

MICROBIAL COMMUNITY RESPONSE TO CONTAMINANTS OF
EMERGING CONCERN IN THE KĀNE‘OHE WATERSHED

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We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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For Dad

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ABSTRACT:

Coastal pollution is an important concern as it affects local ecosystems, public health, and aquifer reserves. Anthropogenic contaminants travel through both surface runoff and groundwater movement in streams and can have a continuous presence through consistent use. A survey on groundwater discharge flux within the Kāneʻohe Watershed (Oʻahu, Hawaiʻi) found that groundwater volumetric contributions equaled surface runoff, supporting the hypothesis that groundwater discharge contributes significantly to water quality in streams and the coastal ocean. This influence includes submarine groundwater discharge (SGD), which has been recently demonstrated to be a major source of Contaminants of Emerging Concern (CEC) to nearshore environments on Oʻahu. This project aims to observe distributions of CECs found in the Northern Kāneʻohe watershed - glyphosate, caffeine, and sulfamethoxazole - and how they may interact with microbial community metabolism. I hypothesized that CEC concentration will differ between streams according to onsite sewage disposal system (OSDS) density, CECs will be differentially attenuated along stream reaches in different areas of the watershed, and that water column microbial degradation would differ among upstream, downstream, and estuarine habitats. Specifically, I examined which areas in the stream system may contain naturally occurring microbial taxa able to attenuate the studied CECs and asked what this would mean for microbial processes in the Kahaluʻu and ʻĀhuimanu streams. To test whether the contaminants of interest could be attenuated by natural aquatic microbial communities I added CECs to water samples and measured changes in contaminant concentrations and associated growth responses by the microbe community. These observations will help us understand the prevalence of CECs in this region and whether their concentrations can be attenuated through microbial interactions affecting their residence time.

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CHAPTER 1:

INTRODUCTION:

1.1 Background

1.1.1 Global impacts of pollution on coastal areas:

A significant issue that affects many coastal ecosystems around the world is the management of pollution sources. Pollution, defined by the United Nations Convention on the Law of the Sea, includes substances specifically introduced by humans that result in deleterious effects such as harm to resources, marine life, and human health (Islam & Tanaka, 2004). There are many types of pollutants that can each create their own specific effects but are often interconnected in their overall impact on the environment. For example, some of the major types of pollutants, fertilizers, and sewage effluent, have been observed to promote rapid algal blooms that decrease dissolved oxygen levels and increase toxin production causing a rise in aquatic organism mortality and risk to public health (Islam & Tanaka, 2004 & Chakraborty, et al., 2017). Toxic algal blooms have been recorded to increase in scale and severity over the last twenty years on coasts around the world (Brunei, Malaysia, South Africa, Hong Kong, Japan, and Thailand) as anthropogenic input to coasts has only increased (Islam & Tanaka, 2004).

There are also specific physiological effects on coastal organisms and humans from pollutants sourced from pharmaceuticals & personal care products (PPCPs). Even at relatively low concentrations, PPCPs can still be a concern due to many of them being designed to be biologically active at low doses, which can lead to adverse effects on non-target organisms once released into the environment (Ebele et al., 2017). These effects on coastal organisms include disruption of the endocrine system (Ebele et al., 2017), neurotoxic effects, cellular damage, etc. (Vieira et al., 2022). As for impacts on public

health, the use of PPCPs, which include several types of antibiotics, may lead to the potential creation of antibiotic-resistant bacteria in natural microbe populations (Ebele et al., 2017). Positive correlations were observed between antibiotic-resistant microorganisms and trace concentrations of aquatic antibiotic contaminants along with producing detrimental effects on other naturally occurring bacteria present in the environment (Novo et al., 2013).

A majority of pollutants from these groups are specifically categorized as Contaminants of Emerging Concern (CECs). CECs are defined as pollutants sourced from human activity that can potentially cause ecological and human health harm, they are also typically not regulated under current environmental laws (EPA, 2015). There are multiple pathways for CECs to enter streams and coastal areas, including surface runoff (Tian et al., 2021), submarine groundwater discharge (Welch et al., 2019), and wastewater effluent (Novo et al., 2013). Some CECs have even been observed to remain in the water for months, which results in chronic exposure to aquatic organisms (Tian et al., 2021). Observing the inventories of CECs in streams along with measuring if it correlates with notable groundwater input can help discover whether CECs present there have the potential to create the impacts described above. The observation of CEC effects on microbial density in water samples can also potentially show the first signs of CECs being biologically active in an environment. This experiment carries out both of these methods to learn more about CEC presence and potential effects on water quality in Kāneʻohe Bay.

1.1.2 Site location

Kāneʻohe Bay covers an area of 46 km² on the Northeast side of Oʻahu. Constant tidal flushing through its two entrance channels gives a short residence time of water from 12-24 days based on location (Laws, 2017). Yet the barrier reef spanning across the

mouth of the bay does restrict circulation between the coast and open ocean. This feature is significant to note when considering how the surrounding Kāneʻohe Bay Watershed runoff potentially impacts the coastal ecosystem. The watershed covers the adjacent land area encircled by a cliff 500-850 m above sea level; runoff flow in this area is distributed among 11 streams. Yet during heavy rain events, streams may overflow causing runoff flow from all shoreline areas (Laws, 2017). This makes the environmental conditions of the bay variable to changes in land use and the subsequent land runoff change.

Prior to the 1940's, the watershed was used for taro cultivation by the Native Hawaiian population, where land runoff into the bay was reduced through diversion into taro patches that minimized the intensity of runoff by the time it reached the shore (Chave and Maragos 1973). After the arrival of western settlers, the Native Hawaiian population experienced a huge drop in population primarily from the introduction of their diseases (Chave and Maragos 1973). The population only began to rise again from the influx of immigrants from China, Japan, etc., leading to a change in agricultural practices as rice terraces replaced taro patches and the keeping of domesticated animals in the northern sector caused higher rates of land runoff from the resulting dried soil (Banner, 1974). Though the 1910's catalyzed the process of urbanization with the construction of Kāneʻohe town. This brought about a significant runoff increase: clearing of land for construction left bare soil vulnerable to erosion by rainwater and the building of more impervious surfaces reduced interaction between runoff and soils (Laws, 2017). This has caused a constant excess of runoff into Kāneʻohe Bay causing detrimental effects on water quality and the species residing there, especially corals (Laws, 2017).

