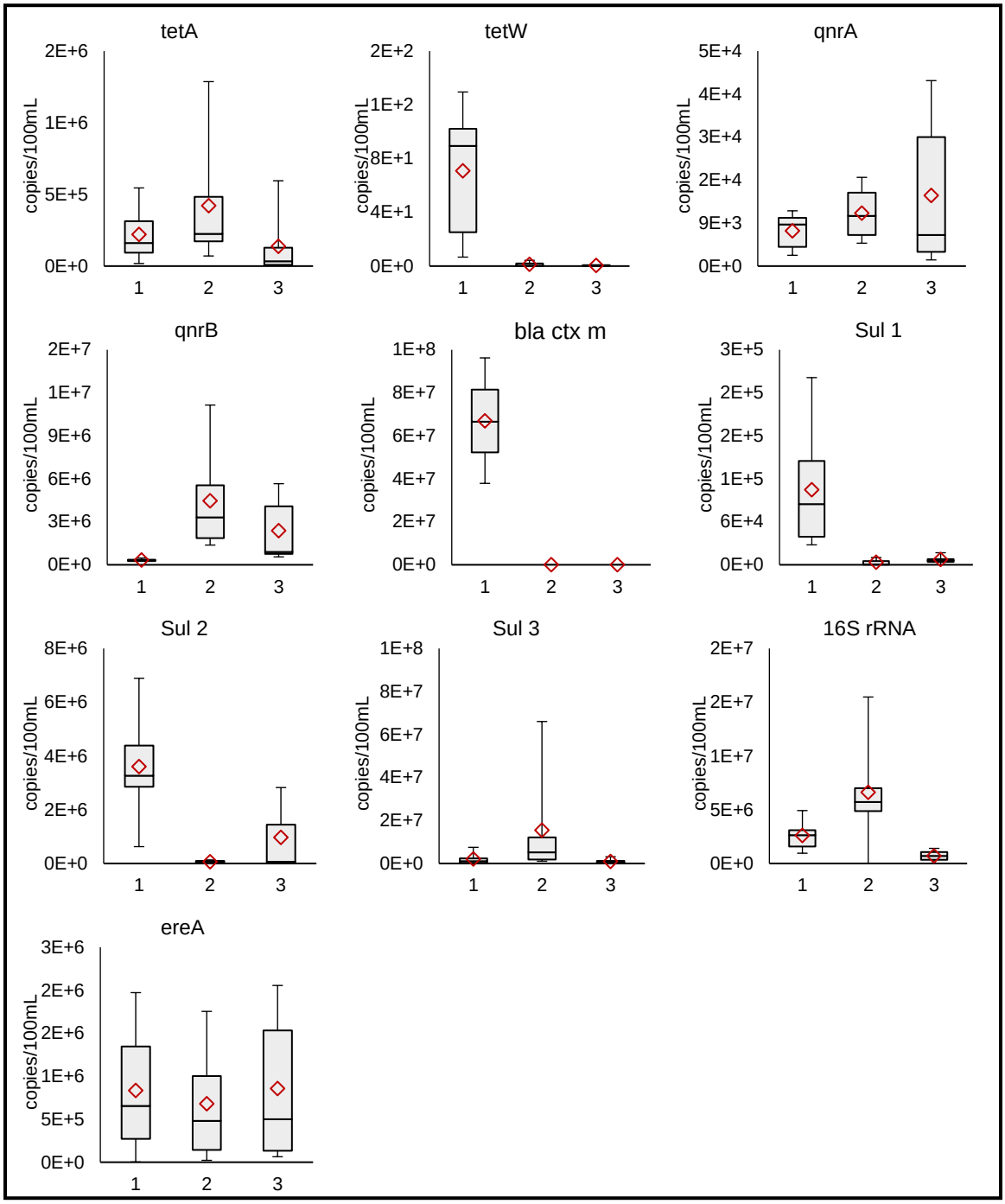


FIGURE 55. Comparison of ARG concentrations for MDC WWTP effluent and FWTP influent (CW 1 and CW 3).

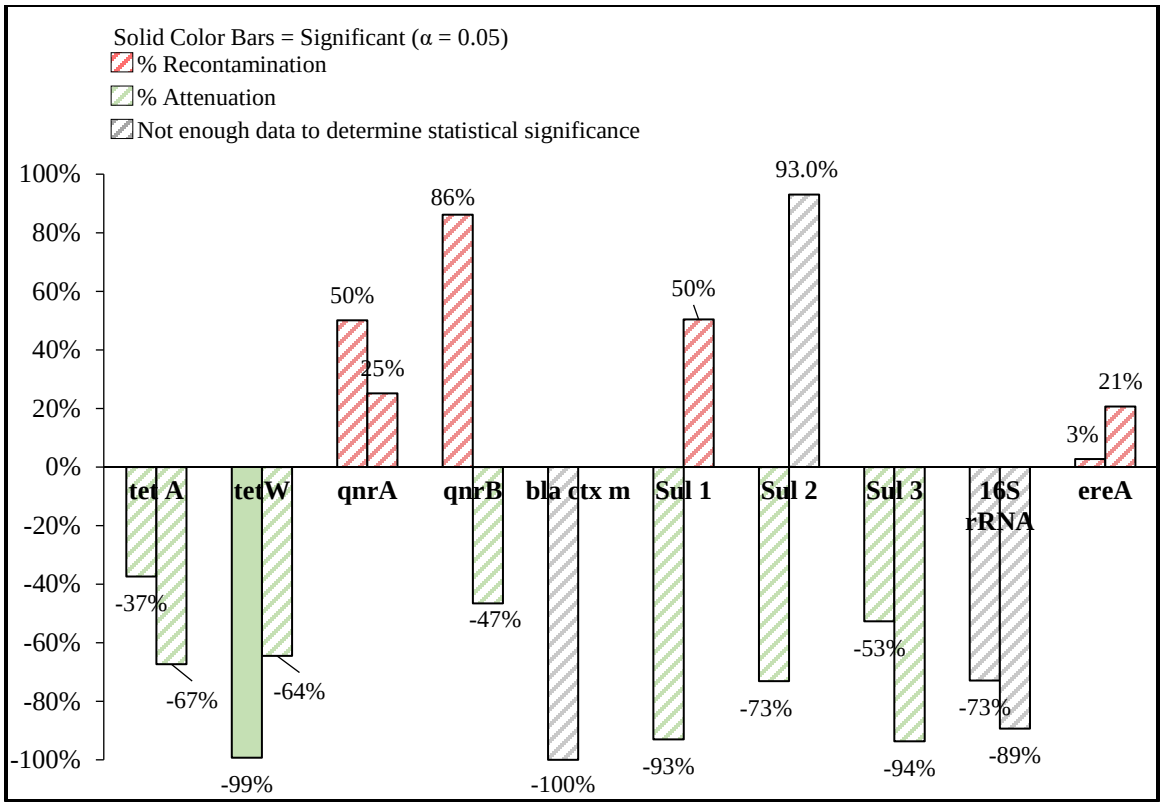


FIGURE 56. Average percent decrease or increase of ARGs in CW samples.

3.4.8 Cost Analysis

The City of Charlotte consumer confidence report in 2017 provided a list of contaminants and their MCL and MCLG. All regulated contaminant concentrations are in compliance with the mandatory health standards published by the United States Environmental Protection Agency (USEPA).

Mountain Island Lake significantly reduced much of the constituents from the WW discharge. However, the contaminant concentration in the WW effluent was under the EPA drinking water standards for metals, anions, and nutrients as shown in Table 30. Therefore, no additional buffer treatment was necessary for the removal of those contaminants from the WW discharge.

However, the microbial load, coliforms, *E. coli*, and *Salmonella spp* measured above the EPA regulatory limit. Additionally, the increase in TSS will need to be addressed as well. This means no alteration to FDWP's current treatment processes: powdered activated carbon (PAC) pre-treatment, coagulation, flocculation, sedimentation, and filtration will all be necessary for the removal of TSS, including TOC and some microbials, from the source water to prevent DBP formation and to meet secondary drinking water standards such as odor and taste. Chlorine disinfection will be necessary to kill/inactivate aquatic pathogens as well as to provide residual disinfection during the distribution process. [134] By bypassing the environmental buffer, the DWTP can potentially save costs on TSS removal, by either reducing or eliminating the maintenance, energy, and chemicals necessary to perform coagulation/flocculation processes. Additionally, the dilution process can also reduce a number of unregulated contaminants and can be considered a source of non-tangible benefit. However, if only regulated parameters are considered, switching to direct potable reuse may not be worth the infrastructure cost when only one contaminant, TSS in this instance, needs to be addressed.

TABLE 30. EPA vs Mountain Island Lake [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (MIL CW1)	Effluent (MIL CW3)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.00 ± 0.00	0.00 ± 0.00
Iron (Fe) [1]	ppm	0.3	NS	0.057 ± 0.01	0.072 ± 0.088
Boron (B) [1]	ppm	NS	NS	0.23 ± 0.05	0.059 ± 0.009
Calcium (Ca) [1]	ppm	NS	NS	44.76 ± 5.45	3.813± 0.79
Magnesium (Mg) [1]	ppm	NS	NS	3.37 ± 0.33	2.04 ± 0.10
Manganese (Mn)	ppm	0.05	NS	0.01 ± 0.00	0.035 ± 0.031
Sodium (Na) [1]	ppm	NS	NS	36.12 ± 3.92	5.03 ± 0.40
Cadmium (Cd)[2]	ppb	2	0.005	0.00 ± 0.00	0.00 ± 0.00
Mercury (Hg) [2]	ppb	2	0.002	0.00 ± 0.00	0.00 ± 0.00
Lead (Pb) [1]	ppb	15	0.2	0.00 ± 0.00	0.00 ± 0.00
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	47.81 ± 6.15	5.71 ± 2.28
Bromide (Br)	ppm	NS	NS	0.117 ± 0.052	0.082 ± 0.053
Sulfate (SO_4^{2-}) [1]	ppm	500	NS	23.9 ± 6.15	4.2 ± 0.27
Iodine (I)	ppm	NS	NS	0.198 ± 0.008	0.211 ± 0
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.006 ± 0.001	0.006 ± 0.001
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	4.8 ± 0.59	0.3 ± 0.06
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.014 ± 0.005	0.012 ± 0.004
Total Phosphorous	mg/L PO_4^--P	NS	NS	0.559 ± 0.172	0.099 ± 0.025
Total Nitrogen	mg/L-N	NS	NS	6.56 ± 3.27	4.08 ± 2.35
Microorganisms					
Total Coliform[2]	MPN/100 mL	MCL[3]	0	47.6 ± 57.2	383.2 ± 382.4
Fecal Coliform[2]	MPN/100 mL	MCL[3]	0	7.84 ± 9.88	31.78 ± 35.99
Escherichia coli[2]	MPN/100 mL	MCL[3]	0	11.15 ± 14.35	15.83 ± 16.19
Enterococci[2]	MPN/100 mL	NS	NS	1 ± 0	15.65 ± 16.7
Salmonella spp. [2]	MPN/100 mL	NS	NS	0.16 ± 0.07	8.53 ± 18.41
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	6.8 ± 4.88	7.02 ± 7.9
Aggregate Water Quality					
Total Dissolved Solids[1]	ppm	500	NS	0.82 ± 0.43	1.91 ± 0.81
pH[1]		6.5 – 8.5	NS	7.29 ± 0.18	6.87 ± 0.06
Total Organic Carbon	mg/L	NS	NS	7.0 ± 1.0	1.84 ± 0.23
Biochemical Oxygen Demand	mg/L-DO	NS	NS	1.34 ± 0.69	1.13 ± 0.82
Conductivity	µS	NS	NS	447.3 ± 42.0	61.1 ± 3.2
Alkalinity	mg/L-$CaCO_3$	NS	NS	98.4 ± 15.1	12.7 ± 1.2
Chemical Oxygen Demand	mg/L -COD	NS	NS	83.1 ± 101.4	14.56 ± 2.66
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	11.8 ± 9.9	2.6 ± 2.3
Benzo[a]pyrene[2]	ppt	200	0.0	119.3 ± 0.1	157.6 ± 0.1

NS: no standard **Red: above MCL**

Green Cells: Significant Reduction Red Cells: Significant Increase

[1] Charlotte Water Annual Drinking Water Quality Report – 2017

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation." - *National Primary Drinking Water Regulations, USEPA* – 2018.

3.5 ADVANCED WATER PURIFICATION FAICLITY (OCWD 2A – 2B)

3.5.1 Orange County Groundwater Replenishment System (OCGR)

The OCSD in Fountain Valley processes municipal wastewater via preliminary screening, settling and floatable removals, then activated sludge, filtration, and clarification. The treated wastewater effluent is transferred directly to the Advanced Water Purification Facility (AWPF) at OCWD adjoining the OCSD facility. The clarified wastewater effluent undergoes advanced purification via microfiltration, RO, and UV/hydrogen peroxide AOP to produce a finished product water that is near distilled water in quality. A total of 6 samples consisting of the UV/AOP product water were collected. AWPF water production is held constant at 100 MGD. The influent into AWPF may be subject to environmental effect. Therefore 3 dry and 3 rain event samples were collected to ensure consistency. Although OCWD offers more a comprehensive database of their constituent output, this study only utilized the results obtained by the UNC-Charlotte environmental laboratory. Therefore, any variations or changes caused by instrumental or human error were applied equally to all samples.

3.5.2 Metals

The advanced purification process removed all detected metal contaminants and achieved significant removal of boron and sodium from the secondary wastewater effluent as seen in Figure 57. The RO process was highly effective in removing chemical contaminants, including metal ions. Although significant, the removal of boron, a nonmetallic element, is quite low compared to the other metal ions at 33.1% removal as seen in Figure 58.

Boron removal via RO has always been challenging, and is considerably impacted by the pH, temperature, and transmembrane pressure of the purification system. In aquatic environments, boron is commonly found in the form of boric acid, which are small, symmetric, and non-polar compounds that interact differently with membranes as compared to its larger, and more polar metal ion counterparts.[193] Margara *et al.* reported that boron rejection for polyaromatic amide membranes was generally observed at 43–78%, which is slightly higher but comparable to findings at ~33% rejection from this study.[194] Also, an increase in pH shifts the boron species to the deprotonated form, thus increasing its polarity.[195] In this study, the pH of the feedwater into AWPF is chemically adjusted to pH 6.9. Boron rejection by RO membranes is considered an important issue mainly for the desalination of seawater. It is not of major concern for freshwater systems as its occurrence is relatively low. Anthropogenic pollution may contribute to the rise in boron related contaminants via industrial uses, such as fertilizers, detergents, metallurgy, and nuclear applications.[196]

Currently, boron is an unregulated chemical with no established MCL from the USEPA. As a macronutrient, boron is vital to bone health, skin regeneration, hormone balance, cognitive functions and has anti-inflammatory properties.[68] The average daily boron intake for adults in the U.S. is roughly 1.11 ± 0.69 mg/L, which is above what was detected in the purified effluent.[197] Clinical symptoms of boron toxicity only start to occur at the 100 mg dose, which varies widely depending on the person's age and body weight. Some symptoms include: inflammation, skin irritation, and edema of body cavities.[198]

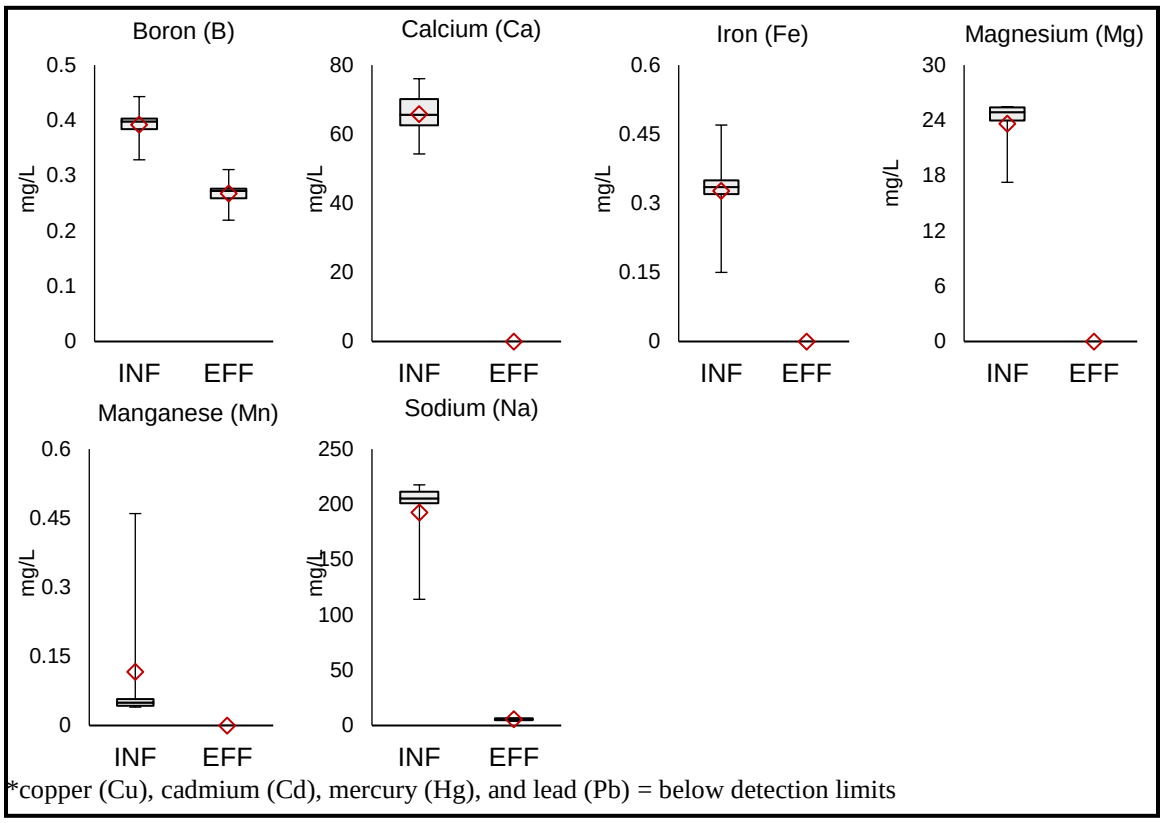


FIGURE 57. Comparison of metal concentrations for pre- and post - AWPF treatment of wastewater.

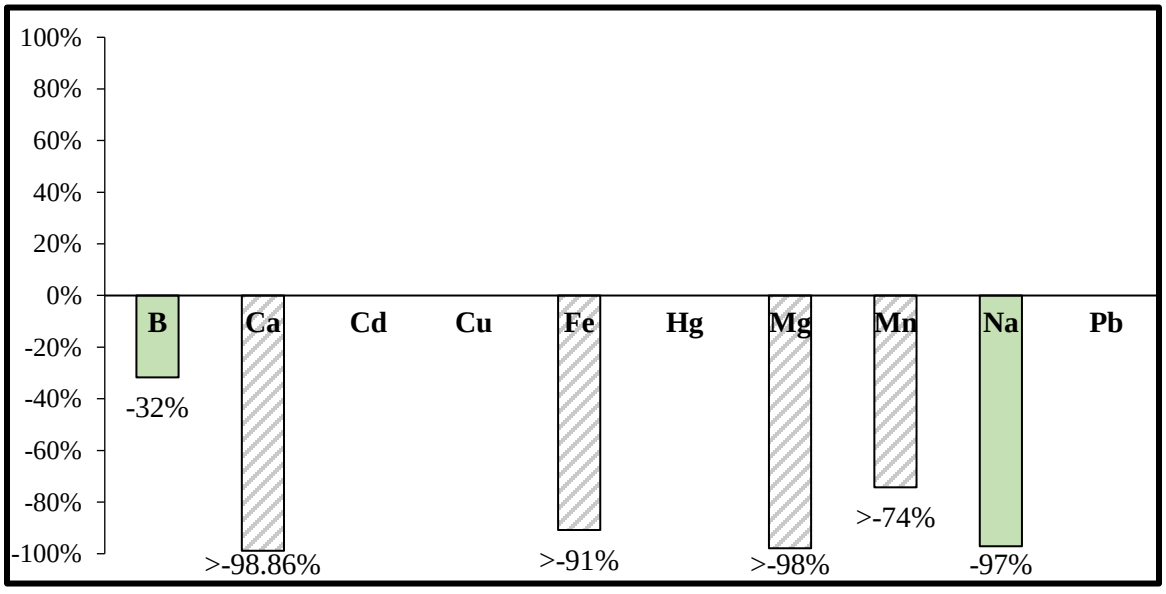


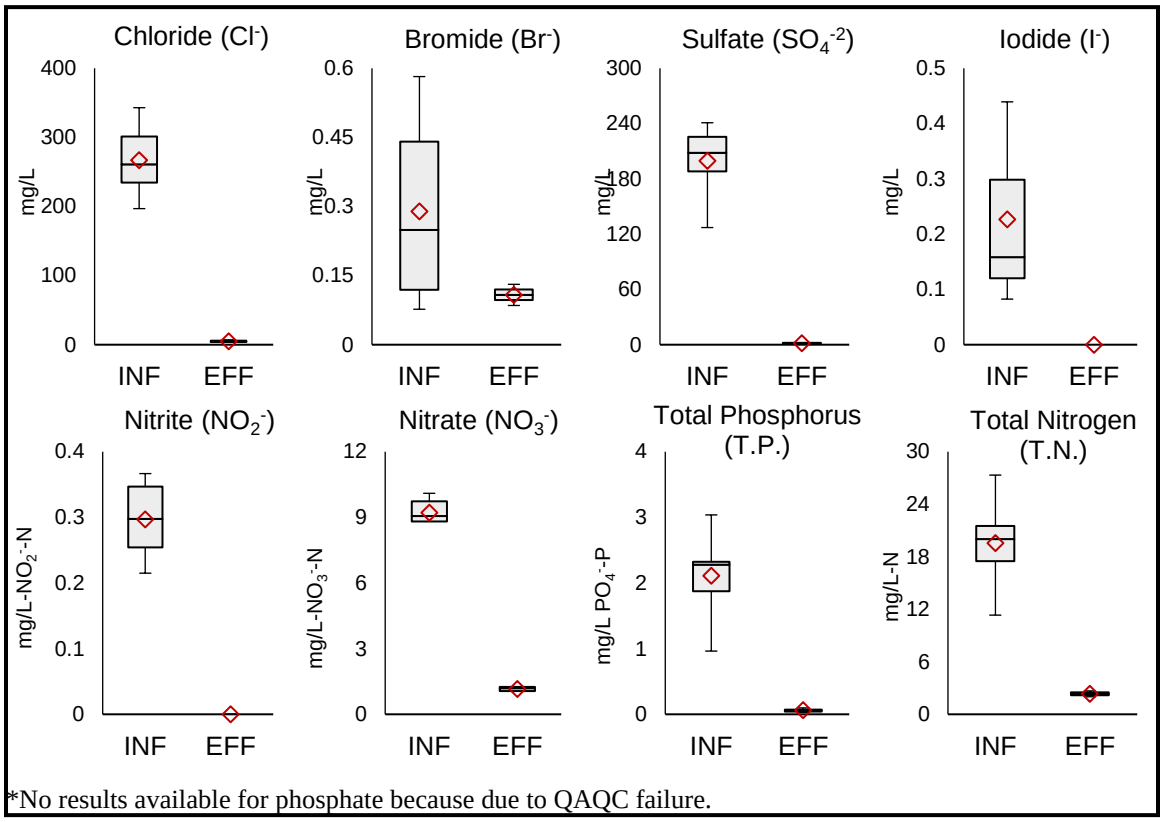
FIGURE 58. Average percent attenuation and recontamination of metal contaminants; AWPF.