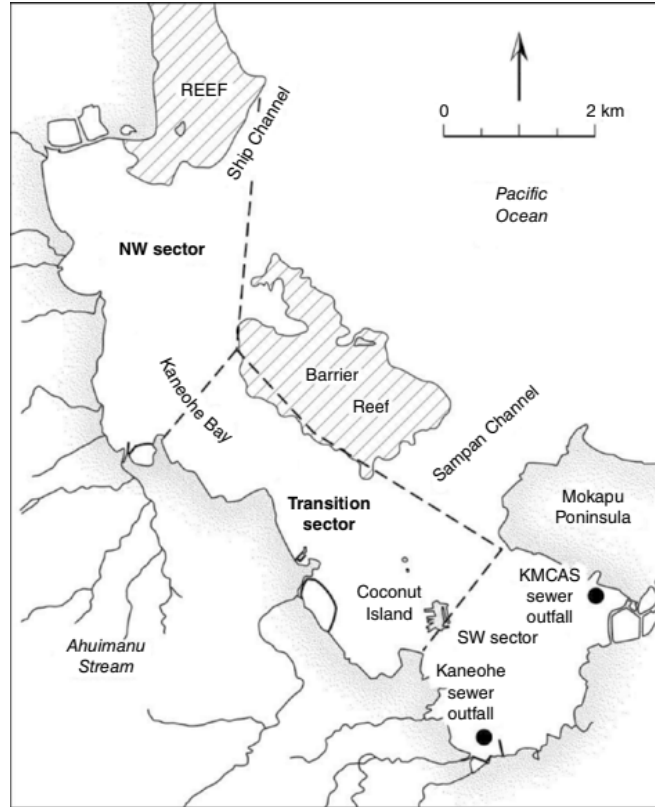
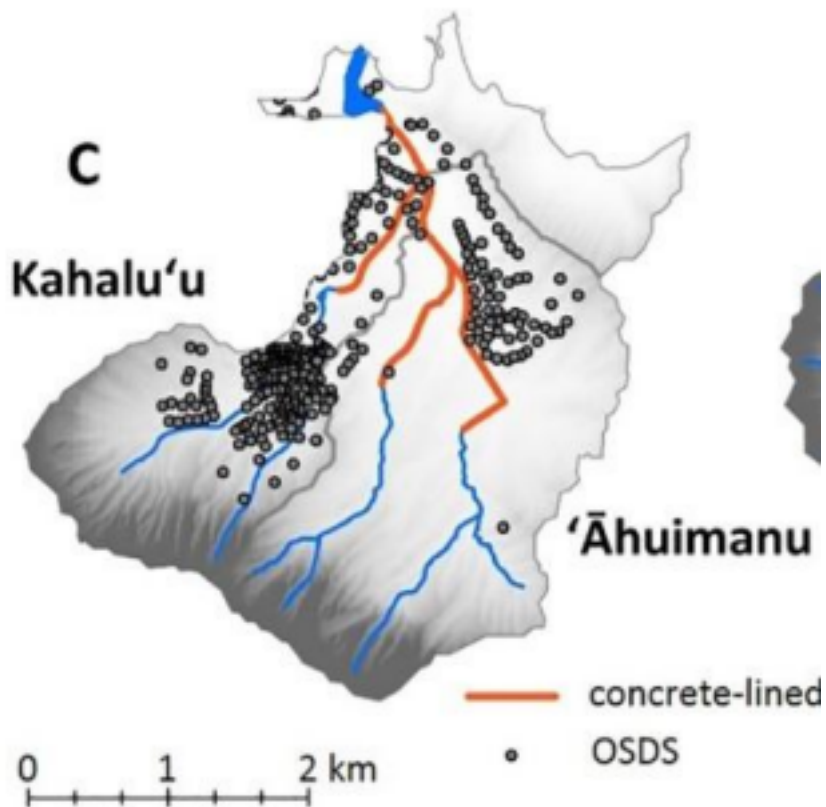


Figure 1: Map of Kāneʻohe Bay outlining stream systems that feed into it. (Laws, 2017)

1.1.3 Redirection of Kāneʻohe sewer system

In addition to general land use change, in 1970, a sewage treatment center in Kahaluʻu Valley was constructed that discharged its effluent into ʻĀhuimanu Stream. The outflow was redirected in 1978 to the Mokapu Peninsula, but it still left the ʻĀhuimanu stream section connected to the sewer system, while the rest of the watershed still depended on on-site sewage disposal systems (OSDS), primarily cesspools (Laws, 2017). Comparing these sections of the watershed, the residential areas surrounding Kahaluʻu stream have a lower population and area than ʻĀhuimanu but have a significantly higher OSDS density. Figure 1 illustrates the density and distribution of OSDS units in the Kahaluʻu and ʻĀhuimanu sub-watersheds (McKenzie, Dulai, Chang,



2019).

Figure 2: Map illustrating Kahalu'u and 'Āhuimanu streams, including Onsite

Sewage Disposal System (OSDS) unit density within each stream (McKenzie, Dulai, Chang, 2019)

1.1.4 OSDS unit leeching and future environmental risk

Onsite sewage disposal systems receive all the wastewater produced from individual properties. The waste undergoes limited treatment in these units, as light and heavy solids are separated by gravity, and requires complete removal to treat pathogens and nutrient concentrations. It is critical to note that these units were designed without the understanding that the unsaturated soil area they were constructed in would gradually decrease as the aquifer below rises due to sea level rise. This has led to the escalated threat of septic system pollution as the surface and groundwater tables are predicted to intersect (Miami-Dade County Department of Regulatory & Economic

Resources, 2018). Current issues involving OSDS units often involve the leaching of cesspools. There are currently 88,000 of them across Hawai'i and their leaching leads to pollutants, i.e. caffeine and sulfamethoxazole, and hazardous materials going into groundwater that eventually travels into the coast (Coleman, 2019).

1.1.5 Residential pesticide/herbicide use

Another threat to water quality here is the application of pesticides and herbicides in both agricultural and residential uses. Since the early 1900's, agricultural lands of O'ahu have experienced intense herbicide and pesticide use, which has led to environmental consequences such as a USGS survey in the 1990's finding the highest concentration levels of pesticides in fish within the nation being found in Honolulu streams (Spengler et al., 2018). While the use of certain pesticides such as Dichlorodiphenyltrichloroethane (DDT) has been outlawed due to threats on wildlife on public health, they still remain a concern due to their extended residence making them still present in the environment almost fifty years after they had been outlawed (Welch et al, 2019). This, paired with the continued use of herbicides, primarily from residential usage (Spengler et al., 2018), adds to the overall concentration of these compounds within streams (Welch et al, 2019). One of the most heavily used herbicides today is glyphosate (brand name Roundup) and, while it has a much shorter residence time than DDT (Welch et al, 2019), it still poses similar environmental threats (Spengler et al., 2018). Glyphosate's ability to adhere to soil particles also makes it mobile through groundwater movement and surface runoff after large rain events (Spengler et al., 2018). This leads to glyphosate eventually being transported into streams and coastal oceans.

1.1.6 CEC prevalence and impact on coastal ecosystems

These pollutant pathways are some well-known examples of how Contaminants

of Emerging Concern (CEC) are transported from residential areas in Kāneʻohe Bay. Glyphosate for example, while being observed as a persistent pollutant throughout streams on Oʻahu, was not included in a pesticide-monitoring program of surface water in Hawaii initiated by the USGS and the Hawaii Department of Agriculture (Spengler et al., 2018). Contaminants from cesspool leaching not only include the bacteria from human waste but also contain consumables and pharmaceuticals that are passed from humans (McKenzie, Dulai, Chang, 2019). These include the compounds caffeine and sulfamethoxazole. All three of these compounds, glyphosate, caffeine, and sulfamethoxazole, are CEC that can be transported to streams and the coast as submarine groundwater discharge (SGD) (McKenzie, Dulai, Chang, 2019 & Spengler et al., 2018). Within Kāneʻohe Bay it has been observed that SGD is a major source of CEC to the nearshore environment (McKenzie, Dulai, Chang, 2019). Supported inventories of CEC are of concern to the environment and public as the effects of high CEC intensity include reproductive effects in aquatic organisms (EPA, 2015) and the potential rise of antibiotic-resistant bacteria in bodies of water (Hernando et al., 2006).

1.2 Project Objective

Through this project, I aim to observe how the CEC inventories of glyphosate, caffeine, and sulfamethoxazole vary across the ʻĀhuimanu and Kahaluʻu stream system. As CEC concentrations are known to decrease through microbial processes, I will also study whether the microbial communities present within the streams and nearshore environment are likely to be breaking down these compounds or if they are likely to accumulate within the coasts. By setting up in-lab experiments of stream and coastal water samples treated with these compounds, it may be possible to observe CEC effects on microbial density as well through the use of flow cytometry. While all compounds covered in this experiment are able to be transported through groundwater movement

caffeine and sulfamethoxazole are expected to show higher concentrations throughout the stream system, especially within the Kahalu‘u stream which has a high OSDS density. Finally, since the streams are divided into different, channelized and natural streambeds, it is hypothesized that CEC concentration and microbe density will differ based on environment type.

CHAPTER 2: METHODS AND MATERIALS

2.1.1: Radon Surveys

Throughout June-August 2021 five sample collections took place throughout the Kahalu‘u-‘Āhuimanu stream system. Samples were collected from the sites shown in Figure 2, with a total of 20 samples collected each outing. Water samples were initially stored from the site in 16 oz glass bottles and then were transported to the laboratory to be filtered through a (.45 um) filter using a pump. Filtered samples were then repackaged in Thermo Scientific™ 4mL Amber Screw Top Vials that had been recently fire-washed. From all sample collections, 100 4mL Amber Vials were obtained for CEC analysis. Additionally, groundwater input was also approximately measured using the radon concentration values of stream samples calculated by a DurrIDGE RAD7 Electronic Radon Detector. Twenty-five samples were taken from multiple sites across the studied area and had radon concentration measured throughout the Summer 2021 collection period.

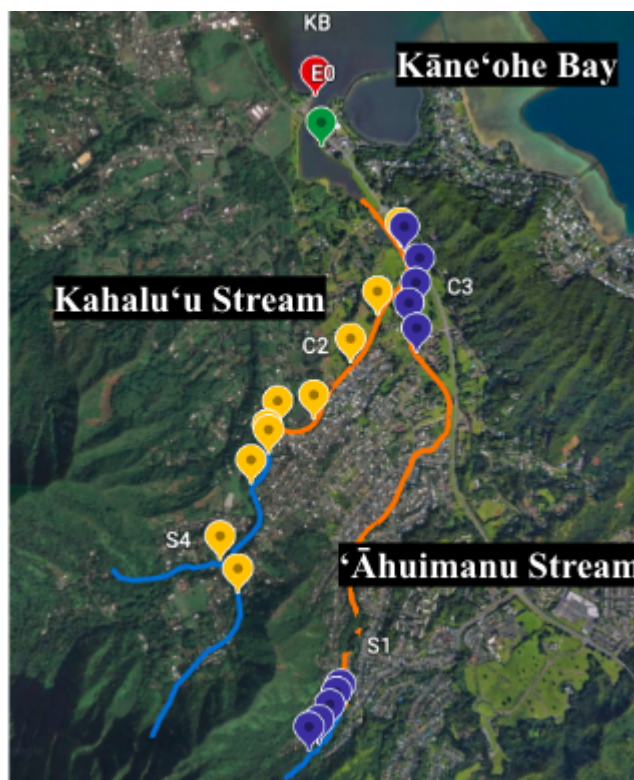


Figure 3: Map of sample collection sites for Summer 2021 CEC analysis.

Kahalu'u stream sites are shown in yellow, while 'Āhuimanu sites are shown in purple. Sites selected for microbial density observation are labeled.

2.1.2: CEC sample analysis

Shortly after the last collection outing, all samples for CEC analysis were gathered to have their concentrations measured using the Eurofins Abraxis Microtiter Plate Kit of each specified contaminant (Glyphosate (GLY), Caffeine (CAF), and Sulfamethoxazole (SMX)). Each kit provided an ELISA assay that generally required adding in the included standards and controls to assigned wells before adding in samples to the rest of the wells. After adding in samples, antibody and enzyme conjugate solutions were added in and incubated at room temperature before cleaning out the plate and adding in the substrate and stop solutions. The last step of the process involved in putting the microtiter plate of the kits into a spectrophotometer to record absorbance values and calculate concentrations from it. Between each day in the lab, samples were

stored in the lab refrigerator.