There are some cost implications associated with the compete removal of metals. The primary concern being that, with such low buffering capacity and low dissolved metal concentration, there is a high potential for toxic metals leaching into the drinking water supply from corroded distribution pipes. The cost of water treatment will increase as remineralization will be required prior to distribution. The wastewater effluent saw significantly higher calcium concentration after rain events. Although not significant, the pH of the wastewater effluent was observed to be lower during rain events. This may slightly affect the formation of calcium precipitates thus increase the amount of calcium ion suspensions measured in the effluent.

3.5.3 Nutrients and Anions

Advanced purification product water quality remained consistent regardless of influent quality. However, in wastewater effluent, bromide concentrations were significantly higher in dry weather samples. Up to 99.3% of certain nutrient and anion contaminants were removed as seen in Figure 59. Traditional drinking water infrastructures often are not able to address the removal of nitrogen contaminants without the addition of expensive tertiary processes like RO or ion exchange units. As part of the advanced purification process, RO was able to significantly reduce sulfate, chloride, nitrate, and total phosphorus. In addition to drinking water treatment, advanced treatment can also be of benefit to wastewater facilities. The Clean Water Act of 1972 established wastewater discharge regulations to centralize, maintain, and restore the health of polluted waterways in the U.S. In order to meet the standards, set by the National Pollutant Discharge Elimination System (NPDES), wastewater facilities must limit the amount of contaminants entering the environment in accordance to the discharge permit.

Figure 60 compares the nutrient and anion concentrations between the OCSD effluent and AWPF final product water.



*No results available for phosphate because due to QAQC failure.

FIGURE 59. Comparison of nutrient and anion concentrations for pre- and post - AWPF treatment of wastewater.

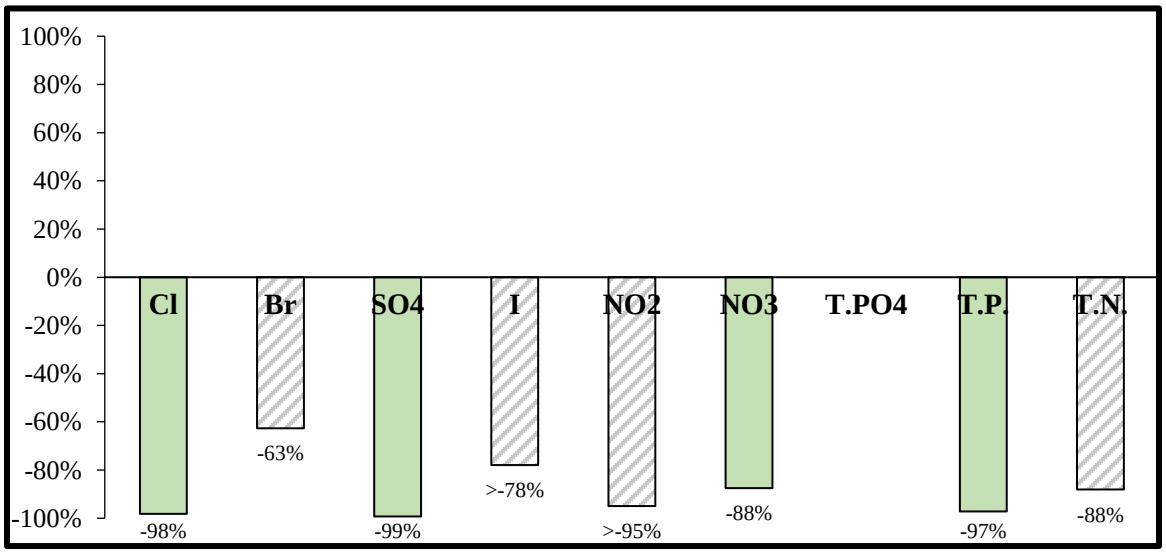


FIGURE 60. Average percent attenuation and recontamination of anion and nutrient contaminants; AWPF.

3.5.4 Microbial Contaminants

All microbial contaminants were all removed, and no significant differences were observed between dry and rain events, seen in Figures 61 and 62. As well, due to the lack of data in post purification as all microbial analytes were below detection limits, it is assumed that > 99.99% removal occurred. Although ineffective against chemical contaminants, microfiltration is effective at removing protozoan and bacteria from the wastewater effluent. The microfiltration at the AWPF operated with hollow fibers with a pore size of 0.2 microns capable of capturing bacteria, which are typically between 1–10 microns. Smaller pathogens such as viruses that pass-through microfiltration pores are subsequently retained and rejected during the RO process.

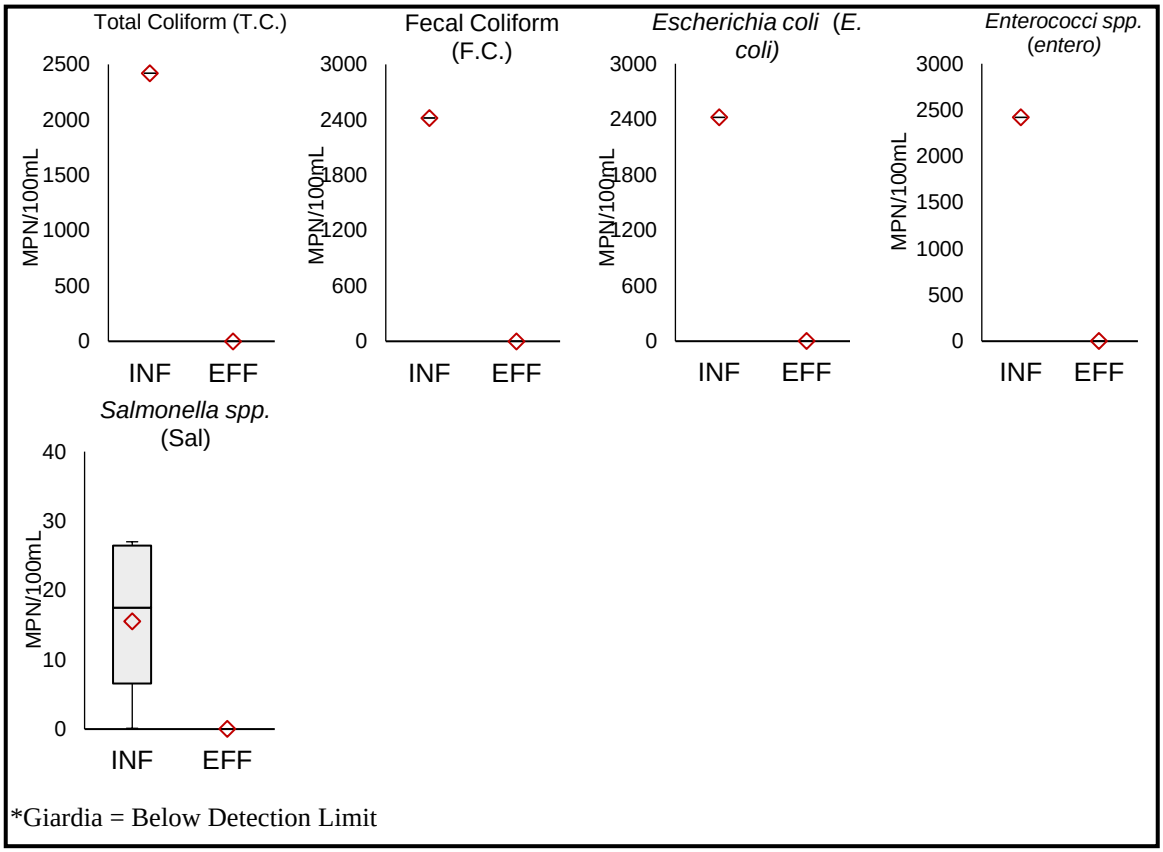


FIGURE 61. Comparison of microbial concentrations for pre- and post- AWPF treatment of wastewater.

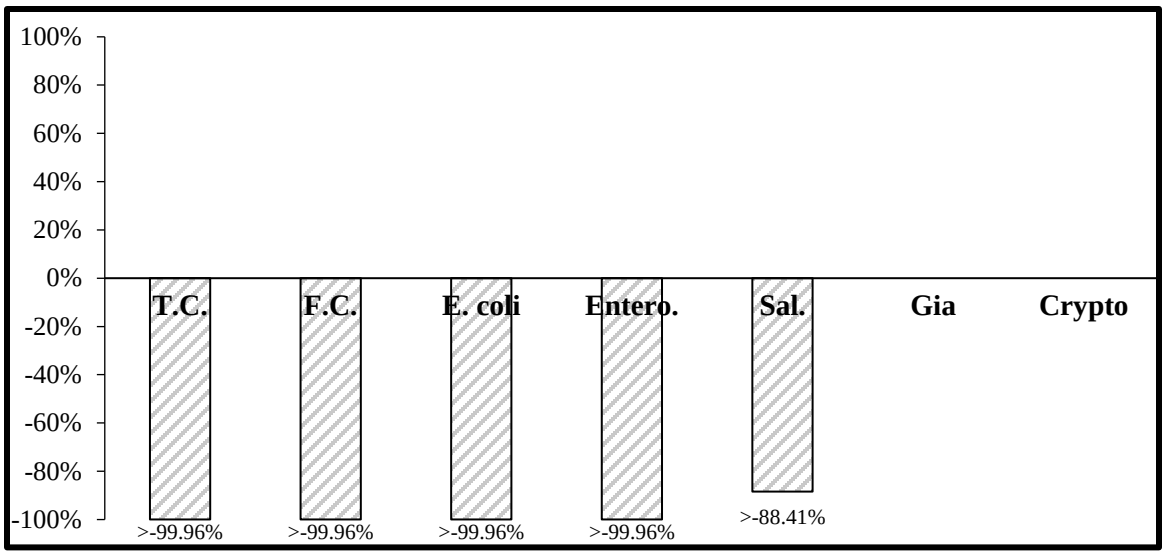


FIGURE 62. Average percent attenuation and recontamination of microbes; AWPF.

3.5.5 Aggregate Water Quality Assessment

No significant differences were observed between dry and rain events. Significant reduction was observed for all aggregate water quality parameters. The removal of suspended solids, microorganisms, and organics consequently reduced the amount of oxygen demand and organic carbon in the effluent. The RO process is capable of reducing alkalinity through demineralization by removing up to 98% of all dissolved minerals measured in this study as seen in Figures 63 and 64. This was supported by the significant reduction in conductivity observed in this study.[199]

As discussed before, due to the removal of alkalinity and dissolved ions during the RO process, dissolved minerals will need to be replenished by the treatment facility prior to distribution, which adds to the cost of treatment. As another consideration, inorganic scaling can occur on the surface of RO membranes, such as the buildup of calcium sulfate and silicates, which can reduce membrane efficiency. To prevent precipitation in the RO system, is its preferable to minimize the alkalinity and hardness of

the feed water before RO treatment. AWPF mitigates scaling by adding antiscalants to the RO feedwater.

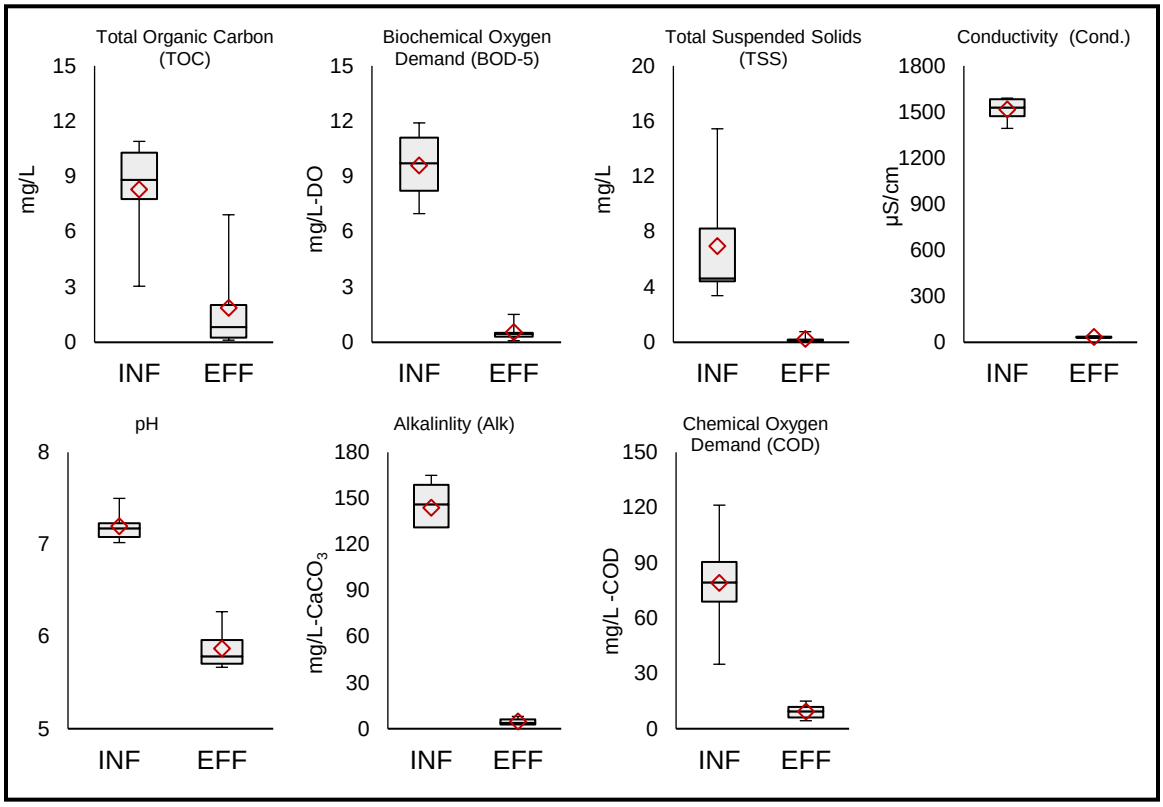


FIGURE 63. Water quality assessment for pre- and post- AWPF treatment of wastewater.

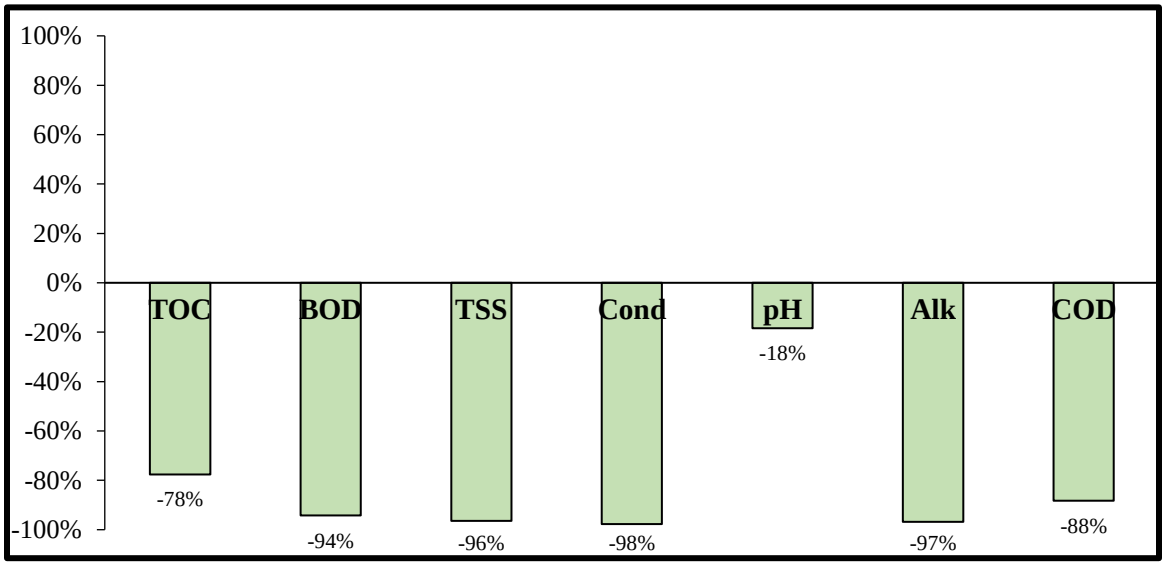
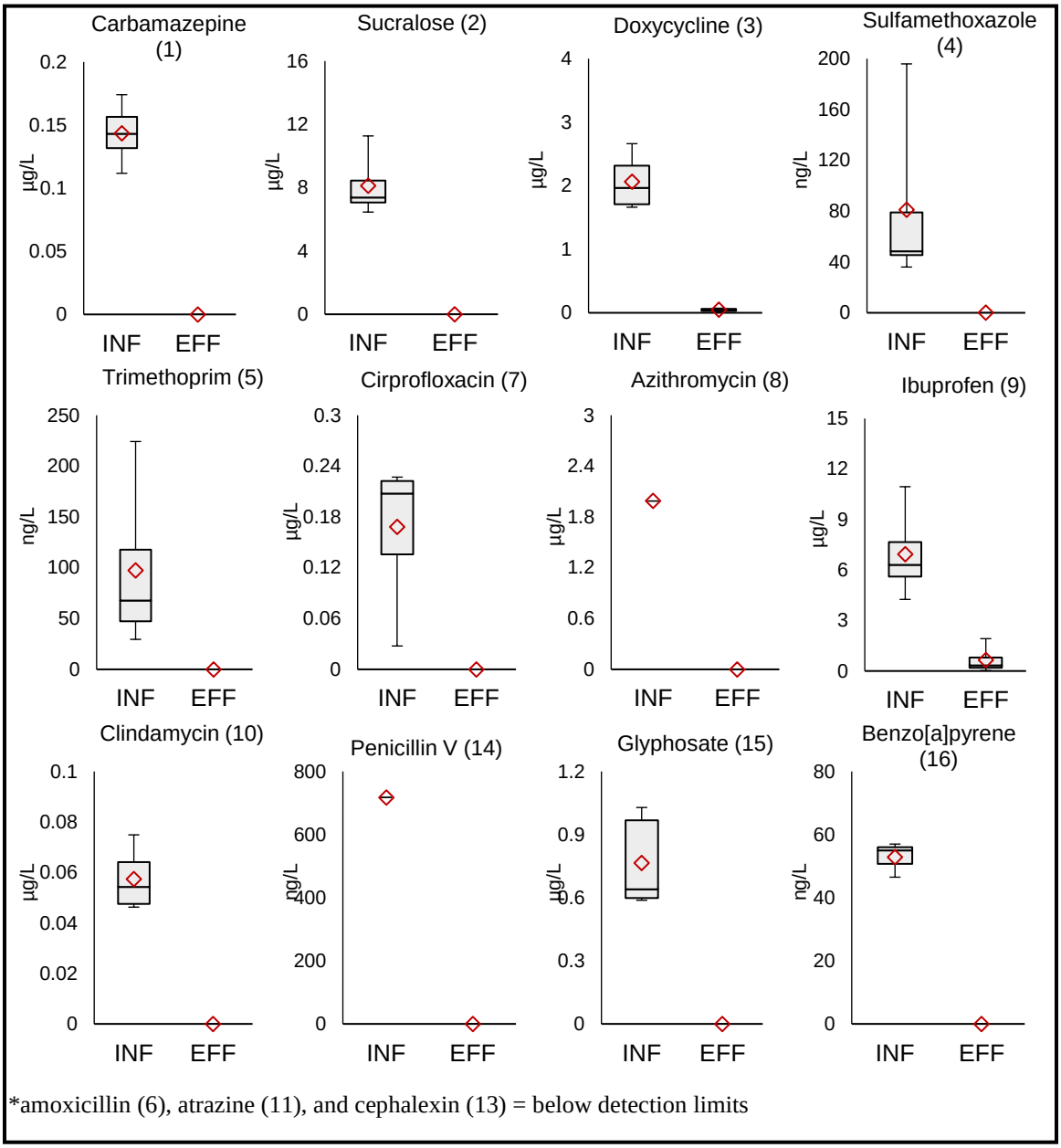


FIGURE 64. Average percent increase or decrease of aggregate water quality parameters; AWPF.

3.5.6 Emerging Contaminants of Concern

All detectable contaminates were removed with significant removal achieved for doxycycline and ibuprofen as shown in Figures 65 and 66. The AWPF utilizes semi-permeable polyamide membranes which can function under a wide variety of pH ranges. However, polyamide membranes are sensitive to chlorine which can result in performance loss through membrane depolymerization.[200]



*amoxicillin (6), atrazine (11), and cephalexin (13) = below detection limits

FIGURE 65. Comparison of emerging contaminant concentrations for pre- and post- AWPF treatment of wastewater.

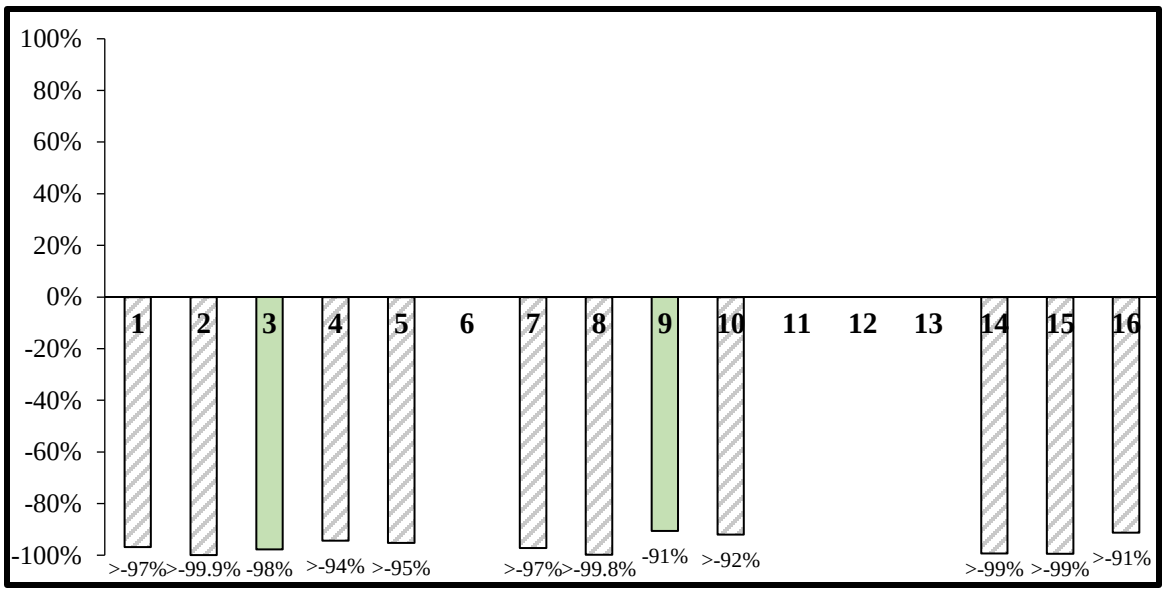


FIGURE 66. Average percent attenuation or recontamination of emerging contaminants; AWPF.
(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

3.5.7 Antibiotic Resistance Genes (ARG)

In general, the levels of ARGs in wastewater effluent were comparable with the levels of ARGs measured in Santa Ana River as shown in Figures 67 and 68. None of the ARGs were detected in the AWPF UVP due to near complete removal of microbes in the treatment processes (no DNA associated with ARGs was recovered from the samples from the six sampling events).

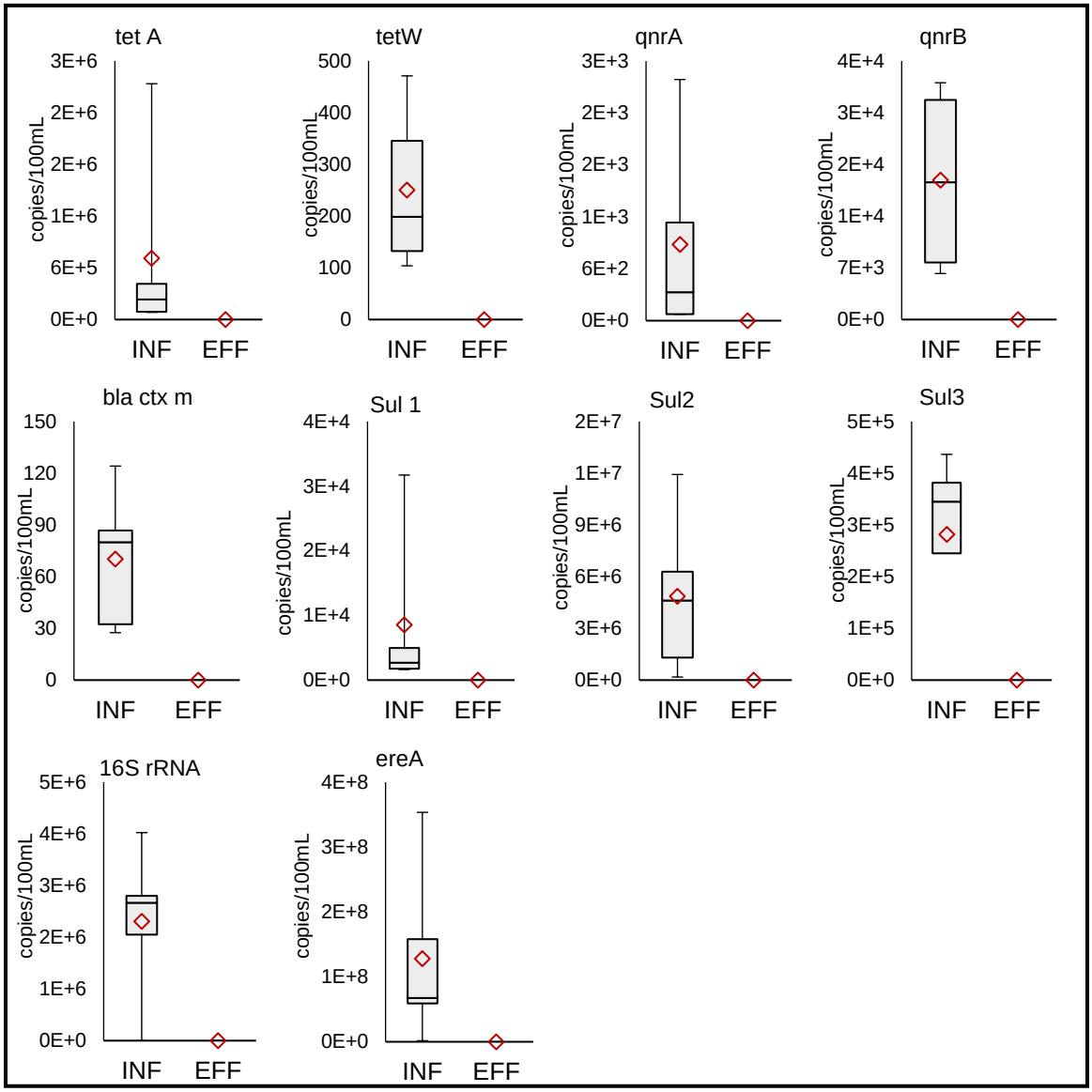


FIGURE 67. Comparison of ARG concentrations for pre- and post- AWPF treatment of wastewater.

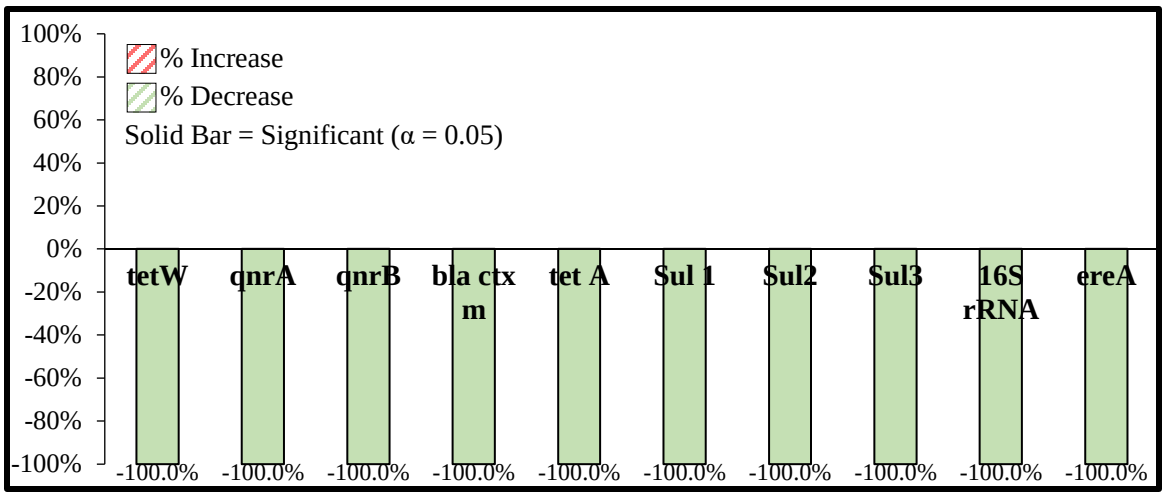


FIGURE 68. Average percent attenuation or recontamination of ARGs; AWPF.

3.5.8 Cost Analysis

Nineteen drinking water treatment facilities within OC provide clean water to supply their 2.4 million residents. The USGS estimates that the average person uses 80 - 100 gallons of water per day. Therefore, an estimated 240 million gallons per day (MGD) is required to sustain the population in Orange County.[201] According to the May 2018 GWRS Technical Report, the AWPF currently produces 70 MGD of high-quality potable water for groundwater replenishment, which is subsequently recharged into the region's groundwater system used by the 19 facilities as a drinking water source via pumping/extraction from the groundwater system and then additional drinking water treatment and disinfection prior to distribution.[39] Table 31 compares the AWPF feedwater and product water to EPA drinking water standards.

The capital cost of bringing the purification facility online was $480.9 million, with the current operational costs at $525 per acre-foot with subsidies provided by the Metropolitan Water District of Southern California (MWDSC) for the Green Acres Project (GAP) Local Resources Program (LRP) or $850 per AF without ($0.26 per 100 gallons).[202] The current processing rate can directly provide for roughly 30 – 35% of the 2.4 million OC resident at 100 MGD production capacity. A conventional DWTP with 100 MGD capacity have an average estimated construction cost of $75 million, with production cost at about $1000 per AF of water for Southern California (S.CA).[203] An additional cost associated with advanced treatment are the expenses related to brine management. There is a potential for recovery of certain metals such as rubidium from the brine solution.[204] However, this is currently not a commercially developed option.

The lower capital cost for bringing a traditional DWTP online may be an incentive to continue with the current conventional treatment methods. However, the

cost of actual potable water production will be cheaper over the long run for areas that import water from distant sources. Especially in regions that suffer from constant shortages of local water supplies. The $1000/AF includes expenses paid by local DWTP to S.CA for the import of source water from distant locations. Areas with sustainable supplies of raw water may avoid the need to import source water. Therefore, recycling water locally can be a more attractive option for such communities as opposed to the higher costs of advanced treatment.

TABLE 31. EPA vs AWPF [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (OCSD)	Effluent (AWPF)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.0 ± 0.0	0.0 ± 0.0
Iron (Fe) [1]	ppm	0.3	NS	0.064 ± 0.03	0.020 ± 0.010
Boron (B) [1]	ppm	NS	NS	0.28 ± 0.04	0.25 ± 0.02
Calcium (Ca) [1]	ppm	NS	NS	23.0 ± 1.6	8.4 ± 0.2
Magnesium (Mg) [1]	ppm	NS	NS	5.48 ± 0.66	2.33 ± 0.28
Manganese (Mn)	ppm	0.05	NS	0.09 ± 0.10	0.00 ± 0.00
Sodium (Na) [1]	ppm	NS	NS	43.1 ± 2.42	7.1 ± 1.9
Cadmium (Cd)[2]	ppb	2	0.005	0.0 ± 0.0	0.0 ± 0.0
Mercury (Hg) [2]	ppb	2	0.002	0.0 ± 0.0	0.0 ± 0.0
Lead (Pb) [1]	ppb	15	0.2	0.0 ± 0.0	0.0 ± 0.0
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	103.9 ± 47.1	6.2 ± 0.5
Bromide (Br)	ppm	NS	NS	0.09 ± 0.05	0.02 ± 0.03
Sulfate (SO_4^{2-}) [1]	ppm	500	NS	49.6 ± 10.4	1.4 ± 0.7
Iodine (I)	ppm	NS	NS	0.2 ± 0.4	0.0 ± 0.0
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.016 ± 0.009	0.005 ± 0.006
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	0.91 ± 0.21	1.54 ± 0.05
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.023 ± 0.004	0.011 ± 0.001
Total Phosphorous	mg/L PO_4^--P	NS	NS	1.87 ± 0.54	0.62 ± 0.13
Total Nitrogen	mg/L-N	NS	NS	4.4 ± 2.9	3.0 ± 2.0
Microorganisms					
Total Coliform[2]	MPN/100 mL	MCL[3]	0	869.4 ± 645.8	0.000 ± 0.0
Fecal Coliform[2]	MPN/100 mL	MCL[3]	0	54.9 ± 52.4	0.000 ± 0.0
Escherichia coli[2]	MPN/100 mL	MCL[3]	0	54.4 ± 44.6	0.000 ± 0.0
Enterococci[2]	MPN/100 mL	NS	NS	51.4 ± 49.9	0.000 ± 0.0
Salmonella spp. [2]	MPN/100 mL	NS	NS	1.6 ± 1.7	0.000 ± 0.0
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	0.000 ± 0.0	0.000 ± 0.0
Aggregate Water Quality					
Total Dissolved Solids[1]	ppm	500	NS	6.6 ± 4.5	0.21 ± 0.28
pH[1]		6.5 – 8.5	NS	7.2 ± 0.17	5.87 ± 0.23
Total Organic Carbon	mg/L	NS	NS	8.3 ± 2.8	1.86 ± 2.61
Biochemical Oxygen Demand	mg/L-DO	NS	NS	9.6 ± 1.9	0.552 ± 0.489
Conductivity	µS	NS	NS	1515.6 ± 78.8	34.1 ± 4.5
Alkalinity	mg/L-$CaCO_3$	NS	NS	143.8 ± 19.2	4.57 ± 2.32
Chemical Oxygen Demand	mg/L -COD	NS	NS	79.1 ± 28.6	9.28 ± 4.07
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	11.8 ± 9.9	2.6 ± 2.3
Benzo[a]pyrene[2]	ppt	200	0.0	119.3 ± 0.1	157.6 ± 0.1

NS: no standard **Red: above MCL**

Green Cells: Significant Decrease Red Cells: Significant Increase

[1] City of Orange Water Division Consumer Confidence Report – 2017

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation. Giardia lamblia: 99.9% removal/inactivation" - *National Primary Drinking Water Regulations, USEPA* – 2018.

3.6 AQUIFER RECHARGE (OCWD 2A – 3)

3.6.1 La Palma Recharge Basin

*Note: the purpose of sampling at this location was not to assess its efficacy as an environmental buffer for the water purification but rather for the observance of recontamination post advanced purification. However, the purported properties of aquifers were discussed in length below for the consideration as an environmental buffer.

The 350 square-mile groundwater basin in OCWD lies beneath the northern and central parts of Orange County and is the source of potable water for more than 20 cities nearby. The basin holds over 40 million AF of water with an annual yield of roughly 300,000 AF and a capacity of 500,000 AF of water per year.[39] The SAR was the predominant source of replenishment for the basin until the 1940's when the extraction of groundwater for use exceeded what nature could recharge. In response, water conservation and recycling efforts were made upstream of the SAR to offset the groundwater depletion occurring downstream. The combination of restoration activities and extreme weather patterns brought about by climate change has caused the SAR to develop a non-uniform flow pattern that fluctuates on an annual basis. OCWD has been compensating for these fluctuations by importing water from the Colorado River and the Sacramento-San Joaquin River Delta to replenish the groundwater system. However, importing water can be costly and energy intensive. Furthermore, the longevity of Colorado River's sustainability is questionable as it is shared between seven US states and Mexico for drinking water, all of whom receive infrequent precipitation and are facing potential population growth.

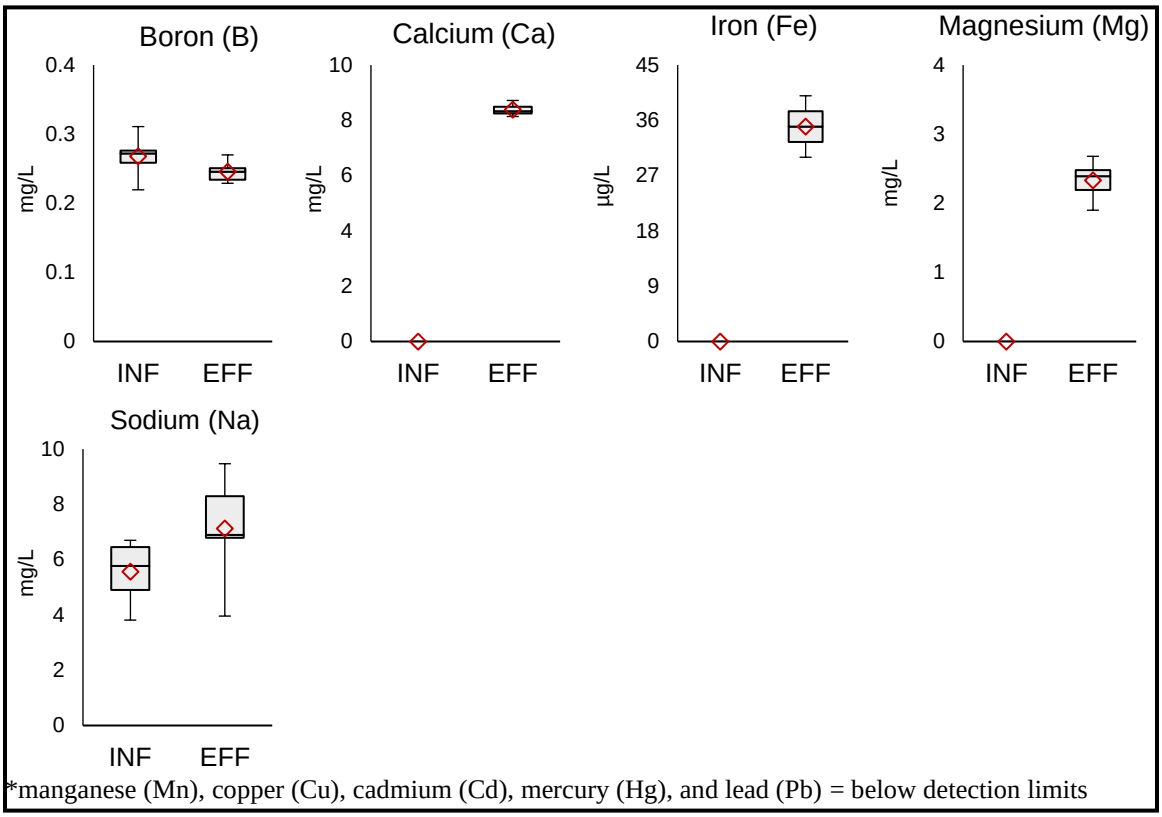
As of 2008, the aquifer recharge has been indirectly receiving 89.6% of the 100 MGD of AWPF product water through recharge basins.[205] The finished water is pumped from the purification facility to an above ground storage basin from where the water percolates into the aquifer over time. OCWD recharge facilities covers a total of 1,100 acres and sends an estimated 280,000 AF per year of AWPF finished water into the groundwater basin. The La Palma basin in the Anaheim location, adjacent to Carbon Creek Diversion Channel, expands 17.7 acres above ground and receives roughly 49.2 MGD of AWPF finished product water from the nearby GWRS distribution pipeline.[205] The basin has an estimated average percolation rate of 45 MGD.[206-208]

Samples from this location were extracted from monitoring well AM-52. The well is located 155 ft below ground and has a hydraulic travel time from the recharge ponds of roughly 30 days. A total number of 6 samples were collected from the well; three samples were collected during the dry season and three during the rainy season. The data for the dry and wet seasons were analyzed together in order to provide a comprehensive assessment that considered environmental fluctuations due to temporal and climate differences. The results from this location represent the impact of soil aquifer treatment on the AWPF product water.