2.1.3: Relative attenuation calculation

The following equation below (Li et al., 2016) was used to calculate the relative attenuation rates observed through large sections of the stream system. Relative attenuation values are used to describe whether a contaminant's concentration is increasing or decreasing over a set stretch of the stream. With positive values indicating loss along the stretch and negative values displaying a constant or potential increase in concentration. The reference or C_{REF} contaminant used for the equation was carbamazepine, a pharmaceutical that had been found to have an overall constant concentration throughout the stream system in previous studies (Mckenzie, Dulai, & Chang, 2019).

$$Att_x = \left(1 - \frac{\frac{C_{x,siteB}}{C_{x,siteA}}}{\frac{C_{REF,siteB}}{C_{REF,siteA}}} \right) \times 100\%$$

Figure 4: Relative attenuation equation from (Li et al., 2016)

2.2.1 Fall 2021: Microbial Community Observation rationale

Following the surveys, I designed an experiment to observe if the microbial communities found within the stream water samples consume selected contaminants by tracking microbial growth rates and contaminant concentrations over time. The occurrence of the increased rate of microbial growth coinciding with the decreased concentration of a CEC would suggest that species within the microbial community are consuming CECs. While a constant concentration throughout the trial would suggest that overall the microbial community present in the stream does not consume the particular

CEC in a significant portion. This observation would also infer that the contaminant is more likely to accumulate in the stream water since microbial consumption is not a likely degradation factor, and travel into nearby coastal areas.

2.2.2 Microbial Community Observation experiment setup

Stream, estuarine and oceanic sites selected for microbial community observation were chosen based on either elevated CEC or radon concentrations. All sites selected for this experiment are labeled within the Figure 3 map. Figures 4 & 5 then outline the various treatment scenarios created for the project. Figure 4 displays the process of how the collected 10 L samples from each site were filtered and then distributed for each contaminant treatment. Filtration and the setup of the experiment required the use of 6 10 L containers for an initial site collection, 3 more 10 L bottles for initial filtration, and 60 1 L bottles for treatment setup. The contaminant treatment process consisted of the following. Figure 5 shows how all these treatments for every site are arranged to occur at the same time. All 10 L samples from each site were collected in mid-January and the entire treatment observation took place in over the course of two weeks. Shortly after field collection, samples were filtered to collect 7.5 L of filtered water that contained the natural solutes from each stream.

The contaminant treatment liter bottles each consisted of a calculated amount of 200 μ l of a 2mM (mmol/L) stock solution made of 33 mL D.I. water and a variable amount of powdered solution of each CEC (CAF= 12.8 mg, GLY= 11.2 mg, SMX=16.72 mg). Calculating the approximate initial concentrations for each treatment scenario results in the following caffeine: 129.30 ppb, glyphosate: 113.13 ppb, and sulfamethoxazole: 168.89 ppb. At the start of the CEC treatment, all samples that had CEC solution added to them had 60 ml collected from each of them in glass amber vials. They were then stored in the lab fridge at C-MORE in order to measure the initial CEC

concentrations at a later date. This was also done at the end of the two-week observation period in order to get the final CEC concentrations.

Control treatments were designated into two groups, one Group A being described in Figure 4 as non-CEC controls, wherein each site has one treatment bottle that has no amount of the 2mM stock CEC solution. These control treatments did not have samples collected for CEC analysis at the beginning and end of the treatment. Control treatments of Group B, also known as CEC-only control samples consisted only of the filtrate and the corresponding CEC stock solution. These treatments were meant to observe if CEC concentrations are able to degrade without the presence of the ambient microbial community.

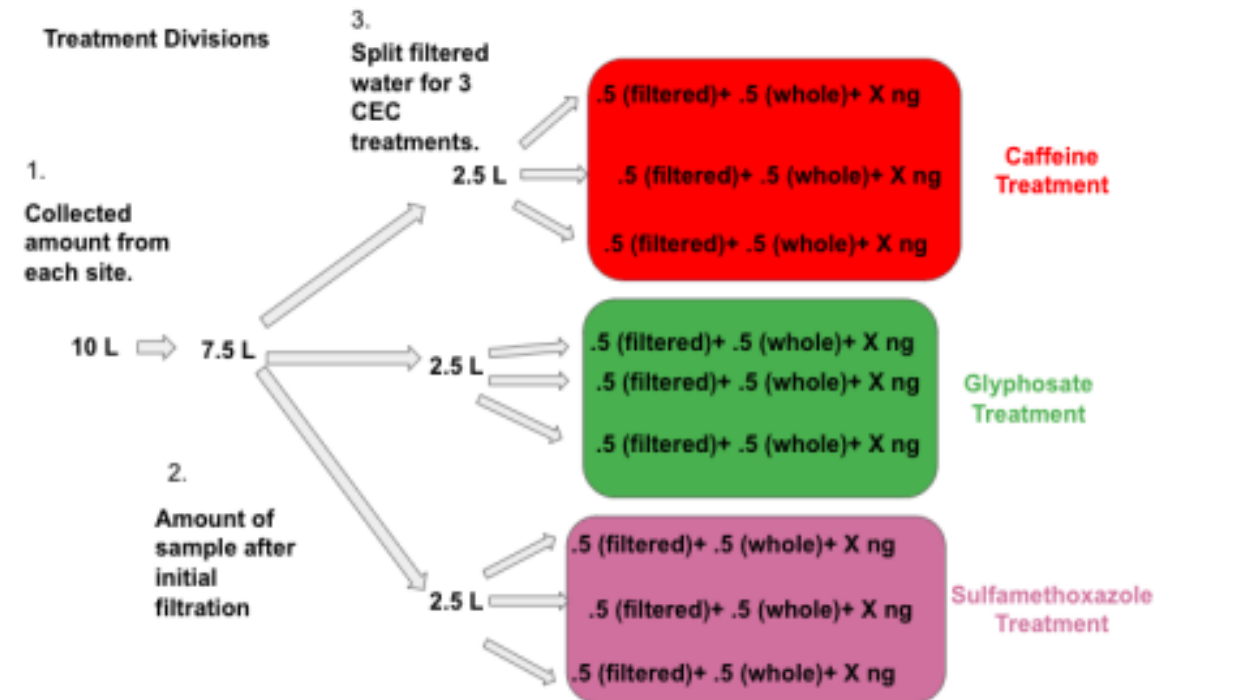


Figure 5: Diagram of how samples were prepared and distributed for CEC microbial degradation experiments.