3.6.2 Metals

The additional boron attenuation post advanced purification may be the result of dilution with ambient groundwater see in Figures 69 and 70. However, partitioning may also have contributed to the reduction of boron as well. Partitioning can be broken down into three different processes. First, the accumulation of metals onto an adsorbent surface via adsorption or absorption of metals. Depending on the surface charge of the

contaminant and the sorption surface, metal ions can accrue on aquifer surfaces, reducing their mobility in the water column. The distribution typically follows the Langmuir isotherm, typical for electrostatic interactions.[209] Common aquifer adsorbent phases are comparable to water treatment adsorbents such as polyciliate clay particles, natural organic matter, and some metal oxyhydroxides.[210] In the second process, depending on the equilibrium solubility of the contaminant, precipitation may occur either as a pure compound or may co-precipitate with another chemical complex.[211, 212] Third, anaerobic, and sometimes aerobic, microbial activity can substitute oxygen with iron and manganese as their metabolic electron acceptor.[29] These microorganisms are similar to what is used in bioremediation projects for pollutant removal of industrial waste contaminated aquifers. As a side benefit, this biological process can inadvertently remove hydrocarbon contaminants as well. Studies have shown that under certain environmental conditions, microorganisms may obtain source of carbon from organic pollutants such as benzene, toluene, and xylene (BTX), and polycyclic aromatic hydrocarbons (PAH). [213, 214]



*manganese (Mn), copper (Cu), cadmium (Cd), mercury (Hg), and lead (Pb) = below detection limits

FIGURE 69. Comparison of metal concentrations; Aquifer Recharge.

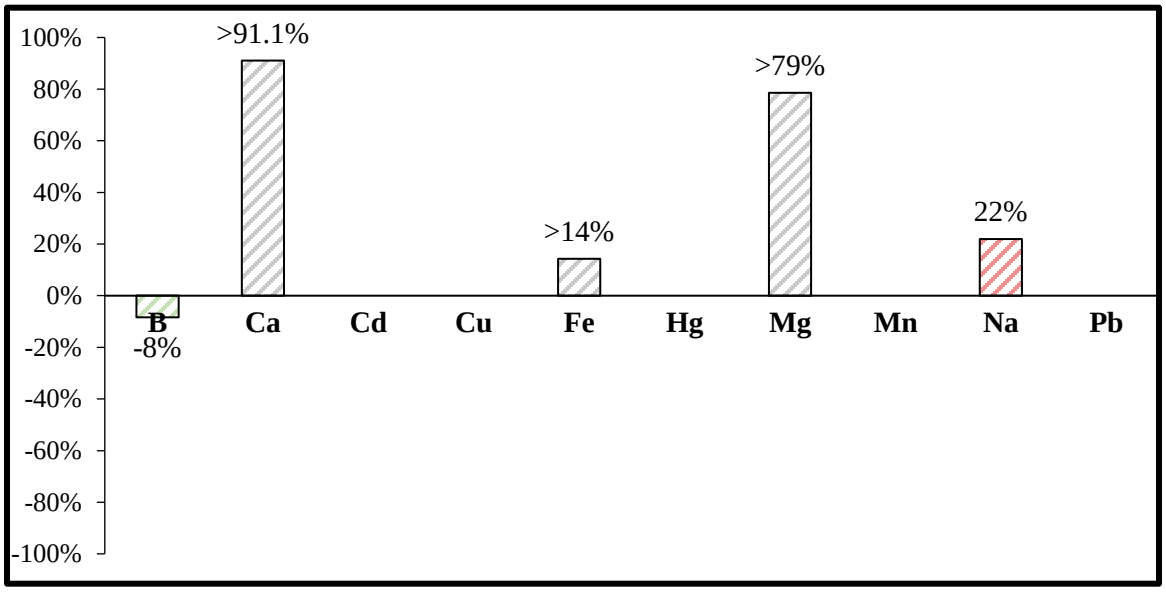


FIGURE 70. Average percent attenuation and recontamination of metal contaminants for aquifer recharge.

Calcium, iron, magnesium, and sodium are some of the most commonly occurring dissolved ions found in nature. This study measured an increase in all three of those metal ions, with calcium and magnesium measuring significantly higher post aquifer treatment. In nature, calcium and magnesium ions and minerals are encountered more often in groundwater than surface water, as the principal sources of calcium and magnesium are dissolved polyvalent metallic ions from sedimentary rocks and soil runoffs.[215] In drinking water treatment, the amount of calcium and magnesium is expressed in terms of calcium carbonate ($CaCO_3$)-mg/L equivalence, or "hardness". Water containing less than 60 $CaCO_3$-mg/L is considered to be soft; 60 – 120 $CaCO_3$-mg/L is moderately hard; 120 – 180 $CaCO_3$-mg/L is hard; and greater than 180 $CaCO_3$-mg/L is considered very hard, with the ideal hardness for drinking water to be between 80 - 100 $CaCO_3$-mg/L. [215] [216] This study measured an average of 0.132 $CaCO_3$-mg/L in AWPF product water, before the remineralization phase, and an average of 35.0 $CaCO_3$-mg/L in the aquifer sample. Comparatively, the average occurrence of calcium is up to and often exceeds 100 mg/L in aquifers. On the other hand, magnesium is present at lower concentrations, from <0.01 mg/L to 50 mg/L and rarely exceeding 100 mg/L.[217] Regardless, the permineralized water and aquifer recharge water samples measured much lower than the previously mentioned typical occurrence values.

In instances of high levels of dissolved minerals, scaling can occur within the water distribution infrastructure by creating a coating the inner surface of pipelines with mineral deposits. On the other hand, soft water can also adversely affect the piping system via corrosion and cause the leaching of heavy metals from the pipes to the water supply.[217] In this case, supplemental chemicals will be necessary to increase the

hardness of the product water from AWPF and the aquifer sample water before it is made available to consumers. In this study, iron also saw a significant increase post aquifer buffer treatment. Excessive iron in water is can cause damage to the plumbing aesthetics and to an extent increased hardness. However, despite the significant increase between the AWPF product water and the groundwater sample, the iron concentration was still below the EPA MCL for drinking water standards, therefore, it is not of major concern for drinking water purposes.

In conclusion, drinking water treatment plants sourcing aquifer recharge water or AWPF product water will need to implement additional treatment steps to render the water usable and safe for consumers.

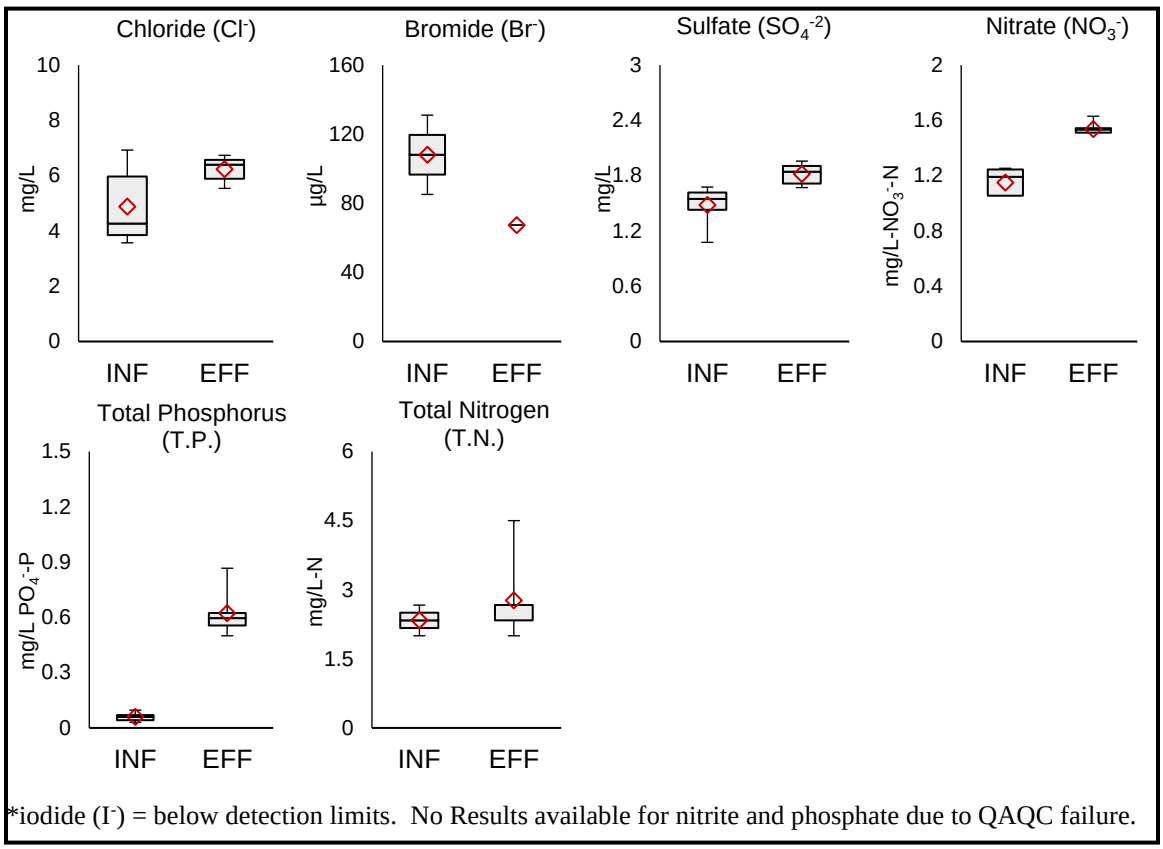
However, groundwater tends to pick up and accumulate dissolved minerals as it percolates through the aquifer column. If more time was allotted for the water to move through the aquifer recharge before samples were taken, the hardness level may have increase above the average of 35.0 $CaCO_3$-mg/L that was measured in this study. Therefore, depending on where drinking water was extracted from the aquifer recharge, remineralization may not be necessary, but instead lime or lime soda softening may be necessary if the groundwater has exceeded the acceptable drinking water hardness level.

3.6.3 Nutrients and anions

Sulfate, nitrate, and total phosphorus were re-contaminated the aquifer recharge as seen in Figures 71 and 72. Microbial degradation has significant impacts on mitigating inorganic contaminants in groundwater. In this instance, the attenuation of bromide in groundwater can also be partially attributed to sorption or microbial uptake.

Due to nitrate's high reduction potential, comparable to that of oxygen's in aerobic conditions, denitrification was expected to be highly efficient in groundwater through microbial absorption processes.[218] However, contrarily, we measured a significant increase in nitrate levels in the aquifer recharge, which is likely indicative of the impact from agricultural seepage on the underlying aquifer. One statistical regression study suggested that average daily precipitation is inversely proportional to nitrate concentration in groundwater, since precipitation stimulates plant growth resulting in higher uptake of nutrients. Therefore, areas with high average daily precipitation may experience lower groundwater nitrate concentration.[219] Although average annual rainfall does vary, Southern California is still considered to be one of the drier regions in the United States. According to surveys conducted by USGS and other research groups, S. California is heavily impacted by nitrate pollution, in some cases exceeding 2.6 mg/L. Compared to results found in literature, the average nitrate measured in this study is lower than what is typically measured. [220, 221]

Due to its negative charge, nitrate has high solubility and high mobility through soil columns. This results in a very high potential for the nitrate to leach into groundwater with minimal interference from soil filtration. [222, 223] However, nitrate in groundwater disperses quickly through horizontal advection, and the highly variable spatial distribution of nitrogen contaminants makes it difficult determine non-point source pollutions.



*iodide (I^-) = below detection limits. No Results available for nitrite and phosphate due to QAQC failure.

FIGURE 71. Comparison of nutrient and anions; Aquifer Recharge.

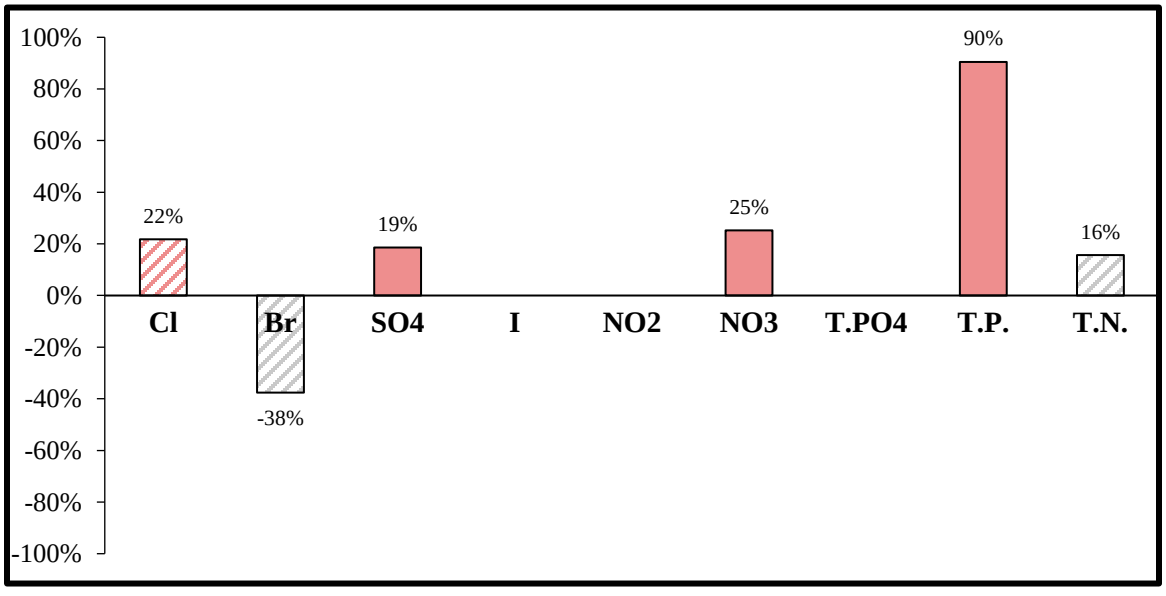
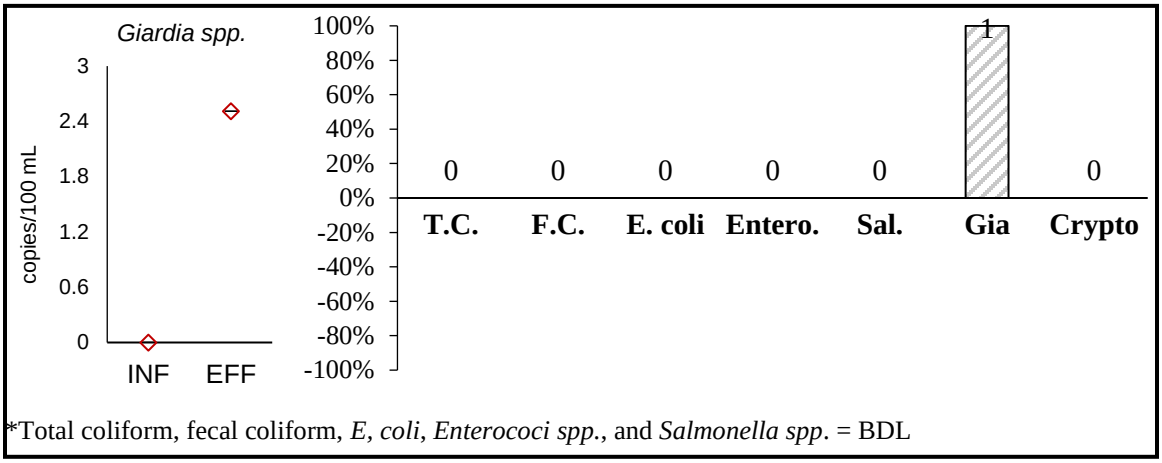


FIGURE 72. Average percent attenuation and recontamination of anion and nutrient contaminants for aquifer recharge.

Studies have shown that groundwater receiving fertilizer and wastewater seepage often measure high levels of nitrate and phosphate pollution. Although phosphorous is a major component of fertilizers, it does not behave like nitrate does in soil. Phosphorous is usually rendered immobile as it is retained in the porous soil layer. A major breach of phosphorus in groundwater often does not occur except under certain quantities, such as soil with low attenuation capacity or facilitated transport through phosphorous-containing wastes.[224] However, when the volume of contaminants exceeds the soil's retention threshold, the nutrients will readily dissolve and move freely into the nearest body of watershed.[225, 226] This is especially apparent in areas located near dairy lagoons where the livestock manure can infiltrate the soil column into the groundwater below.[227] AWPF product water serves as La Palma basin's only source of replenishment with negligible levels of nutrient contaminants. Therefore, the numerous cattle ranch and dairy farms situated upstream of SAR and the residential and commercial areas located along the SAR is speculated to be the main contributor to the significantly high concentration of phosphorus and phosphate measured in the aquifer.

3.6.4 Microbial Contaminants

The microbial contaminants were below detection limits for both the AWPF final product water and the groundwater. The only exception is the presence of *Giardia spp.* which commonly occur in environmental aquatic systems. Due to insufficient sample size, was unable to perform statistical analysis on the percent change, see Figure 73.



*Total coliform, fecal coliform, *E, coli*, *Enterococi spp.*, and *Salmonella spp*. = BDL

FIGURE 73. Comparison of microbial concentrations; Aquifer Recharge.

3.6.5 Aggregate Water Quality Assessment

The post advanced purification samples were collected before remineralization. Which may explain the 40% increase in TSS and the significant increase in conductivity, pH, and alkalinity measured post La Palma basin percolation, shown in Figures 74 and 75.

Although not statistically significant, the attenuation of BOD and COD levels in the aquifer as compared to AWPF water is considered unusual. The reintroduction of AWPF effluent to the environment should exposed it to biotic and abiotic oxygen demanding species. A positive correlation typically exists between BOD and COD as well as to nutrient inputs. La Palma saw a disproportional decrease in oxygen-demand and organic carbon concentrations despite the significant increase in eutrophicating nutrients.[228] The 2018, City of La Palma reported their average TOC level to be "not detected" with the lowest detection range at 1.8 ppm; comparable to the findings in this study where the average concentration of TOC was measured to be around 1.8 ppm.[207]

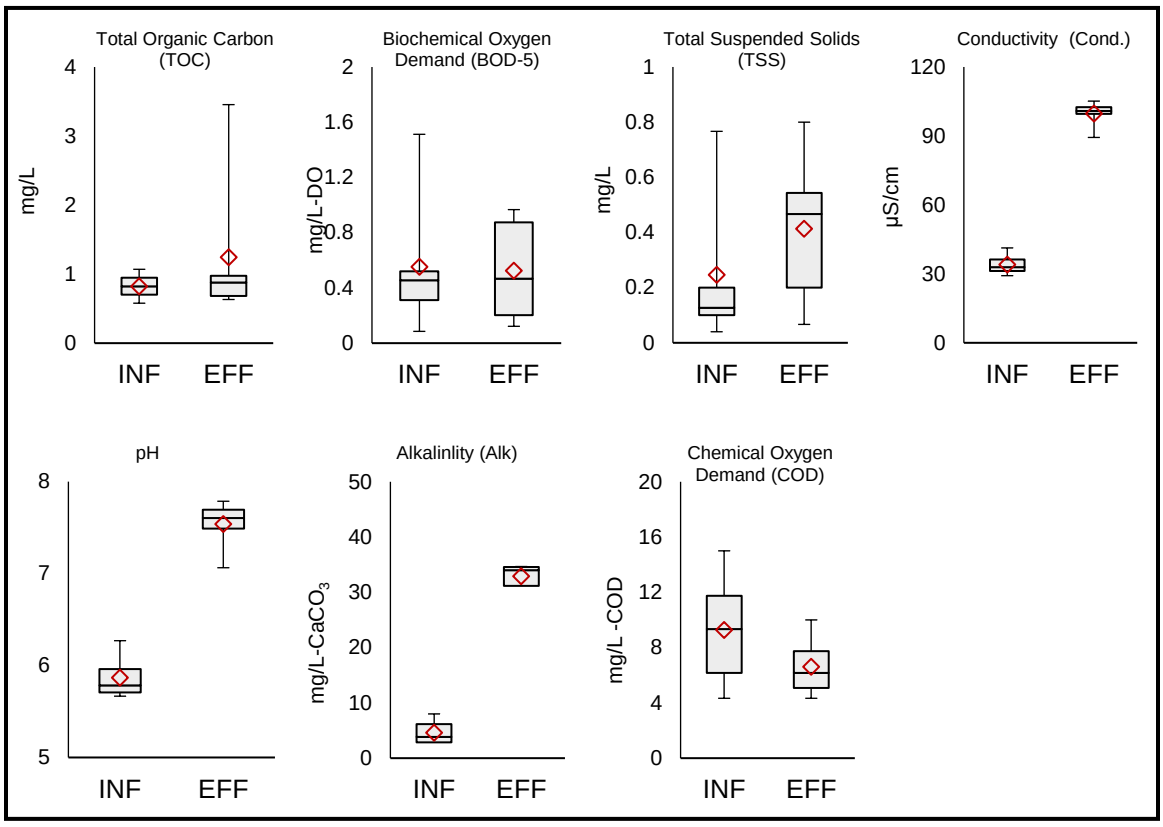


FIGURE 74. Water quality assessment; Aquifer Recharge.

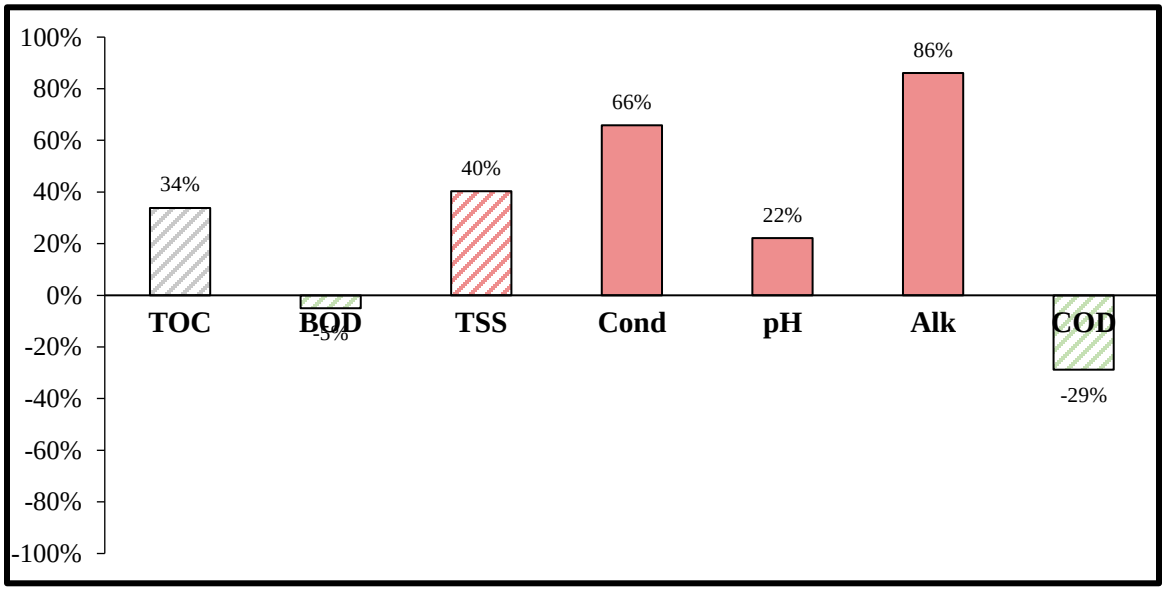
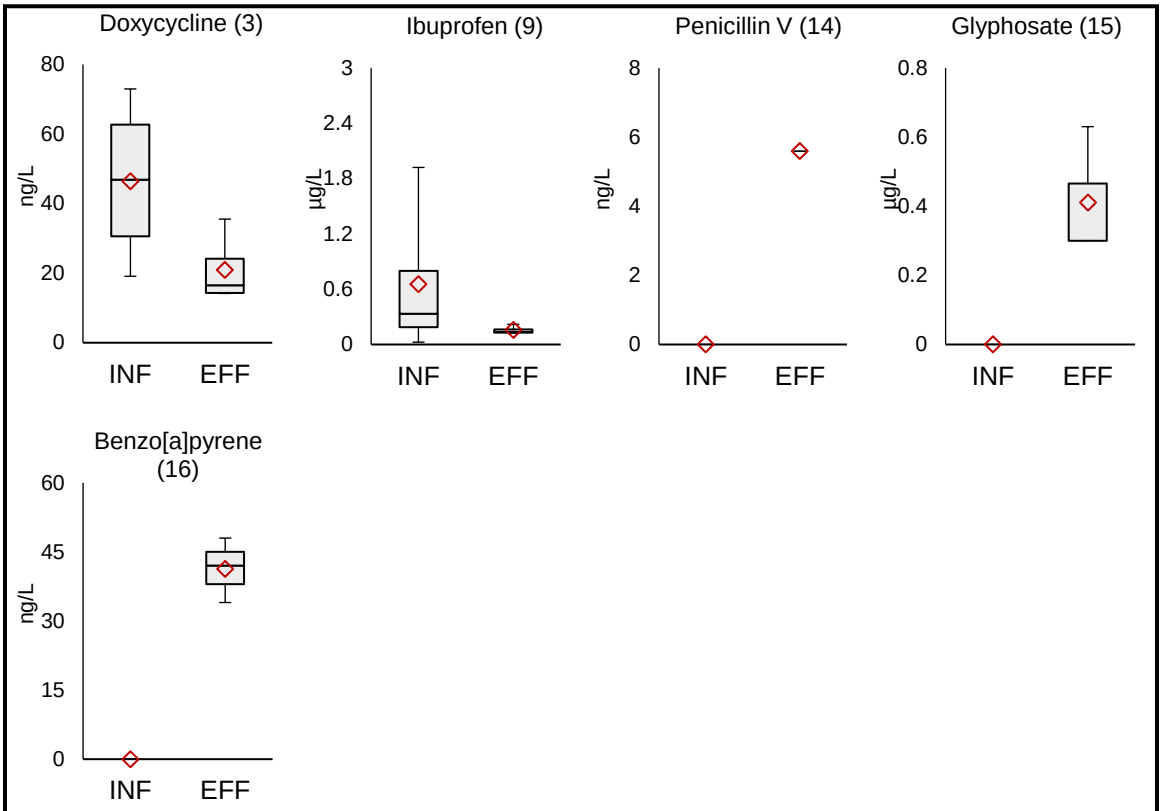


FIGURE 75. Average percent increase or decrease of aggregate water quality parameters for aquifer recharge.

3.6.6 Emerging Contaminants of Concern

Attenuation was observed for doxycycline and ibuprofen at 55% and 76% respectively while penicillin V, glyphosate, and benzo[a]pyrene was re-introduced in aquifer recharge; at >18%, >99%, and >89% respectively, see Figures 76 and 77 for more details.

The attenuation of ibuprofen suggests the occurrence of biodegradation. However, adsorption is likely to have occurred as well in addition to microbial degradation since ibuprofen is also susceptible to adsorption in the soil column.[229] The study is unable to offer a definitive answer for the occurrence of adsorption since the adsorption pathway indicator, carbamazepine, was below detection limits in both the AWPF effluent and the groundwater. However, as biodegradation is minimal and the lack of light source inhibits photolysis, adsorption was likely the dominant attenuation processes within the aquifer system. Advection may also occur via dispersion, allowing for significant attenuation via dilution within the system. The fate and transport of antibiotics are heavily influenced by the physiochemical make up of groundwater which may have varying effects on different contaminants depending on their hydro-chemical properties.[230, 231]



*(1) carbamazepine, (2) sucralose, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (10) clindamycin, (11) atrazine, (12) cephalexin, and (13) levofloxacin = BDL

FIGURE 76. Comparison of emerging contaminant concentrations; Aquifer Recharge.

(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

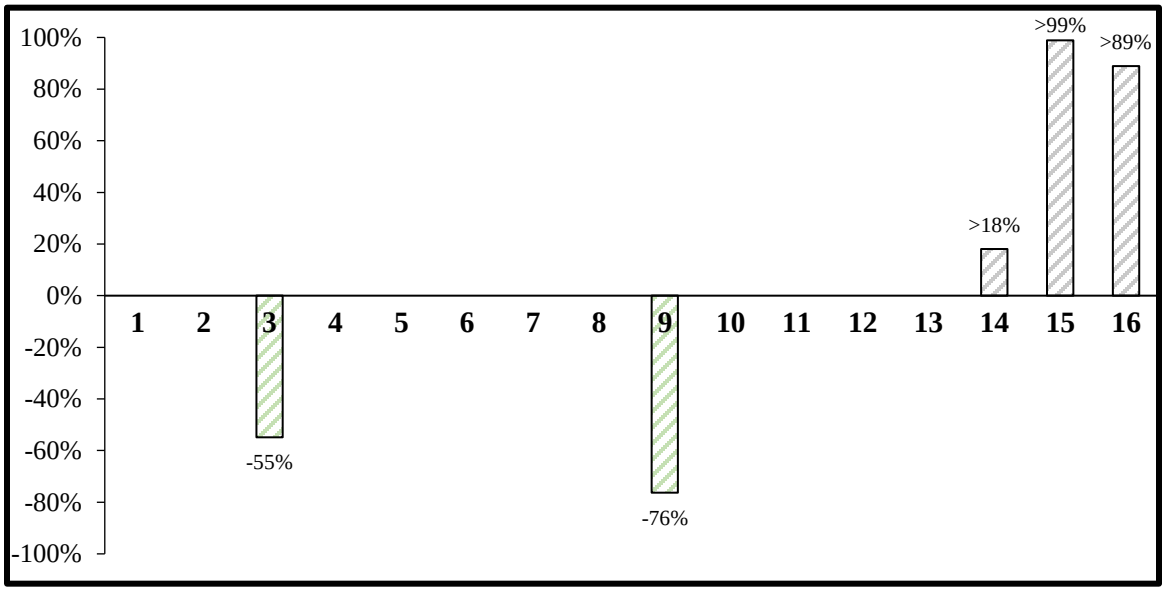


FIGURE 77. Average percent attenuation or recontamination of emerging contaminants for aquifer recharge.

(1) carbamazepine, (2) sucralose, (3) doxycycline, (4) sulfamethoxazole, (5) trimethoprim, (6) amoxicillin, (7) ciprofloxacin, (8) azithromycin, (9) ibuprofen, (10) clindamycin, (11) atrazine, (12) cephalexin, (13) levofloxacin, (14) penicillin V, (15) glyphosate, (16) benzo[a]pyrene.

Although not detected in this study, clindamycin, if it were present, are susceptible to adsorption through the soil and sediments in groundwater aquifers. They exhibit characters of hydrophobicity with low solubility and high sorption potential between pH levels 6.0 - 8.0.[232, 233] . Tetracyclines, including doxycycline, forms complexes and adhere to suspended organic matter in the same pH levels. These organic complexes are negatively charged and are retained on topsoil during basin and riverbank filtration. [234] β-lactams, like penicillin and cephalexin are extremely unstable in aquatic conditions due to the 4-member cycloalkane ring and are highly sensitive to shifts in pH and temperature. The fragile ring strain comes from the extreme electrophilicity of the carbonyl carbon which breaks open upon contact with bacterial hydroxyl active site and functions as bacterial transpeptidase, a cell wall growth molecule, thus inducing bacterial death by inhibiting bacterial growth activity.[185] The increase of penicillin V in the

aquifer could be attributed to sample contamination or excessive use in local animal husbandry or human populations.

Atrazine is a common herbicide for the suppression of broadleaf weeds and grass growth in corn and sorghum agriculture. Both biotic and abiotic pathway of atrazine degradation to be of equal occurrence in the environment.[235, 236] Primary chemical degradation occurs via hydrolysis. In the presence of organic matter, adsorption-catalyzed hydrolysis is triggered when hydrogen bonds form between carboxyl groups in organic matter and nitrogen rings in atrazine.[237, 238] Therefore, the chemical degradation of atrazine is enhanced in soil columns with high organic content. Microbial degradation pathway is dominated by the N-dealkylation of atrazine and is stimulated by increased moisture, temperature, and organic matter.[239, 240] Regardless, atrazine is usually persistent in aquatic environments.[241] The increase in atrazine concentration measured in this study may be attributed to the lack of microbial activity and low organic matter in the aquifer recharge. Regardless of the increase, the concentration of atrazine detected int his study was sub-ng/L level, much lower than many surveying studies. Comparatively, glyphosate is the preferred choice of herbicides for Californian residents, this is reflected in this study where the concentration of glyphosate in water measured much higher than the levels of atrazine.

According to a national groundwater survey conducted by USGS, sulfamethoxazole and trimethoprim are some of the most prevalent antibiotic contaminants found in groundwater; however, sulfonamides were below detection limit for both AWPF product water and the extracted aquifer recharge sample.[242 243] Aside from the prevalence of use, sulfonamides are persistent in the aquifer recharge because

they are less sensitive to environmental degradation than most other antibiotics. Sulfonamides have low micro-degradability and exhibit hydrophilic and weak sorption characteristics, which are exacerbated at lower temperatures. These compounds are mainly adsorbed through pH dependent electrostatic interactions, which is consistent with laboratory batch studies that showed the Langmuir isotherm fit.[244] Additionally, they are often outcompeted by dissolved organic matter for adsorbent sites which minimizes the capacity of soil to adsorb sulfonamides. Those combined factors augment the transport of sulfonamides through the groundwater system.

Sucralose is generally resistant to most environmental fates, however, a groundwater plume study showed that sucralose was reserved within the initial injection site, however, the concentration reduced from 20 ug/L to less than 1 ug/L within 20 meters of injection site.[231] The study theorized biodegradation to be the main process restricting the plume expansion; groundwater microbial populations would acclimate to specific organic contaminants, which can efficiently metabolize it as their main carbon source, as long as the source is constant.

Glyphosate, commonly referred to by its trade name Roundup©, is a broad-spectrum herbicide widely used to control the growth of broadleaf weeds and grasses in agricultural and commercial enterprises, and residential areas. The popularity of glyphosate tripled since the introduction of genetically modified Roundup Ready® crops in 1997, such as glyphosate resistant corn and soybeans. One of the main appeals of using glyphosate is its apparently non-toxicity to humans, and the prevalence of glyphosate use can be attested by its now virtually ubiquitous presence in the environment.[245, 246] A 23-year study conducted by the University of California San Diego

School of Medicine measured the level of glyphosate in the urine of 100 individuals living in Southern California. From 1994 to 2014, the global use of glyphosate was estimated to have risen 15-fold; from 1993 to 2016, the excretion levels of glyphosate in the 100 participants increased about 18-fold from 0.025 ug/L in 1993 – 1996 to 0.449 ug/L in 2014 – 2016.[247]

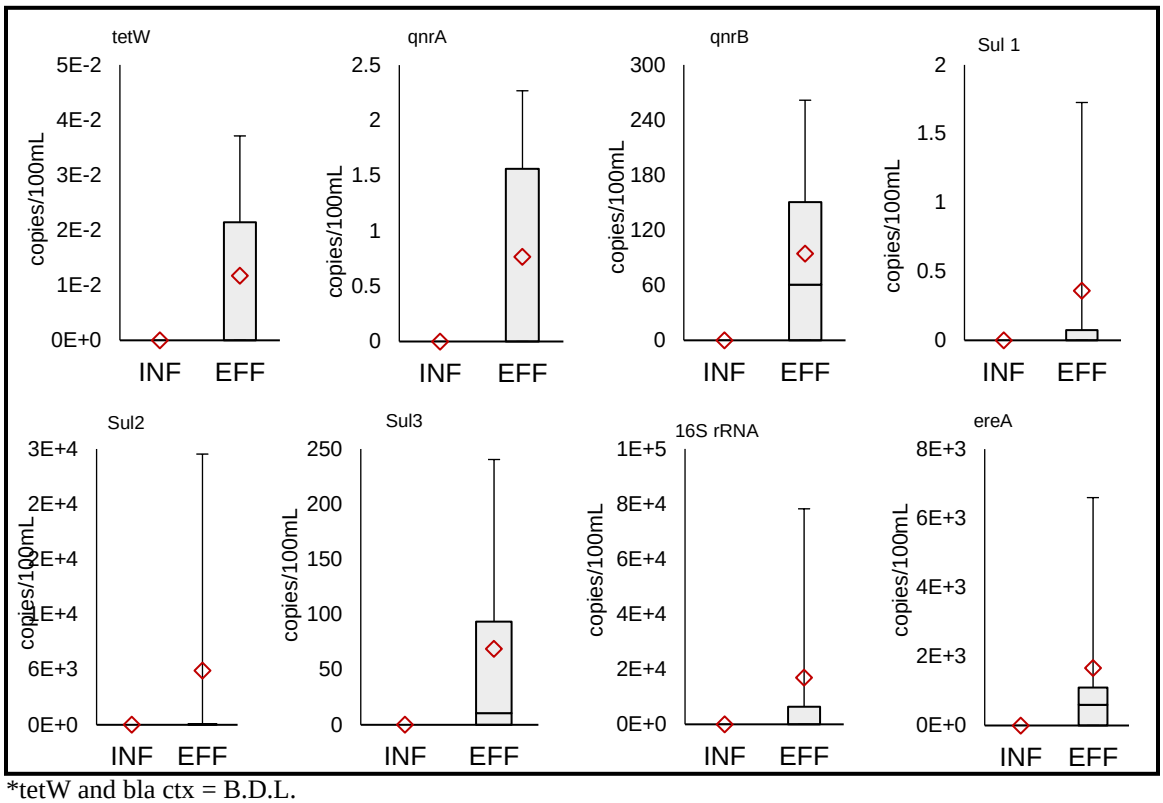
The persistence of glyphosate in the environment can be attributed to its resistance to degradation, which may explain the significant increase measured in this study. The compound has a hydrolysis half-life >35 days, is highly soluble in water at 11,600 mg/L at 25C and stable across a wide range of pH levels from pH 3 to 9.[248] It is unaffected by photodegradation under natural sunlight and has little proclivity for hydrolytic decomposition.[249] The dominant environmental fate for glyphosate in aquatic systems is through adsorption onto the soil column where it is only considered moderately persistent.[250] In soil medium, glyphosate is still resistant to chemical degradation and photolysis by sunlight; it is still relatively immobile once adsorbed onto soil particles and less than one percent is absorbed via the roots.[251] Glyphosate's primary environmental degradation pathway is through microbial uptake.[252] A study conducted by the USDA observed up to 55% metabolization of glyphosate into CO_2 in 4 weeks with little effect on the microbial population and no interference by the presence of nitrogen fixation, nitrification or any denitrification activity[253]

The occurrence of benzo[a]pyrene in groundwater differs with location, typically associated with specific anthropogenic activities such as motorways, manufacturing, energy facilities, and rural residential areas.[254] By far, the highest concentration of BaPs found in groundwater are those in close proximity to automotive activity.[254, 255] The

OCWD aquifer is situated underneath large cities like Anaheim and Santa Ana CA, that supports a large population in dense urban areas. Specifically, La Palma Recharge Basin is situated near the CA-91 Hwy which may have caused the presence of BaP measured in the monitoring well samples.

3.6.7 Antibiotic Resistance Genes (ARG)

Purified water from AWPF contained no detectable ARGs. However, a few samples taken from the aquifer into which it was discharged, contained some ARGs as shown in Figures 78 and 79. Three of the samples did not have detectable microbial 16S rRNA indicating low microbial presence in general. ARGs were mainly detected in the samples that had measurable 16S rRNA. Those that were detectable are shown in Figure 37. ARGs tetW, tetA, and bla ctx m were not detected in any samples. While the number of copies per unit volume were lower in groundwater than in wastewater, it was higher than in AWPF UVP, which contained no detectable ARGs. This indicates that release of highly purified water into the environment exposes it to environmental microorganisms, some of which carry ARGs.



*tetW and bla ctx = B.D.L.

FIGURE 78. Comparison of ARGs before and after aquifer recharge.

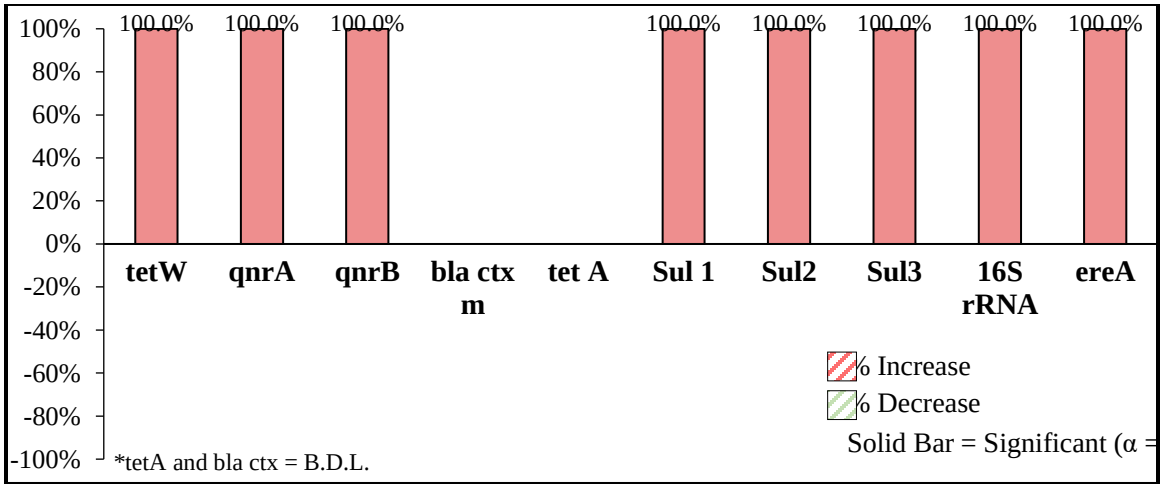


FIGURE 79. Average percent decrease or increase of ARGs in aquifer recharge.

3.6.8 Cost analysis

Recontamination of AWPF product water occurred through the aquifer recharge process. Some metals such as calcium, sodium, iron, and magnesium were higher post percolation from the recharge basin. Sulfate, nitrate, phosphorous, conductivity, and alkalinity had risen significantly after the recharge process. Despite the increase, those contaminants were below the EPA MCL, see Table 32, therefore no additional treatment is required. Theoretically, the aquafer buffer system may reduce the cost of water treatment with the attenuation of TOC. This can potentially cut the operational and chemical costs associated with coagulation, filtration, and disinfection. The attenuation of TOC also reduces the formation potential of DBPs by reducing the number of organics in the influent.