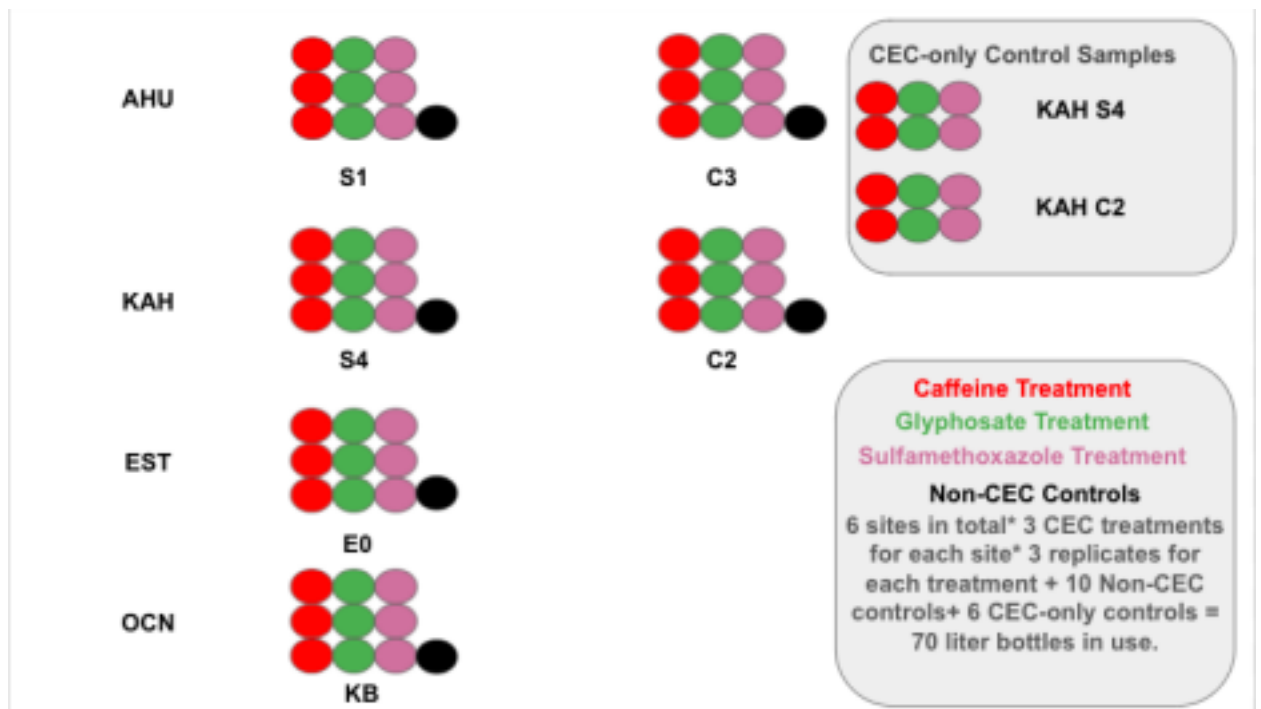


Figure 6: CEC Treatment bottle set up with outlined contaminant type, site location, and concentration amount.

2.2.3 Flow Cytometry Analysis

After collecting unfiltered sample water for each site and the initial project setup, the process of recording potential microbial density change began. By using the flow cytometry method bacterial cell population was tracked throughout the two-week period of observation. For the first 48 hours, sample collections were taken around every six hours. Then for the rest of the period, samples meant for flow cytometry measurements were taken every other day for the remainder of the two-week period. These samples, 60 for each timepoint, were then analyzed through an Attune NxT Acoustic focusing cytometer. 200 μ l of each sample from a specified timepoint were transferred to a 96-well assay and had the dye sybr-green added to them before being placed into the connected Auto-Sampler. As the sybr-green dye bonded to any DNA within the sample, while also recording the relative size of a particle, bacterial populations were able to be recorded

within the sample. This data was analyzed at a later date. All FCM recordings took place from mid-February to early March.

2.2.4 Final CEC analysis:

Soon after the use of the FCM, the CEC analysis of the microbial experiment samples took place. The same ELISA kits from the summer analysis were used for the total 132 samples, half being taken at the start of the experiment period, and the other at the end. Samples taken at the beginning and end of the observation period were measured for initial and final CEC concentrations of CAF, GLY, & SMX in order to observe if any change had taken place.

CHAPTER 3: RESULTS

3.1.1 CEC & Groundwater Input Survey: May-August 2021

The Summer 2021 survey had average CEC concentrations for glyphosate, caffeine, & sulfamethoxazole calculated for each of the 10 sites that were collected along with average radon concentrations for selected sites. The presence of positive radon concentration values at all observed sites supported the hypothesis of groundwater input in multiple areas of the stream system, whether the area consisted of a natural streambed or not. Along with this relative attenuation values were calculated to observe if concentrations were subject to degradation along individual streams and if varying attenuation rates between channelized and natural stream bed sections could support the hypothesis of stream environment influencing residence time of contaminants.

3.1.2: Average Radon Concentrations within the stream system

Overall, radon concentrations (Figures 6 & 7) varied for each stream and did not exhibit any notable pattern, though the 'Āhuimanu stream exhibited higher values than Kahalu'u at similar areas inland, about 1-2 km from the coast. Measured radon concentrations ranged from values of 0.05 to 83 dpm/L. The averages for selected sites are listed by their corresponding stream section in Figure 13. For sites with the Kahalu'u channelized section the averages totaled to 15.3 ± 13.7 and 14.6 ± 9.7 dpm/L. This differed from Kahalu'u streambed section averages that totaled to 4.6 and 6.5 dpm/L. This indicates that groundwater input may actually be higher at the channelized portion of Kahalu'u despite have reduced hypoheric flow. As for 'Āhuimanu, averages for sites within the channelized portion were even lower values at 4.0 & 3.9 dpm/L. Though for the streambed section one site, S5, averaged at 4.0 dpm/L while the other

site S3 had an average value of 13.3 dpm/L.