Although not regulated by the EPA drinking water standards, high levels of dissolved calcium and magnesium can cause pipeline scaling and water palatability, the taste threshold for calcium is similar to the common occurrence in groundwater at 100 to 300 $CaCO_3$-mg/L. Water utilities will often treat for hardness as part of its secondary drinking water standards to maintain the integrity of the distribution infrastructure and to preserve customer satisfaction. Iron, however, is recognized as a secondary contaminant and is regulated by the EPA. Excessive soluble ferrous iron in water can produce a metallic taste, stain plumbing fixtures, and encourage the bacterial growth. Although not immediately health threatening, the side-effects of having high iron content in water is more prominent in its adverse aesthetic impacts. The significant increase in calcium, magnesium, and iron in this instance will require treatments such as chemical addition of lime or lime-soda ash for water softening, physical exclusion via filtration, or catalytic reactions. These processes can initiate a domino effect for subsequent treatment units

requiring additional resources and time to address, such as change in pH from chemical additions and remineralization of the product water.

Regardless if the contaminants exceeded regulation levels, the AWPF product water was still significantly re-contaminated in multiple categories of contaminants. The additional process of sending the AWPF effluent to the aquifer recharge served to undo the purification process by introducing contaminants back into the high-quality water; meaning additional treatment cost may be necessary to convert the water back to drinking water quality level. The infrastructure necessary to recharge the aquifer can be costly as well, the three-year La Palma project to build a 17-acre recharge basin had a capital cost of $5,279,894 not including long-term maintenance and risk management.[202] Direct transport of AWPF product water for potable reuse will require a complete retrofit of the current drinking water distribution system, which may upstage the cost of the recharge facilities depending on current infrastructure and scale.

TABLE 32. Orange county contaminant regulatory levels vs measured concentration in aquifer recharge [mean ($\bar{X}$) ± 1 standard deviation (σ)].

Contaminant	Unit	MCL	MCLG	Influent (AWPF)	Effluent (La Palma)
Metals					
Copper (Cu)[1]	ppm	1.3	0.3	0.00 ± 0.0	0.00 ± 0.0
Iron (Fe) [1]	ppm	0.3	NS	0.01 ± 0.004	0.02 ± 0.006
Boron (B) [1]	ppm	NS	NS	0.28 ± 0.010	0.25 ± 0.007
Calcium (Ca) [1]	ppm	NS	NS	0.002 ± 0.002	8.43 ± 0.098
Magnesium (Mg) [1]	ppm	NS	NS	0.01 ± 0.009	2.42 ± 0.101
Manganese (Mn)	ppm	0.05	NS	0.00 ± 0.0	0.00 ± 0.0
Sodium (Na) [1]	ppm	NS	NS	5.72 ± 0.60	7.12 ± 1.92
Cadmium (Cd)[2]	ppb	2	0.005	0.00 ± 0.0	0.00 ± 0.0
Mercury (Hg) [2]	ppb	2	0.002	0.00 ± 0.0	0.00 ± 0.0
Lead (Pb) [1]	ppb	15	0.2	0.00 ± 0.0	0.00 ± 0.0
Anions and Nutrients					
Chloride (Cl) [1]	ppm	500	NS	4.878 ± 0.721	6.236 ± 0.241
Bromide (Br)	ppm	NS	NS	0.044 ± 0.026	0.019 ± 0.015
Sulfate (SO_4^{2-}) [1]	ppm	500	NS	1.480 ± 0.110	1.422 ± 0.359
Iodine (I)	ppm	NS	NS	0.000 ± 0.0	0.000 ± 0.0
Nitrite (NO_2^-) [1]	mg/L-NO_2^--N	1	1	0.002 ± 0.0	0.005 ± 0.003
Nitrate (NO_3^-) [1]	mg/L-NO_3^--N	10	10	1.149 ± 0.059	1.537 ± 0.026
Total Phosphate (PO_4)	mg/L PO_4^{-3}	NS	NS	0.007 ± 0.001	0.011 ± 0.001
Total Phosphorous	mg/L PO_4^--P	NS	NS	0.059 ± 0.012	0.623 ± 0.064
Total Nitrogen	mg/L-N	NS	NS	7.444 ± 2.93	3.000 ± 0.994
Microorganisms					
Total Coliform[2]	MPN/100 mL	MCL[3]	0	0.000 ± 0.0	0.000 ± 0.0
Fecal Coliform[2]	MPN/100 mL	MCL[3]	0	0.000 ± 0.0	0.000 ± 0.0
Escherichia coli[2]	MPN/100 mL	MCL[3]	0	0.000 ± 0.0	0.000 ± 0.0
Enterococci[2]	MPN/100 mL	NS	NS	0.000 ± 0.0	0.000 ± 0.0
Salmonella spp. [2]	MPN/100 mL	NS	NS	0.000 ± 0.0	0.000 ± 0.0
Giardia spp.[2]	Copies/100 mL	MCL[3]	0	0.000 ± 0.0	2.510 ± 0.0
Aggregate Water Quality					
Total Dissolved Solids[1]	ppm	500	NS	0.21 ± 0.142	0.41 ± 0.138
pH[1]		6.5 – 8.5	NS	5.87 ± 0.117	7.54 ± 0.129
Total Organic Carbon	mg/L	NS	NS	1.86 ± 1.31	1.25 ± 0.546
Biochemical Oxygen Demand	mg/L-DO	NS	NS	0.55 ± 0.249	0.52 ± 0.191
Conductivity	µS	NS	NS	34.06 ± 2.24	99.7 ± 2.75
Alkalinity	mg/L-$CaCO_3$	NS	NS	4.57 ± 1.16	32.9 ± 1.12
Chemical Oxygen Demand	mg/L -COD	NS	NS	9.28 ± 2.04	6.61 ± 1.07
Emerging Contaminants					
Glyphosate[2]	ppb	700	NS	0.00 ± 0.00	0.41 ± 0.19
Benzo[a]pyrene[2]	ppt	200	0.0	0.00 ± 0.00	31.0 ± 21.0

NS: no standard **Red: above MCL**

Green Cells: Significant Decrease Red Cells: Significant Increase

[1] City of Orange Water Division Consumer Confidence Report – 2017

[2] National Primary Drinking Water Regulations, *USEPA* - 2018

[3] "A routine sample that is fecal coliform-positive or *E. coli*-positive triggers repeat samples- -if any repeat sample is total coliform-positive, the system has an acute MCL violation. A routine sample that is total coliform-positive and fecal coliform-negative or *E. coli* negative triggers repeat samples--if any repeat sample is fecal coliform-positive or *E. coli*-positive, the system has an acute MCL violation. Giardia lamblia: 99.9% removal/inactivation" - *National Primary Drinking Water Regulations, USEPA* – 2018.

CHAPTER 4. CONCLUSION

4.1 EFFECTIVENESS OF ENVIRONMENTAL BUFFERS

The effectiveness of several environmental buffers for attenuation of various classes of contaminants was evaluated in the context of direct vs. indirect potable water reuse (acknowledged or de facto). The following environmental buffer systems were considered: constructed wetlands, river, lake, groundwater recharge with purified water, and RBF and aquifer recharge with a conventional water source.

Wetlands provided significant attenuation to nutrients such as nitrate and total phosphorus and some microbes. And although not significant, it did retain metals such as iron and reduced TSS by an average of 94%. Some of the CECs susceptible to adsorption and hydrolysis were attenuated as well. Other CECs, however, were concentrated via loss of water to evapotranspiration in the wetlands. Photolysis and biodegradation did not play a role in attenuation of CECs in the evaluated wetland system. Wetlands system evaluated in this study was susceptible to occasional increases in livestock related contaminants, such as antibiotic clindamycin and antiseptic iodide, due to the proximity of livestock operations. There was also a significant increase in manganese from the naturally occurring minerals in the soil with elevate levels exceeding secondary drinking water standards.

Discharge of effluent into a river resulted in significant recontamination with suspended solids and microbes, especially *E.coli* with an average of 85% increase and although not significant, *Salmonella spp.* saw 90% increase. However, turbulent river environment, compared to lake and wetlands environment, where less mixing occurs in short time scales, provided favorable conditions for attenuation of CECs susceptible to

photolysis and hydrolysis. Additionally, because of the nitrate levels in the river, indirect photolysis of CECs was also contributing to their attenuation. In the field study used for this project, discharge of effluent into a river was followed by RBF and aquifer recharge and recovery (ARR). This additional buffer mitigated the increase in TSS and microbe counts that occurred in the river and provided attenuation of ARGs, likely via removal of carrier microbes.

Lake environment was susceptible to runoff, in particular that related to soil erosion. Urban setting next to several coal-burning power plants also resulted in increased levels of BaP, which can serve as an indicator of overall anthropogenic pollution. Occasional spikes in TSS and pathogens, and overall variability of water quality are some of the downsides of a lake as an environmental buffer in potable water reuse. Some concentration of persistent CECs in the lake via evapotranspiration was also observed.

One of the contrasting properties of the lake and river systems evaluated in this study is in the amount of dilution they were able to provide. The lake had a very large flow compared to the flow of effluent it received (less than 0.3% effluent), and was able to provide a considerable level of dilution to CECs and to the salt and mineral content in wastewater resulting from water use and treatment. In comparison, the river that was 40–95% WWTP effluent did not provide the benefit of dilution. Dilution can be of benefit in potable water reuse. However, areas that are purposefully practicing or considering potable water reuse typically do not have the luxury of water bodies pristine enough to provide considerable dilution. Therefore, it is an unlikely benefit from a typical environmental buffer in a water reuse scenario.

In two aquifer recharge field studies considered, the field study where aquifer was recharged with purified water that underwent advanced purification treatment showed recontamination with agricultural nutrients and pesticides, and impact of road runoff (even though the well was 155 ft deep). Urban and agricultural impacts on aquifers are challenging to control in ARR. The other field study used river water for ARR. In that instance, filtration provided by RBF and ARR improved water quality with respect to suspended solids and microbial quality and stabilized the range of values for many water quality parameters, making downstream drinking water treatment more predictable and easier to control. In this field study a pesticide was removed from the river water rather than reintroduced. As mineral content of the water increased in ARR, it underscored the importance of the aquifer formation, as the increase in hardness or in nuisance constituents, such as iron and manganese, can increase the cost of downstream water treatment. However, attenuation of TOC, TSS and microbial counts can provide significant savings for water treatment. It is important to factor in the cost of pumping associated with ARR when evaluating the impact of this environmental buffer on the cost of water treatment.

4.2 CONSIDERATIONS FOR CHOSING BETWEEN DRP AND IPR

When selecting between DPR and IPR there are the following factors that are important to consider:

If the goal of the environmental buffer to provide additional treatment to effluent, wetlands can certainly provide additional treatment to effluent impacted streams and are of benefit to IPR. In implementation of wetlands, it is important to consider impacts of

runoff and mineral leaching from soil. In constructed wetlands, those factors can sometimes be controlled. Release into a lake or a river does not provide additional treatment that would be of economic benefit to a wastewater treatment plant. It can only be of benefit if the lake or a river provides considerable dilution to the effluent by baseflow that is not heavily impacted by upstream wastewater discharges and other human activity, which is rarely available in the areas where potable reuse is being considered.

While RBF and ARR can improve water quality and stabilize the range of values for a number of water quality parameters, it is only true when these processes applied to environmental waters, as was the case with river water. In the case of AWPF effluent being used for ARR, the only benefit to such arrangement is if purified water can be released without remineralization, as subsurface environment can provide effective remineralization and pH balancing. However, it is only possible if purified water does not need to be transported to the ARR location. If ARR cannot be located in the immediate vicinity of the purification facility, remineralization is necessary for transport to prevent pipe corrosion. In that scenario, ARR provides no additional benefit to treated water. In fact, depending on the urban or agricultural impacts on groundwater, such practice can introduce or reintroduce contaminants. Often, those are unregulated contaminants, e.g. pesticides, and would not impact the cost of water treatment. However, in agriculture impacted areas nitrate can approach regulatory levels. Additionally, hardness, iron and manganese are also of consideration.

CHAPTER 5. REFERENCES

1. NDMC, Percent Population in U.S. Drought Monitor Categories. In *Tabular Data Archive*, 2018 ed.; National Drought Mitigation Center: Lincol, NE, 2019.
2. Census, U., In *State Population Totals and Components of Change: 2010-2018*, U.S. Department of Commerce: 2019.
3. Charlotte - Recent Annual Temperatures, Rain & Snow.
4. Schmidt, C. S., The yuck factor: when disgust meets discovery. *Environmental Health Perspe* **2008,** *116* (12), A524-7.
5. Schmidt, C. W., The yuck factor: when disgust meets discovery. *Environ Health Perspect* **2008,** *116* (12), A524-7.
6. Painter, M. M. B., M. A.; Julius, M. L.; Vajda, A. M.; Norris, D. O.; Barber, L. B.; Furlong, E. T.; Schultz, M. M.; Schoenfuss, H. L., Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas). *Environmental Toxicology & Chemistry* **2009,** *28* (12), 2677-2684.
7. Langer, G., POLL: What Americans Eat for Breakfast. In *ABC News*, 2005.
8. Conners, D. E. R., E. D.; Armbrust, K. L.; Jeong-Wook, K.; Black, M. C.,, Growth ans development of tadpoles (Xenopus laevis) exposed to selective serotonin peuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. *Environmental Toxicology & Chemistry* **2009,** *28* (12), 2671-2676.
9. Akiyama, T.; Savin, M. C., Populations of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream. *Science of The Total Environment* **2010,** *408* (24), 6192-6201.
10. Chait, R.; Palmer, A. C.; Yelin, I.; Kishony, R., Pervasive selection for and against antibiotic resistance in inhomogeneous multistress environments. *Nature Communications* **2016,** *7*, 10333.
11. Goñi-Urriza, M.; Capdepuy, M.; Arpin, C.; Raymond, N.; Caumette, P.; Quentin, C., Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp. *Applied and Environmental Microbiology* **2000,** *66* (1), 125-132.
12. Goñi-Urriza, M. C., M.; Arpin, C.; Raymond, N.; Caumette, P.; Quentin, C., Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp. *Applied and Environmental Microbiology* **2000,** *66* (1), 125-132.
13. Akiyama, T. S., M. C., Populations of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream. *Science of The Total Environment* **2010,** *408* (24), 6192-6201.
14. Bai, J.; Cui, B.; Cao, H.; Li, A.; Zhang, B., Wetland Degradation and Ecological Restoration. *The Scientific World Journal* **2013,** *2013*, 523632.
15. District, O. C. W., Prado Wetlands Fact Sheet.
16. Nag, S. K.; Liu, R.; Lal, R., Emission of greenhouse gases and soil carbon sequestration in a riparian marsh wetland in central Ohio. *Environmental Monitoring and Assessment* **2017,** *189* (11), 580.
17. *Wetland Functions and Values*; Watershed Academy Web.

18. Giácoman-Vallejos, G.; Ponce-Caballero, C.; Champagne, P., Pathogen removal from domestic and swine wastewater by experimental constructed wetlands. *Water Science and Technology* **2015,** *71* (8), 1263-1270.
19. Hansen, A. T.; Dolph, C. L.; Foufoula-Georgiou, E.; Finlay, J. C., Contribution of wetlands to nitrate removal at the watershed scale. *Nature Geoscience* **2018,** *11* (2), 127-132.
20. Irwin, N. B.; Irwin, E. G.; Martin, J. F.; Aracena, P., Constructed wetlands for water quality improvements: Benefit transfer analysis from Ohio. *Journal of Environmental Management* **2018,** *206*, 1063-1071.
21. Tao, W.; Sauba, K.; Fattah, K. P.; Smith, J. R., Designing constructed wetlands for reclamation of pretreated wastewater and stormwater. *Reviews in Environmental Science and Biotechnology* **2017,** *16* (1), 37-57.
22. Kadlec, R. H., Comparison of free water and horizontal subsurface treatment wetlands. *Ecological Engineering* **2009,** *35* (2), 159-174.
23. Lin, Y.-F.; Jing, S.-R.; Lee, D.-Y.; Wang, T.-W., Nutrient removal from aquaculture wastewater using a constructed wetlands system. *Aquaculture* **2002,** *209* (1), 169-184.
24. Crites RW, M. E., Bastian RK, Reed SC, *Natural wastewater treatment systems*. 2nd ed.; CRC Press: Boca Raton, 2014.
25. Ghermandi, A.; Bixio, D.; Thoeye, C., The role of free water surface constructed wetlands as polishing step in municipal wastewater reclamation and reuse. *Science of The Total Environment* **2007,** *380* (1), 247-258.
26. Kadlec RH, W. S., *Treatment wetlands*. 2nd ed.; CRC Press: Boca Raton, 2009.
27. *Groundwater Ecology*. Academic Press: 1994; p 571.
28. Kebede, S., *Functions of Groundwater*. 2013; p 205-220.
29. *Redox Conditions in Contaminated Ground Water*; U.S. GEOLOGICAL SURVEY: Washington D.C., 2006.
30. Kvitsand, H. M. L.; Myrmel, M.; Fiksdal, L.; Østerhus, S. W., Evaluation of bank filtration as a pretreatment method for the provision of hygienically safe drinking water in Norway: results from monitoring at two full-scale sites. *Hydrogeology Journal* **2017,** *25* (5), 1257-1269.
31. USEPA *Potable Reuse Compendium*; 2017.
32. Drewes, J.; Heberer, T.; Rauch, T.; Reddersen, K., Fate of pharmaceuticals during groundwater recharge. *Groundw. Monit. Remediat* **2002,** *23*, 64-72.
33. Bouwer, H., Role of Groundwater Recharge in Treatment and Storage of Wastewater for Reuse. *Water Science and Technology* **1991,** *24* (9), 295-302.
34. McCabe, D., Rivers and Streams: Life in Flowing Water. *Nature Education and Knowledge* **2011,** *3* (10), 19.
35. Gren, I.-M.; Groth, K.-H.; Sylvén, M., Economic values of Danube floodplains. *Journal of environmental management* **1995,** *45*, 333-345.
36. Costanza, R.; d'Arge, R.; groot, R. d.; Farber, S.; Grasso, M.; Hannon, B.; Limburg, K.; Naeem, S.; O'Neill, R. V.; Paruelo, J.; Raskin, R. G.; Sutton, P.; Belt, M. v. d., The value of the world's ecosystem services and natural capital. *Nature* **1997,** *387*, 253-260.
37. Atwood, D.; Paisley-Jones, C., Pesticides industry sales and usage: 2008-2012 market estimates. *U.S. Environmental Protection Agency* **2017**, 1-32.

38. Hoffman, E. J.; Mills, G. L.; Latimer, J. S.; Quinn, J. G., Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. *Environmental Science & Technology* **1984,** *18* (8), 580-587.
39. Groundwater Replenishment System Technical Brochure. District, O. C. W., Ed. 2018.
40. Saphr, N. E. B., S.R.; and Hammond, S.E. *SELECTED HYDROLOGIC DATA FOR THE SOUTH PLATTE RIVER THROUGH DENVER, COLORADO*; USGS, U.S. Department of the Interior: Lakewood, CO, 1985.
41. Deere and Ault Consultants, I. In *Aurora's Prarie Waters Project*, American Ground Water Trust Conference, 2012.
42. NCDWQ *Chapter 4*; 2010.
43. USEPA *Practical Methods for Data Analysis*; USEPA: Washington DC, 2000.
44. Croghan, C. A. P. P. E., METHODS OF DEALING WITH VALUES BELOW THE LIMIT OF DETECTION USING SAS. In *Southeastern SAS User Group, St.* , Petersburg, FL, 2003.
45. Babcsányi, I.; Imfeld, G.; Granet, M.; Chabaux, F., Copper Stable Isotopes To Trace Copper Behavior in Wetland Systems. *Environmental Science & Technology* **2014,** *48* (10), 5520-5529.
46. Lung, W.-S.; Light, R. N., Modelling copper removal in wetland ecosystems. *Ecological Modelling* **1996,** *93* (1), 89-100.
47. Knox, A. S.; Nelson, E.; Halverson, N.; Gladden, J., Long-Term Performance of a Constructed Wetland for Metal Removal. *Soil and Sediment Contamination: An International Journal* **2010,** *19* (6), 667-685.
48. Knox, A. S.; Dunn, D.; Paller, M.; Nelson, E. A.; Specht, W. L.; Seaman, J. C., Assessment of Contaminant Retention in Constructed Wetland Sediments. *Engineering in Life Sciences* **2006,** *6* (1), 31-36.
49. Donahoe, T. J. L., C.; Dobson, K.; Granham, E., Cycling of iron and manganese in a Riparian wetland In *Goldschmidt Conference Edinburgh 1994*, Edinburgh, Schottland, 1994.
50. Fuge, R.; Johnson, C. C., Iodine and human health, the role of environmental geochemistry and diet, a review. *Applied Geochemistry* **2015,** *63*, 282-302.
51. Board, C. R. W. Q. C., Treatment Wetlands and Sea Level Rise: Ensuring the San Francisco Bay Water Board's
Wetland Protection Policies are Climate Change Ready. Control, S. F. B. R. W. Q., Ed. Oakland, CA 94612, 2018.
52. Zhang, S.; Ho, Y.-F.; Creeley, D.; Roberts, K. A.; Xu, C.; Li, H.-P.; Schwehr, K. A.; Kaplan, D. I.; Yeager, C. M.; Santschi, P. H., Temporal Variation of Iodine Concentration and Speciation (127I and 129I) in Wetland Groundwater from the Savannah River Site, USA. *Environmental Science & Technology* **2014,** *48* (19), 11218-11226.
53. Gunnarsdottir, I.; Dahl, L., Iodine intake in human nutrition: a systematic literature review. *Food & nutrition research* **2012,** *56*, 10.3402/fnr.v56i0.19731.
54. Gurtle, J. C. B. A. A. F. R. B. B. H. W. G. H. H. a. G. C., Sources and Content of Iodine in California Milk and Dairy Products. *Journal of Food Protection* **1982,** *46* (1), 41-46.