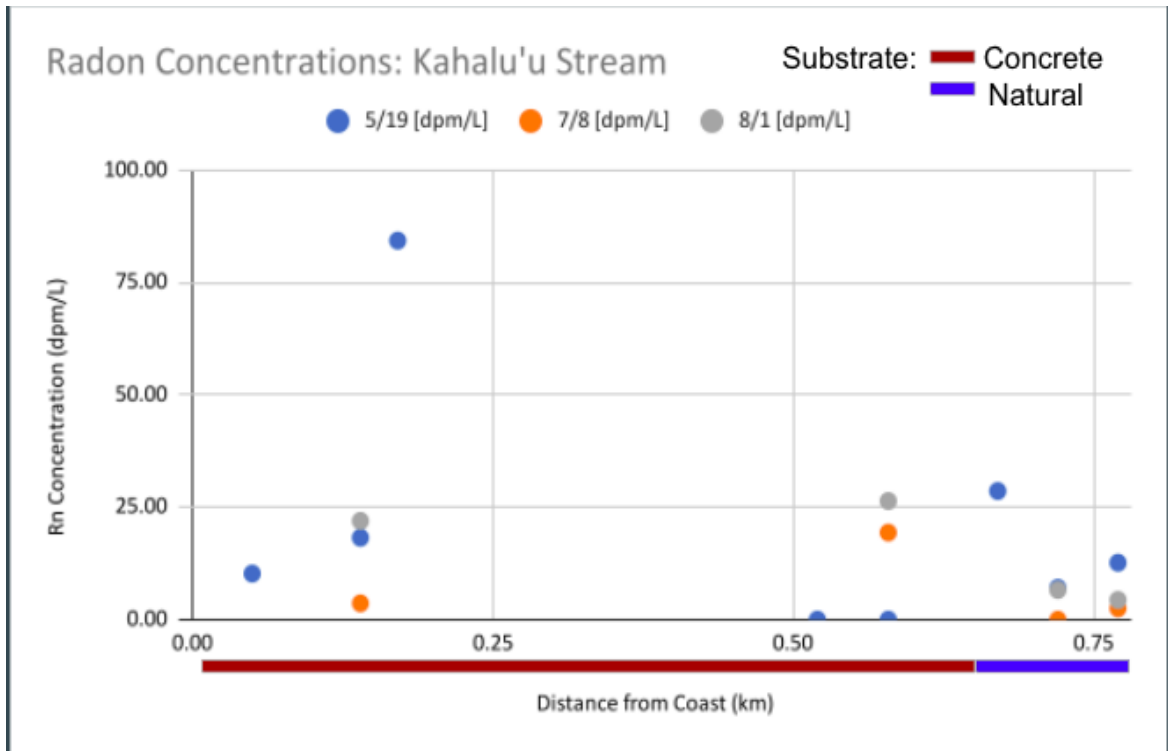


Figure 7: Radon concentrations measured as a groundwater proxy at sites across Kahalu'u Stream.

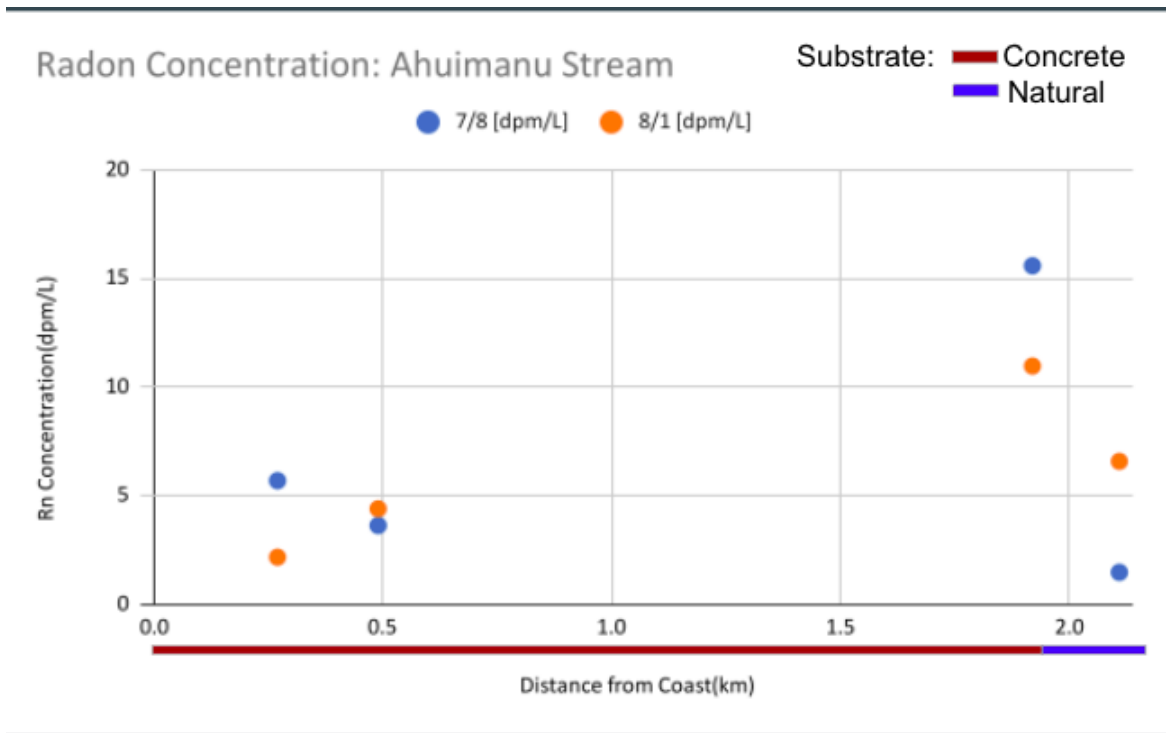


Figure 8: Radon concentrations measured from water samples collected along ‘Āhuimanu stream.