55. Hladik, M. L.; Hubbard, L. E.; Kolpin, D. W.; Focazio, M. J., Dairy-Impacted Wastewater Is a Source of Iodinated Disinfection Byproducts in the Environment. *Environmental Science & Technology Letters* **2016,** *3* (5), 190-193.
56. Postigo, C.; Richardson, S. D.; Barceló, D., Formation of iodo-trihalomethanes, iodo-haloacetic acids, and haloacetaldehydes during chlorination and chloramination of iodine containing waters in laboratory controlled reactions. *Journal of Environmental Sciences* **2017,** *58* (C), 127-134.
57. Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; Demarini, D. M., Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research-Reviews in Mutation Research* **2007,** *636* (1), 178-242.
58. Ackerson, N. O. B.; Machek, E. J.; Killinger, A. H.; Crafton, E. A.; Kumkum, P.; Liberatore, H. K.; Plewa, M. J.; Richardson, S. D.; Ternes, T. A.; Duirk, S. E., Formation of DBPs and halogen-specific TOX in the presence of iopamidol and chlorinated oxidants. *Chemosphere* **2018,** *202*, 349-357.
59. Cancho, B.; Ventura, F.; Galceran, M.; Diaz, A.; Ricart, S., Determination, synthesis and survey of iodinated trihalomethanes in water treatment processes. *Water Research* **2000,** *34* (13), 3380-3390.
60. Ikari, M.; Matsui, Y.; Suzuki, Y.; Matsushita, T.; Shirasaki, N., Removal of iodide from water by chlorination and subsequent adsorption on powdered activated carbon. *Water Research* **2015,** *68*, 227-237.
61. Ibekwe, A. M. L., S.R.; Leddy, M., Jacobson-Meyers, M., Impact of Plant Density and Microbial Composition on Water Quality from a Free Water Surface Constructed Wetland. *Journal of Applied Microbiology* **2006,** *102*, 921-936.
62. Buckley, K. M., M., Phosphorus in Livestock Manures. In *Advanced Silage Corn Management: A Production guide for coastal British Columbia and the Pacific Northwest.*, Bittman, S. K., C.G., Ed. The Pacific Field Corn Association: 2004.
63. Li, G. L., H.; Leffelaar, P.A.; Shen, J., Zhang, F. , Characterization of Phosphorus in Animal Manures Collected from Three (Dairy, Swine, and Broiler) Farms in China. *PLoS ONE* **2014,** *9* (7).
64. Knowlton, K. F., Kohn, R. In *Feeding management to reduce phosphorus losses from dairy farms*, Proceedings of the Mid Atlantic Dairy Management Conference 1999; pp 24–25.
65. G., T., *The Ecophysiology of Plant-Phosphorus Interactions*. Springer, Dordrecht: 2008; Vol. 7.
66. Ibekwe, A. M.; Murinda, S. E.; Graves, A. K., Microbiological evaluation of water quality from urban watersheds for domestic water supply improvement. *International journal of environmental research and public health* **2011,** *8* (12), 4460-4476.
67. Pant, H. K. R., K. R., Hydrologic influence on stability of organic phosphorus in wetland detritus. *Journal of Environmental Quality* **2001,** *30* (2), 668-674.
68. Rittmann, B. E. M., P. L., *Environmental biotechnology: Principles and applications*. McGraw-Hill: Boston, 2000.
69. Thompson, L. M., *Soils and soil fertility*. Oxford University Press: New York, 1993.

70. Turner, B. L. N., S.; Newman, J. M., Organic Phosphorus Sequestration in Subtropical Treatment Wetlands. *Environmental Science & Technology* **2006,** *40* (3), 727-733.
71. Julian II, P. G., S.; Bhomia, R.; King, J.; Osborne, T.S.; Wright, A.L., Evaluation Of Nutrient Stoichiometric Relationships Amongst Ecosystem Compartments Of A Subtropical Treatment Wetland. Fine-Scale Analysis Of Wetland Nutrient Stoichiometry. *bioRxiv* **2017**.
72. Gusewell, S. K., W., Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology, Evolution and Systematics* **2002,** *5* (1), 37-61.
73. Cheesman, A. W. T., B.L.; Reddy, K. R., Forms of Organic Phosphorus in Wetland Soils. *Biogeosciences* **2014,** *11*, 6697-6710.
74. Mathews, L. C., N., The Ratios of Carbon, Nitrogen, and Phosphorus in a Wetland Coastal Ecosystem of Southern India. *International Review of Hydrobiology* **2003,** *88* (2), 179-186.
75. O'Green, A. T. B., M. L. *Using Wetlands to Remove Microbial Pollutants from Farm Discharge Water*; University of California, Agricultural and Natural Resources: Richmond, CA, 2015.
76. Water, O. o., Wastewater Technology Fact Sheet Free Water Surface Wetlands. Agency, U. S. E. P., Ed. Washington D.C., 2000.
77. Characklis, G. W.; Dilts, M. J.; Simmons, O. D.; Likirdopulos, C. A.; Krometis, L.-A. H.; Sobsey, M. D., Microbial partitioning to settleable particles in stormwater. *Water Research* **2005,** *39* (9), 1773-1782.
78. Karim, M. G. E. P. G., C. P., The effect of wetland vegetation on the survival of Escherichia coli, Salmonella typhimurium, bacteriophage MS-2 and polio virus. *Journal of Water and Health* **2008,** *6* (2), 167-175.
79. Whitman, R. L. N., M. B.; Korinek, G. C., Byappanahalli, M. N., Solar and temporal effects on Escherichia coli concentration at a Great Lakes swimming beach. *Applied and Environmental Microbiology* **2004,** *70*, 4276-4285.
80. Khatiwada, N. R. P., C., Kinetics of Fecal Coliform Removal in Constructed Wetlands. *Water Science and Technology* **1999,** *40* (3), 109-116.
81. Zedler, J. B., Wetlands at your service: Reducing impacts of agriculture at the watershed scale. *Frontiers in Ecology and the Environment* **2003,** *1*, 65-72.
82. Huang, J. Y.; Patrick, M. E.; Manners, J.; Sapkota, A. R.; Scherzinger, K. J.; Tobin-D'Angelo, M.; Henao, O. L.; Cole, D. J.; Vieira, A. R., Association between wetland presence and incidence of Salmonella enterica serotype Javiana infections in selected US sites, 2005–2011. *Epidemiology and Infection* **2017,** *145* (14), 2991-2997.
83. Quiñónez-Dìaz, M. D. J.; Karpiscak, M. M.; Ellman, E. D.; Gerba, C. P., REMOVAL OF PATHOGENIC AND INDICATOR MICROORGANISMS BY A CONSTRUCTED WETLAND RECEIVING UNTREATED DOMESTIC WASTEWATER. *Journal of Environmental Science and Health, Part A* **2001,** *36* (7), 1311-1320.
84. Thurston, J.; Gerba, C.; E Foster, K.; M Karpiscak, M., *Fate of indicator microorganisms, Giardia and Cryptosporidium in subsurface flow constructed wetlands*. 2001; Vol. 35, p 1547-51.

85. DiGiorgio, C. L.; Gonzalez, D. A.; Huitt, C. C., Cryptosporidium and giardia recoveries in natural waters by using environmental protection agency method 1623. *Applied and environmental microbiology* **2002,** *68* (12), 5952-5955.
86. R Karim, M.; D Manshadi, F.; M Karpiscak, M.; Gerba, C., *The persistence and removal of enteric pathogens in constructed wetlands*. 2004; Vol. 38, p 1831-7.
87. García-R, J.; French, N.; Grinberg, A.; Pita, A.; Velathanthiri, N.; Hayman, D., *Local and Global genetic diversity of protozoan parasites: Spatial distribution of Cryptosporidium and Giardia genotypes*. 2017.
88. Feng, Y.; Xiao, L., Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clinical microbiology reviews* **2011,** *24* (1), 110-140.
89. Jasper, J. T.; Nguyen, M. T.; Jones, Z. L.; Ismail, N. S.; Sedlak, D. L.; Sharp, J. O.; Luthy, R. G.; Horne, A. J.; Nelson, K. L., Unit Process Wetlands for Removal of Trace Organic Contaminants and Pathogens from Municipal Wastewater Effluents. *Environmental engineering science* **2013,** *30* (8), 421-436.
90. Storrs, C., Designing Wetlands to Remove Drugs and Chemical Pollutants. *Yale Environment 360* 2015.
91. M.W., L. a. A., K. *Water Treatment and Pathogen Control*; London, UK, 2004.
92. Committee., N. R. C. U. S. D. W. *Disinfection Methods and Efficacy*; National Academies Press (US): Washington D.C., 1987.
93. Granados-Chinchilla, F.; Rodríguez, C., Tetracyclines in Food and Feedingstuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. *Journal of analytical methods in chemistry* **2017,** *2017*, 1315497-1315497.
94. Administration, F. a. D. *Antimicrobials Sold or Distributed for Use in Food-Producing Animals*; Department of Health and Human Services: 2014.
95. Rabolle, M. S., N. H., Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* **2000,** *40* (7), 715-722.
96. Pyörälä, S.; Baptiste, K. E.; Catry, B.; van Duijkeren, E.; Greko, C.; Moreno, M. A.; Pomba, M. C. M. F.; Rantala, M.; Ružauskas, M.; Sanders, P.; Threlfall, E. J.; Torren-Edo, J.; Törneke, K., Macrolides and lincosamides in cattle and pigs: Use and development of antimicrobial resistance. *The Veterinary Journal* **2014,** *200* (2), 230-239.
97. Huang, C.-H. R., J.E.; Smeby,K.L.; Pinkston, K.;Sedlak.D.L. , Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis. *Journal of Contemporary Water Research and Eduction* **2001,** *120*, 30-40.
98. Githinji, L. J. M. M., M.K.; Ankumah, R.O. , Evaluation of the fate of ciprofloxacin and amoxicillin in domestic wastewater. *Water Air Soil Pollution* **2011,** *219*, 191-201.
99. Pikkemaat, M. G. Y., H.; van dr Fels-Klerx, H.J.; Berendsen, B.J.A *Antibiotic Residues and Resistance in the Environment*; 124.73.148.01; RIKILT Wageningen UR: Wageningen, 2016.
100. Shi, J., ; Gardinali, P.R., Photodegradation of caffeine, coprostanol and cephalexin by simulated solar radiation: Implications for environmental fate. In *NATIONAL MEETING- AMERICAN CHEMICAL SOCIETY DIVISION OF ENVIRONMENTAL CHEMISTRY*, American Chemical Society Division of Environmental Chemistry: Atlanta, GA, 2006; Vol. 46, pp 128-132.

101. Lam, M. W. T., K.; Mabury, S. A, PhotoFate: A new approach in accounting for the contribution of indirect photolysis of pesticides and pharmaceuticals in surface waters. *Environmental Science & Technology* **2003,** *37*, 899-907.
102. Hidalgo, M. E. P., C.; Fern´andez, E.; C´ardenas, A. M., Comparative determination of photodegradation kinetics of quinolones. *Photochemistry and Photobiology* **1993,** *73*, 135-138.
103. Tornianen, K., Tammilehto, S. and Ulvi, V, The effect of pH, buffer type and drug concentration on the photochemistry of ciprofloxacin. *International Journal of Pharmaceutics* **1996,** *132*, 53-61.
104. Nowara, A. B., J.; Spiteller, M., Binding of fluoroquinolonecarboxylic acid derivatives to clay minerals. *Journal of Agriculture and Food Chemistry* **1997,** *45*, 1459-1463.
105. Liao, X.; Li, B.; Zou, R.; Dai, Y.; Xie, S.; Yuan, B., Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. *Environmental Science and Pollution Research* **2016,** *23* (8), 7911-7918.
106. Cardoza, L.; Knapp, C.; Larive, C.; Belden, J.; Lydy, M.; Graham, D., Factors Affecting the Fate of Ciprofloxacin in Aquatic Field Systems. *Water, Air, and Soil Pollution* **2005,** *161* (1), 383-398.
107. Zhu, F.; Storey, S.; Ashaari, M.; Clipson, N.; Doyle, E., Benzo(a)pyrene degradation and microbial community responses in composted soil. *Environmental Science and Pollution Research* **2017,** *24* (6), 5404-5414.
108. Sushkova, S.; Deryabkina, I.; Antonenko, E.; Kizilkaya, R.; Rajput, V.; Vasilyeva, G., Benzo[a]pyrene degradation and bioaccumulation in soil-plant system under artificial contamination. *Science of The Total Environment* **2018,** *633*, 1386-1391.
109. Lodovici M, V. M., Marini E, et al, Polycyclic aromatic hydrocarbons air levels in Florence,Italy, and their correlation with other air pollutants. *Chemosphere* **2003,** *50* (377-382).
110. Crampon M, B. F., Akpa-Vinceslas M, Bodilis J, Machour N, Le; Derf F, P.-K. F., Correlations between PAH bioavailability, degrading bacteria, and soil characteristics during PAH biodegradation in five diffusely contaminated dissimilar soils. . *Environmental Science and Pollution Research* **2014,** *21* (13), 8133-8145.
111. Kanaly RA, H., Advances in the field of high molecular weight polycyclic aromatic hydrocarbon biodegradation by bacteria. *Microbial Biotechnology* **2010,** *3* (2), 136-164.
112. Menzie C A; Potocki B B; Santodonato J, Exposure to carcinogenic PAHs in environment. *Environmental Science & Technology* **1992,** *26*, 1278-1384.
113. Monteiro, S. C. B., A.B.A, Occurrence and Fate of Human Pharmaceuticals in the Environment. *Reviews of Environmental Contamination and Toxicology* **2010,** *189*, 53-154.
114. Oppenheimer, J. E., A.; Badruzzaman, M.; Haghani, A. W.; Jacangelo, J.G., Occurrence And Suitability Of Sucralose As An Indicator Compound Of Wastewater Loading To Surface Waters In Urbanized Regions. *Water Research* **2011,** *45* (13), 4019-4027.
115. Zhou, H.; Ying, T.; Wang, X.; Liu, J., Occurrence and preliminarily environmental risk assessment of selected pharmaceuticals in the urban rivers, China. *Scientific Reports* **2016,** *6*, 34928.

116. Kent, R. B., K.; Altmann, A.J.; Wright, M.T.; Mendez, G.O. *Occurrence and Distribution of Pesticide Compounds in Surface Water of the Santa Ana Basin, California, 1998–2001*; U.S. Geological Survey: Sacramento, CA, 2005.
117. Fatta-Kassinos, D. M., S.; Nikolaou, A., Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical Biochemistry* **2010,** *399*, 251-275.
118. Laboratory, A. E. A. *Pharmaceuticals and Personal Care Products in Surface Water - Occurrence, Fate and Transport, and Effect on Aquatic Organisms*; University of California, Davis Aquatic Ecosystems Analysis Laboratory: 2009.
119. Primost, J. E. M., D.J.G.; Aparicio, V.C.; Costa, J.L.; Carriquiriborde, P., Glyphosate and AMPA, "pseudo-persistent" pollutants under realworld agricultural management practices in the Mesopotamic Pampas agroecosystem, Argentina. *Environmental Pollution* **2017,** *229*, 771-779.
120. Nagy, A. S.; Szabó, J.; Vass, I., Occurrence and distribution of polycyclic aromatic hydrocarbons in surface water of the Raba River, Hungary. *Journal of Environmental Science and Health, Part A* **2013,** *48* (10), 1190-1200.
121. Du, J. *Drinking Water Health Advisory for Manganese*; U.S. Environmental Protection Agency Office of Water: Washington, D.C., 2004.
122. Kuo, J.; Chen, C. L.; Nellor, M., Discussion of "Standardized Collimated Beam Testing Protocol for Water⁄Wastewater Ultraviolet Disinfection" by Jeff Kuo, Ching-lin Chen, and Margaret Nellor. *Journal of Environmental Engineering* **2005,** *131129* (5), 827-827.
123. Gong, T.; Zhang, X.; Liu, W.; Lv, Y.; Han, J.; Choi, K. C.; Li, W.; Xian, Q., Tracing the sources of iodine species in a non-saline wastewater. *Chemosphere* **2018,** *205*, 643-648.
124. Pantelaki, I.; Voutsa, D., Formation of iodinated THMs during chlorination of water and wastewater in the presence of different iodine sources. *Science of The Total Environment* **2018,** *613-614*, 389-397.
125. Moreda-Piñeiro, A.; Romarís-Hortas, V.; Bermejo-Barrera, P., A review on iodine speciation for environmental, biological and nutrition fields. *Journal of Analytical Atomic Spectrometry* **2011,** *26* (11), 2107-2152.
126. Muramatsu, Y.; Yoshida, S.; Fehn, U.; Amachi, S.; Ohmomo, Y., Studies with natural and anthropogenic iodine isotopes: iodine distribution and cycling in the global environment. *Journal of Environmental Radioactivity* **2004,** *74* (1), 221-232.
127. Sturini, M.; Speltini, A.; Maraschi, F.; Profumo, A.; Pretali, L.; Irastorza, E. A.; Fasani, E.; Albini, A., Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight. *Applied Catalysis B: Environmental* **2012,** *119-120*, 32-39.
128. Wang, X.-H.; Lin, A. Y.-C., Phototransformation of Cephalosporin Antibiotics in an Aqueous Environment Results in Higher Toxicity. *Environmental Science & Technology* **2012,** *46* (22), 12417-12426.
129. Morillo, E.; Undabeytia, T.; Maqueda, C.; Ramos, A., Glyphosate adsorption on soils of different characteristics.: Influence of copper addition. *Chemosphere* **2000,** *40* (1), 103-107.
130. DoWQ *Lake & Reservoir Assessments Catawba River Basin*; Environmental Sciences Section, Intensive Survey Unit, Division of Water Quality: 2013.

131. Downey, J., State holding open house tonight to present options for closing coal-ash pond at local Duke Energy plant. *Charlotte Buisness Journal* 2019.
132. Okioga, I. T. McDowell Creek Wastewater Improvements Project.
133. Water, C. *Wastewater Performance Report*; Charlotte, NC, 2016.
134. Sasser, G. *Water Treatment Overview*; Charlotte Water: 2016.
135. Wells, J., A rare look inside Cowans Ford dam. *Illumination* 2016.
136. Cotton FA, W. G., Murillo CA, et al.,, *Boron*. 6th ed.; John Wiley & Sons, Inc.: New York, NY, 1999.
137. Wofford, W. T.; Gloyna, E. F.; Johnston, K. P., Boric Acid Equilibria in Near-Critical and Supercritical Water. *Industrial & Engineering Chemistry Research* **1998,** *37* (5), 2045-2051.
138. Hunt, C. D., *Dietary Boron Deficiency and Supplementation*. U.S. Department of Agrifulture: Grand Forks, N.D., 1996.
139. USEPA *Chapter 3: Boron*; USEPA: 2008.
140. Hammes, F.; Seka, A.; de Knijf, S.; Verstraete, W., A novel approach to calcium removal from calcium-rich industrial wastewater. *Water Research* **2003,** *37* (3), 699-704.
141. Evans, M. a. F., C. *The effects of road salts on aquatic ecosystems*; National Water Research Institute and University of Saskatchewan: Saskatoon, Canada, 2001.
142. Roberts, D., Groundwater at Duke Energy coal ash sites faulted for high radioactivity levels. *The Charlotte Observer* March, 20th 2018, 2018.
143. Energy, D. *Marshall Steam Station Ash Basin*; Duke Energy and HDR: 2014.
144. Holleman, F. G., D.J.; and Sullivan, K. *NC coal ash bill leaves 2.6M unprotected from risks*; Souther Environmental Law Center: Charlottesville, VA, 2019.
145. USEPA *Ambient Aquatic Life Water Quality Criteria for Chloride*; 1988.
146. Thompson, G.; Swain, J.; Kay, M.; Forster, C. F., *The Treatment of Pulp and Paper Mill Effluent: A Review*. 2001; Vol. 77, p 275-286.
147. Degryse, F.; da Silva, R. C.; Baird, R.; Beyrer, T.; Below, F.; McLaughlin, M. J., Uptake of elemental or sulfate-S from fall- or spring-applied co-granulated fertilizer by corn—A stable isotope and modeling study. *Field Crops Research* **2018,** *221*, 322-332.
148. Toran, L., Sulfate contamination in groundwater from a carbonate-hosted mine. *Journal of Contaminant Hydrology* **1987,** *2* (1), 1-29.
149. USEPA *National Primary and Secondary Drinking Water Regulations; Synthetic Organic Chemicals and Inorganic Chemicals; Proposed Rule.*; 1990.
150. Drever, J. I., *The Geochemistry of Natural Waters*. 2nd Ed. ed.; Prentice Hall: New Jersey, 1988.
151. Fareleira, P.; S Santos, B.; António, C.; Moradas-Ferreira, P.; LeGall, J.; V Xavier, A.; Santos, H., *Response of a strict anaerobe to oxygen: Survival strategies in Desulfovibrio gigas*. 2003; Vol. 149, p 1513-22.
152. USEPA *Contaminant Candidate List Regulatory Determination Support Document for Sulfate*; USEPA, Standards and Risk Management Division: Washington D.C., 2003.
153. BCJ, Z. *Sensory assessment of water quality*; Pergamon Press. : New York, NY, 1980.
154. NAS *Drinking water and health.* ; National Research Council, National Academy of Sciences. : Washington, DC, 1977.

155. Chien, L.; Robertson, H.; Gerrard, J. W., Infantile gastroenteritis due to water with high sulfate content. *Canadian Medical Association journal* **1968,** *99* (3), 102-104.
156. Davidson, K.; Gowen, R. J.; Harrison, P. J.; Fleming, L. E.; Hoagland, P.; Moschonas, G., Anthropogenic nutrients and harmful algae in coastal waters. *Journal of Environmental Management* **2014,** *146*, 206-216.
157. Anderson, D. M. G., P.M.; Burkholder, J.M., Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* **2002,** *25* (4), 704-726.
158. USEPA *Contaminant occurrence support document for category 2 contaminants for the second six-year review of National Primary Drinking Water Regulations*; United States Environmental Protection Agency, Office of Water Washington D.C., 2009.
159. Jiménez, E.; Giménez, J. B.; Ruano, M. V.; Ferrer, J.; Serralta, J., Effect of pH and nitrite concentration on nitrite oxidation rate. *Bioresource Technology* **2011,** *102* (19), 8741-8747.
160. Grunditz, C.; Dalhammar, G., Development of nitrification inhibition assays using pure cultures of nitrosomonas and nitrobacter. *Water Research* **2001,** *35* (2), 433-440.
161. Organization, W. H. *Nitrate and Nitrite in Drinking-water*; World Health Organization: Geneva, Switzerland, 2011.
162. Groeneweg, J.; Sellner, B.; Tappe, W., Ammonia oxidation in nitrosomonas at NH3 concentrations near km: Effects of pH and temperature. *Water Research* **1994,** *28* (12), 2561-2566.
163. Le, T. T. H.; Fettig, J.; Meon, G., Kinetics and simulation of nitrification at various pH values of a polluted river in the tropics. *Ecohydrology & Hydrobiology* **2018**.
164. Diab, S. S., M. Arch. , Effect of adhesion to particles on the survival and activity of Nitrosomonas sp. and Nitrobacter sp. *Archives of Microbiology* **1988,** *150* (4), 387-393.
165. Gee, C. S.; Pfeffer, J. T.; Suidan, M. T., Nitrosomonas and Nitrobacter Interactions in Biological Nitrification. *Journal of Environmental Engineering* **1990,** *116* (1), 4-17.
166. Hua, G.; Reckhow, D. A.; Kim, J., Effect of Bromide and Iodide Ions on the Formation and Speciation of Disinfection Byproducts during Chlorination. *Environmental Science & Technology* **2006,** *40* (9), 3050-3056.
167. Allard, S.; Nottle, C. E.; Chan, A.; Joll, C.; von Gunten, U., Ozonation of iodide-containing waters: Selective oxidation of iodide to iodate with simultaneous minimization of bromate and I-THMs. *Water Research* **2013,** *47* (6), 1953-1960.
168. Umar, M., Variability in the Concentration of Indicator Bacteria in Landfill Leachate ‐ A Comparative Study. *Water Environment Research* **2015,** *87* (3), 223-226.
169. Fisher, K.; Phillips, C., *The Ecology, Epidemiology and Virulence of Enterococcus*. 2009; Vol. 155, p 1749-57.
170. L.M., B. A. B. S., *Enterococci as Indicators of Environmental Fecal Contamination*. 2014.
171. Polo, F. F. M. J. I., I.; Sala, J.; Fleisher, J.M.; Guarro, J., Relationship between presence of Salmonella and indicators of faecal pollution in aquatic habitats. *FEMS Microbiology Review* **1998,** *160*, 253-256.
172. USEPA 5.11 Fecal Bacteria.

173. Soh, L.; Connors, K. A.; Brooks, B. W.; Zimmerman, J., Fate of Sucralose through Environmental and Water Treatment Processes and Impact on Plant Indicator Species. *Environmental Science & Technology* **2011,** *45* (4), 1363-1369.
174. Mawhinney, D. B.; Young, R. B.; Vanderford, B. J.; Borch, T.; Snyder, S. A., Artificial Sweetener Sucralose in U.S. Drinking Water Systems. *Environmental Science & Technology* **2011,** *45* (20), 8716-8722.
175. Tolls, J., Sorption of Veterinary Pharmaceuticals in Soils: A Review. *Environmental Science & Technology* **2001,** *35* (17), 3397-3406.
176. Kunkel, U.; Radke, M., Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at favorable attenuation conditions. *Water Research* **2012,** *46* (17), 5551-5565.
177. Löffler, D.; Römbke, J.; Meller, M.; Ternes, T. A., Environmental Fate of Pharmaceuticals in Water/Sediment Systems. *Environmental Science & Technology* **2005,** *39* (14), 5209-5218.
178. Sassman, S. A.; Lee, L. S., Sorption of Three Tetracyclines by Several Soils: Assessing the Role of pH and Cation Exchange. *Environmental Science & Technology* **2005,** *39* (19), 7452-7459.
179. Oka, H.; Ikai, Y.; Kawamura, N.; Yamada, M.; Harada, K.; Ito, S.; Suzuki, M., Photodecomposition products of tetracycline in aqueous solution. *Journal of Agricultural and Food Chemistry* **1989,** *37* (1), 226-231.
180. Florence, A. T., Attwood, D., *Physicochemical Principles of Pharmacy.* Chapman and Hall: New York, 1981.
181. Wu, Y. C., D.-H.; Kookana, R. , Aqueous photodegradation of selected antibiotics under different conditions. *Energy Procedia* **2011,** *11*, 2098-2103.
182. Michael, I.; Hapeshi, E.; Osorio, V.; Perez, S.; Petrovic, M.; Zapata, A.; Malato, S.; Barceló, D.; Fatta-Kassinos, D., Solar photocatalytic treatment of trimethoprim in four environmental matrices at a pilot scale: Transformation products and ecotoxicity evaluation. *Science of The Total Environment* **2012,** *430*, 167-173.
183. Guo, J.; Selby, K.; Boxall, A. B. A., Assessment of the Risks of Mixtures of Major Use Veterinary Antibiotics in European Surface Waters. *Environmental Science & Technology* **2016,** *50* (15), 8282-8289.
184. Barra Caracciolo, A., *Persistence of the antibiotic sulfamethoxazole in river water alone or in the co-presence of ciprofloxacin*. 2018.
185. Bush, K.; Jacoby, G. A.; Medeiros, A. A., A functional classification scheme for b-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy* **1995,** *39*, 1211-1233.
186. Corporation, D. E. *Applicant's Environmental Report Operating License Renewal Stage, Applicant's Environmental Report*; 2001.
187. Kenny, D. V. a. O., S.V., Extraction of Lignite Coal Fly Ash for Polynuclear Aromatic Hydrocarbons: Modified and Unmodified Supercritical Fluid Extraction, Enhanced-Fluidity Solvents, and Accelerated Solvent Extraction *Journal of Chromatographic Science* **1998,** *36*, 59 - 65.
188. Masala, S.; Bergvall, C.; Westerholm, R., Determination of benzo[a]pyrene and dibenzopyrenes in a Chinese coal fly ash certified reference material. *Science of The Total Environment* **2012,** *432*, 97-102.

189. Blowes, D. W.; Ptacek, C. J.; Jambor, J. L.; Weisener, C. G., 9.05 - The Geochemistry of Acid Mine Drainage. In *Treatise on Geochemistry*, Holland, H. D.; Turekian, K. K., Eds. Pergamon: Oxford, 2003; pp 149-204.
190. Yung Lu, P.; L. Metcalf, R., *Environmental Fate and Biodegradability of Benzene Derivatives as Studied in a Model Aquatic Ecosystem*. 1975; Vol. 10, p 269-84.
191. Y Lu, P.; L. Metcalf, R.; Plummer, N.; Mandel, D., *The environmental fate of three carcinogens: Benzo-(α)-pyrene, benzidine, and vinyl chloride evaluated in laboratory model ecosystems*. 1977; Vol. 6, p 129-42.
192. Yang, Y. S., W.; Lin, H.; Wang, W.; Du, L.; Xing, W., Antibiotics and antibiotic resistance genes in global lakes: A review and meta-analysis. *Environment International* **2018,** *116*, 60-73.
193. Kim, J. W., M.; Park, J.W.; Brown, J. *Boron Rejection by Reverse Osmosis Membranes: National Reconnaissance and Mechanism Study*; U.S. Department of the Interior Bureau of Reclamation 2009.
194. Magara, Y.; Aizawa, T.; Kunikane, S.; Itoh, M.; Kohki, M.; Kawasaki, M.; Takeuti, H., The behavior of inorganic constituents and disinfection by products in reverse osmosis water desalination process. *Water Science and Technology* **1996,** *34* (9), 141-148.
195. Prats, D.; Chillon-Arias, M. F.; Rodriguez-Pastor, M., Analysis of the influence of pH and pressure on the elimination of boron in reverse osmosis. *Desalination* **2000,** *128* (3), 269-273.
196. Parks, J. L.; Edwards, M., Boron in the Environment. *Critical Reviews in Environmental Science and Technology* **2005,** *35* (2), 81-114.
197. Rainey, C. N., L.; Casterline, J.; and Herman, N.D., Estimation of Dietary Boron Intake in Six Countries: Egypt, Germany, Great Britain, Kenya, Mexico, and the United States. *Journal of Trace Elements in Medicine and Biology* **1999,** *12*, 263-270.
198. Bakirdere, S.; Orenay Boyacioglu, S.; Korkmaz, M., *Effect of Boron on Human Health*. 2010; Vol. 3, p 54-59.
199. Wimalawansa, S. J., Purification of Contaminated Water with Reverse Osmosis: Effective Solution of Providing Clean Water for Human Needs in Developing Countries. *International Journal of Emerging Technology and Advanced Engineering* **2013,** *3* (12), 75-89.
200. Do, V. T.; Tang, C. Y.; Reinhard, M.; Leckie, J. O., Degradation of Polyamide Nanofiltration and Reverse Osmosis Membranes by Hypochlorite. *Environmental Science & Technology* **2012,** *46* (2), 852-859.
201. Engineer's Report on Groundwater Conditions, Water Supply and Basin Utilization in The Orange County Water District, 2016-2017. District, O. C. W., Ed. 2018.
202. Bilodeau, D. *Orange County Water District Budget Report Fiscal Year 2018-19*; 2018.
203. Marie, S. S., What Will Be the Cost of Future Sources of Water for California? *California Pulic Utilities Commission* 2016.
204. Jeppesen, T.; Shu, L.; Keir, G.; Jegatheesan, V., Metal recovery from reverse osmosis concentrate. *Journal of Cleaner Production* **2009,** *17* (7), 703-707.
205. Burris, D. L. *2017 Annual Report - Groundwater Replenishment System*; Orange County Water District: Irvine, CA, 2018.

206. Scott-Roberts, S. *Engineer's Report for the Groundwater Replinishment System Final Expansion Project*; Orange County Water District: Orange County, CA, 2016.
207. Palma, C. o. L. *2018 Water Quality Report*; City of La Palma Community Services Department: La Palma, CA, 2018.
208. Palma, C. o. L. *City of La Palma Water Quality Report*; City of La Palma Community Services Department: La Palma, CA, 2015.
209. Balouch, A.; Kolachi, M.; Talpur, F. N.; Khan, H.; Bhanger, M. I., Sorption Kinetics, Isotherm and Thermodynamic Modeling of Defluoridation of Ground Water Using Natural Adsorbents. *American Journal of Analytical Chemistry* **2013,** *4* (5), 221-228.
210. *Soil Mineralogy with Environmental Applications*. Soil Science Society of America: Madison, Wisconsin, 2002.
211. Fujita, Y. R., G. D.; Ingram, J. C.; Cortez, M. M.; Ferris, F. G.; Smith, R. W. , Strontium incorporation into calcite generated by bacterial ureolysis. *Geochimica et Cosmochimica Acta* **2004,** *68*, 3261-3270.
212. Roden, E. E. L., M. R.; Ferris, F. G.;, Immobilization of strontium during iron biomineralization coupled to dissimilatory hydrous ferrous oxide reduction. *Geochimica et Cosmochimica Acta* **2002,** *66*, 2823-2839.
213. Dyreborg, S.; Arvin, E.; Broholm, K., Biodegradation of NSO-compounds under different redox-conditions. *Journal of Contaminant Hydrology* **1997,** *25* (3), 177-197.
214. Schulze, S.; Tiehm, A., Assessment of microbial natural attenuation in groundwater polluted with gasworks residues. *Water science and technology : a journal of the International Association on Water Pollution Research* **2004,** *50* (5), 347-53.
215. *Hardness in Drinking-water* World Health Organization Geneva, Switzerland, 2011.
216. *Hardness in Groundwater*; British Columbia Ground Water Association: 2007.
217. Council, N. R. *Drinking water and health*; National Academy of Sciences: Washington, DC, 1977.
218. Papaspyrou R.; Smith, C. J. D., L.F.; Whitby, C.; Dumbrell, A.J.; Nedwell D.B., Nitrate Reduction Functional Genes and Nitrate Reduction Potentials Persist in Deeper Estuarine Sediments. Why? *PLoS ONE* **2014,** *9* (4).
219. Wick, K.; Heumesser, C.; Schmid, E., Groundwater nitrate contamination: factors and indicators. *Journal of environmental management* **2012,** *111* (3), 178-186.
220. Burow, K. R. N., B.T.; Rupert, M.G.; Dubrovsky, N.M., Nitrate in Groundwater of the United States, 1991-2003. *Environmental Science & Technology* **2010,** *44*, 4988-4997.
221. Nolan, B. T. R., B.C.; Hitt, K.J.; Helsel D.H., A National Look at Nitrate Contamination of Ground Water. *Water Conditioning and Purification* **1998,** *39* (12), 76-79.
222. Almasri, M. N., Nitrate contamination of groundwater: A conceptual management framework. *Environmental Impact Assessment Review* **2007,** *27* (3), 220-242.
223. Chowdary, V. M.; Rao, N. H.; Sarma, P. B. S., Decision support framework for assessment of non-point-source pollution of groundwater in large irrigation projects. *Agricultural Water Management* **2005,** *75* (3), 194-225.
224. Phosphorus in Minnesota's Ground Water. Division, E. O., Ed. Minnesota Pollution Control Agency: Saint Paul, MN, 1999.

225. Capel, P. D. H., P. A.; and Erwin, M. L., Studies by the U. S. Geological Survey on sources, transport, and fate of agricultural chemicals. *U. S. Geological Survey Fact Sheet* **2004**, 2004-3098.
226. Domagalski, J. L. a. J., H. M. ;, Comparative Study of Phosphorus Transport in the Unsaturated Zone, Groundwater, Streams, and Tile Drains at Five Agricultural Watersheds, U.S.A. *Journal of Hydrology* **2011**, 2009-3042.
227. MacQuarrie, K. T. B.; Sudicky, E. A.; Robertson, W. D., Numerical simulation of a fine-grained denitrification layer for removing septic system nitrate from shallow groundwater. *Journal of Contaminant Hydrology* **2001,** *52* (1), 29-55.
228. Crawford, J. L. Effects of Inorganic Nutrients and Dissolved Organic Carbon on Oxygen Demand in Select Rivers in Northern Utah. Utah State University, Logan, UT, 2013.
229. Jodeh, S.; Staiti, H.; Haddad, M.; Rinno, T.; Zaid, A. n.; Jaradat, N.; Kharoaf, M., *The fate of leachate of pharmaceuticals like amoxicillin, ibuprofen and caffeine in the soil using soil columns*. 2012; Vol. 3, p 480-484.
230. Boy-Roura, M.; Mas-Pla, J.; Petrovic, M.; Gros, M.; Soler, D.; Brusi, D.; Menció, A., Towards the understanding of antibiotic occurrence and transport in groundwater: Findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain). *Science of The Total Environment* **2018,** *612*, 1387-1406.
231. Robertson, W. D.; Van Stempvoort, D. R.; Spoelstra, J.; Brown, S. J.; Schiff, S. L., Degradation of sucralose in groundwater and implications for age dating contaminated groundwater. *Water Research* **2016,** *88*, 653-660.
232. Vazquez-Roig, P.; Andreu, V.; Blasco, C.; Picó, Y., Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego–Oliva Marshlands (Valencia, eastern Spain). *Science of The Total Environment* **2012,** *440*, 24-32.
233. Tolls, J., Sorption of veterinary pharmaceuticals in soils: a review. *Environmental Science & Technology* **2001,** *35*, 3397-3406.
234. Boxall, B.; Fogg, L.; Blackwell, P. K.; Pmberton, E. J.; Croxford, A., Review of veterinary medicines in the environment. *Reviews of Environmental Contamination and Toxicology* **2002,** *180*, 1-91.
235. Héquet, V.; Gonzalez, C.; Le Cloirec, P., Photochemical processes for atrazine degradation: methodological approach. *Water Research* **2001,** *35* (18), 4253-4260.
236. El-Bestawy, E.; Sabir, J.; Mansy, A. H.; Zabermawi, N., Isolation, identification and acclimatization of Atrazine-resistant soil bacteria. *Annals of Agricultural Sciences* **2013,** *58* (2), 119-130.
237. Prosen, H. Z.-K., L., Evaluation of photolysis andhydrolysis of atrazine and its first degradation products in the presence of humicacids. *Environmental Pollution* **2005,** *133*, 517-529.
238. Gamble, D. S. K., S. U., Atrazine hydrolysis in soils:Catalysis by the acidic functional groups of fulvic acid. *Canadian Journal of Soil Science* **1985,** *65*, 435-443.
239. Neumann, G. T., R.; Monson, L.; Kivisaar, M.; Schauer, F.; Heipieper, H. J., Simultaneous degradation of atrazine and phenol by Pseudomonas spstrain ADP: Effects of toxicity and adaptation. *Applied and Environmental Microbiology* **2004,** *70*, 1907-1912.

240. Radosevich, M. T., O. H., Microbial degradation ofatrazine in soils, sediments, and surface water. *Pesticide Decontaminationand Detoxification: ACS Symposium Series* **2004,** *863*, 129-139.
241. Schwab, A. P.; Splichal, P. A.; Banks, M. K., Persistence of Atrazine and Alachlor in Ground Water Aquifers and Soil. *Water Air Soil Pollution* **2006,** *171* (1-4), 203-235.
242. Underwood, J. C.; Harvey, R. W.; Metge, D. W.; Repert, D. A.; Baumgartner, L. K.; Smith, R. L.; Roane, T. M.; Barber, L. B., Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. *Environmental Science & Technology* **2011,** *45*, 3096-3101.
243. Chen, H.; Gao, B.; Li, H.; Ma, L. Q., Effects of pH and ionic strength on sulfamethoxazole and ciprofloxacin transport in saturated porous media. *Journal of Contaminant Hydrology* **2011,** *126*, 29-36.
244. Nam, S. W.; Choi, D. J.; Kim, S. K.; Her, N.; Zoh, K. D., Adsorption characteristics of selected hydrophilic and hydrophobic micropollutants in water using activated carbon. *Journal of Hazardous Materials* **2014,** *270*, 144-152.
245. Rendon-von Osten, J.; Dzul-Caamal, R., Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchén, Campeche, Mexico. *International journal of environmental research and public health* **2017,** *14* (6), 595.
246. la Cecilia, D.; Tang, F. H. M.; Coleman, N. V.; Conoley, C.; Vervoort, R. W.; Maggi, F., Glyphosate dispersion, degradation, and aquifer contamination in vineyards and wheat fields in the Po Valley, Italy. *Water Research* **2018,** *146*, 37-54.
247. Mills, P. J.; Kania-Korwel, I.; Fagan, J.; McEvoy, L. K.; Laughlin, G. A.; Barrett-Connor, E., Excretion of the Herbicide Glyphosate in Older Adults Between 1993 and 2016Urinary Excretion of the Herbicide Glyphosate in Older Adults, 1993-2016Letters. *JAMA* **2017,** *318* (16), 1610-1611.
248. Kollman, W. S., R. *Interim report of the pesticide chemistry database*; Department of Pesticide Regulation.: 1995.
249. Bronstad, J. O. F., H.O., *The Herbicide Glyphosate*. Butterworth and Co. Ltd: Toronto, Canada, 1985.
250. Kirkwood, R. C., *Advance in Pesticide Science*. Pergamon Press: Oxford, 1979.
251. Ghassemi, M. F., L.; Painter, P.; Quinlivan, S.; Scofield, R.; Takata, A. *Environmental fates and impacts of major forest use pesticides*; U.S. EPA: Washington D.C., 1981.
252. Franz, J. E. M., M.K.; Sikorski, J.A. *Glyphosate: A Unique Global Herbicide*; American Chemical Society: 1997; pp 65-97.
253. Rueppel, M. L., Metabolism and degradation of glyphosate in soil and water. *Journal of Agriculture and Food Chemistry* **1977,** *25* (3), 517-528.
254. Twardowska, I.; Kolodziejczyk, A. M., Benzoapyrene in soils and ground water: Occurrence, sources, distribution, interrelation. *Toxicological & Environmental Chemistry* **1998,** *66* (1-4), 127-144.
255. Abdel-Shafy, H. I.; Mansour, M. S. M., A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* **2016,** *25* (1), 107-123.