3.1.3: Average CEC Concentrations between channelized and streambed portions

Average concentrations for measured CEC along the Kahalu‘u stream were observed to vary between CEC type. A common range of 0.05-0.15 ng/mL were observed for averages of glyphosate and sulfamethoxazole (Figures 9 & 11). With glyphosate averages being above 0.05 ng/mL at 3 sites (C4, C5, & S5) and SMX passing this value only at one site (C4). As for caffeine (Figure 9) this range is expanded, though this is primarily due to S4 average concentration being an outlier at 1.30 ng/mL. Four out of the five collected samples for the S4 site showed higher averages than any other site, which supports that this outlier is not from instrumental error.

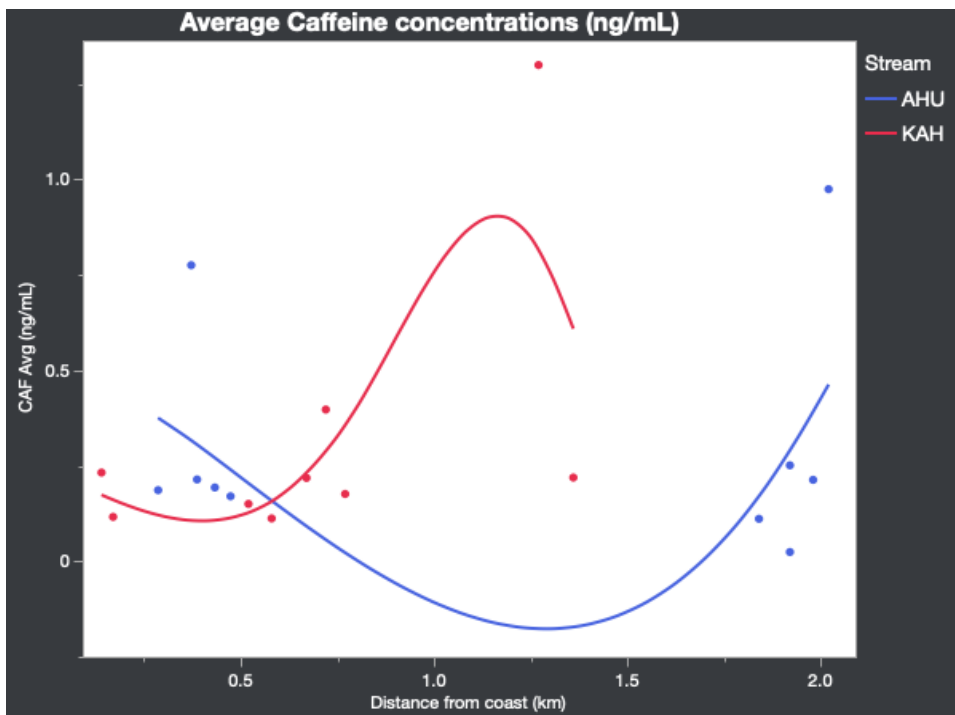


Figure 9: Average (n=5) concentrations of caffeine from sites along Kahalu‘u &

‘Āhuimanu streams, samples were collected throughout Summer 2021.

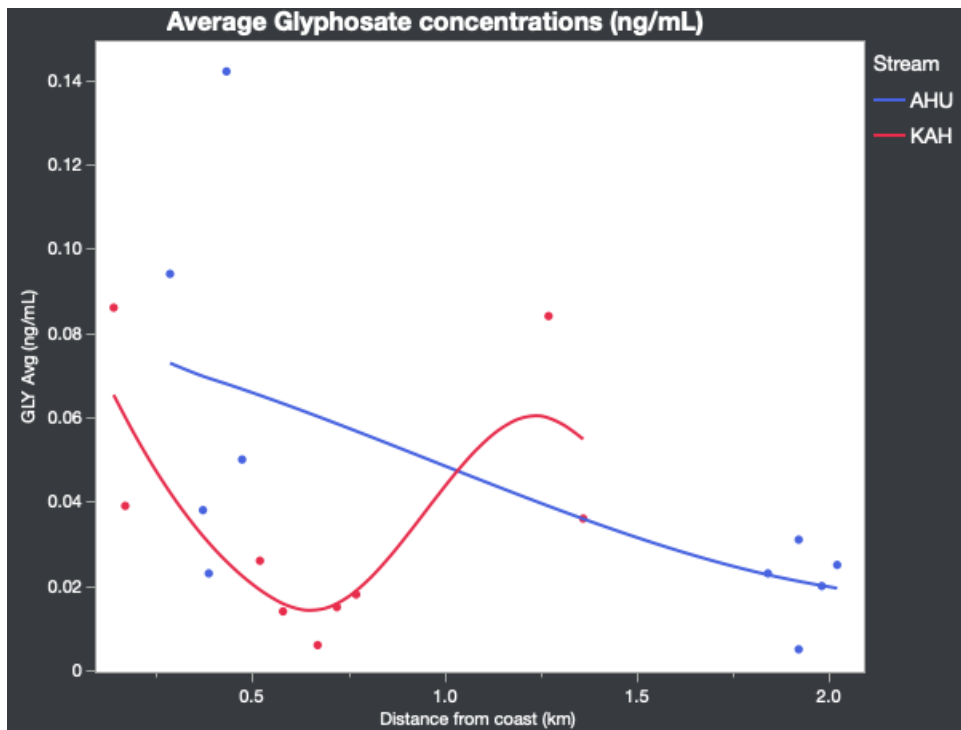


Figure 10: Average glyphosate concentrations through Kahalu‘u & ‘Āhuimanu stream sites.

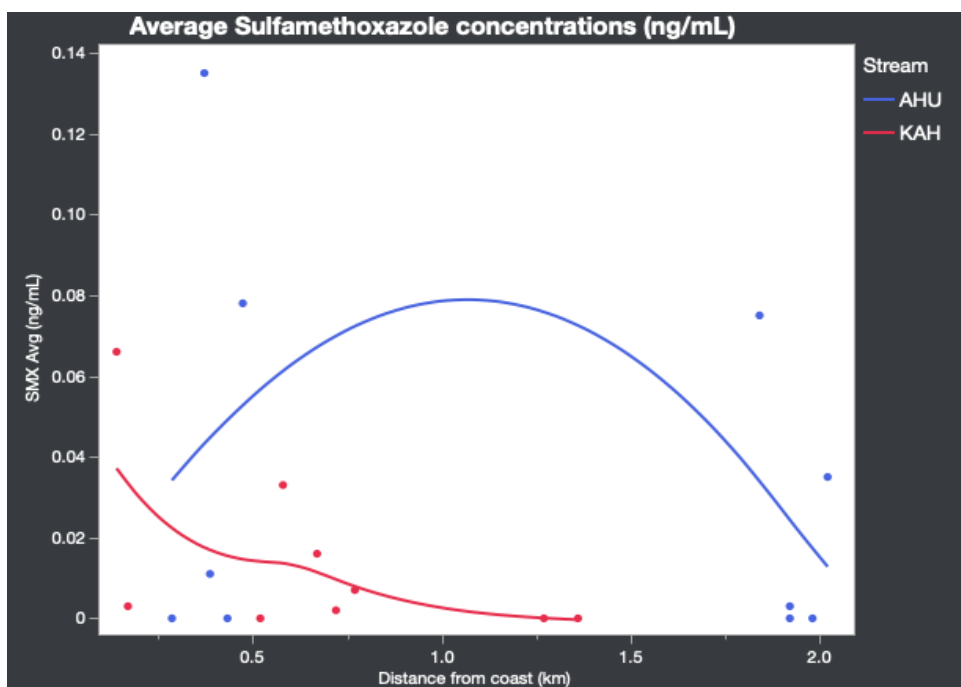


Figure 11: Average sulfamethoxazole concentrations through Kahalu‘u & ‘Āhuimanu

stream sites

As for average CEC concentrations for 'Āhuimanu, average values exceeding 0.5 ng/ml are observed in caffeine concentrations at two sites at both streambed (S5) and channelized sections (C2). This stands out from the other two CEC types that have their highest average concentrations still below 0.15 ng/ml. Figure 10 shows that both streams had their highest glyphosate concentrations closer to the coasts while generally declining as the streams became more inland. Sulfamethoxazole concentrations (Figure 12) were observed to have the lowest averages of CEC, except for four areas, three close to the coast, while the other was near the end of the 'Āhuimanu stream bed portion, which stood out for being the only values, ≥ 0.05 ng/mL.

3.1.4: Relative attenuation rates through individual streams

Relative attenuation, defined as whether a contaminant's concentration is increasing or decreasing over a set stretch of the stream was calculated using the formula displayed in Figure 4 and measured carbamazepine concentrations as a reference compound. This was done in order to observe the influence of the stream bed environment on CEC inventories along with average groundwater input perceived through radon concentrations. Positive values, indicating an overall decrease along the defined stretch, were found for glyphosate and caffeine in the natural streambed portion of the Kahalu'u stream while negative values were observed for the CEC through the channelized portion. Though for 'Āhuimanu stream caffeine exhibits positive values for both channelized and stream bed portions, glyphosate had negative values for these sections. Finally, sulfamethoxazole exhibited positive attenuation for the channelized portion of Kahalu'u stream and the inland section of 'Āhuimanu, while having a negative value for channelized 'Āhuimanu. The concentration values for the Kahalu'u stream bed section were unable to calculate an attenuation value using the

formula in Figure 4.

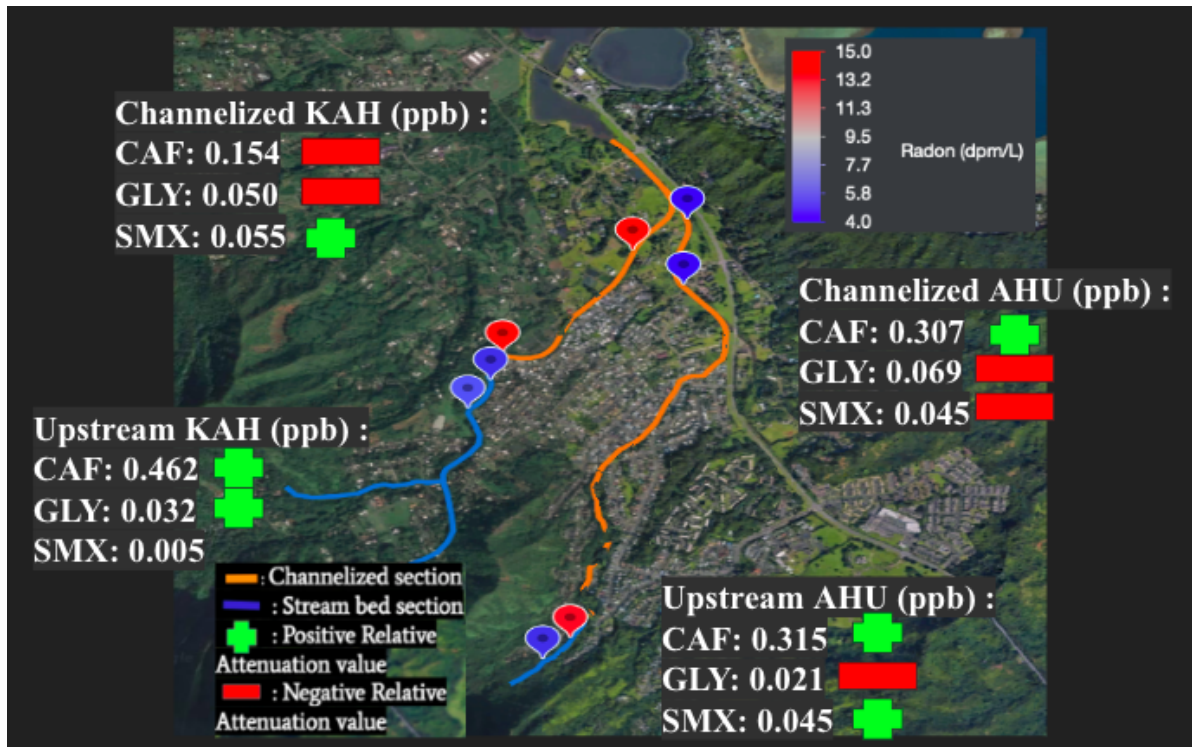


Figure 12: Map of 'Āhuimanu-Kahalu' stream system with channelized and streambed portions outlined. A summary of collected data from the Summer 2021 survey of the stream system is illustrated here through relative attenuation values, average radon, and CEC concentrations.

3.2: Flow Cytometry and CEC measurement observation

3.2.1: CEC inventories: 0 hrs vs 336 hours

At the beginning and end of the microbial community observation experiment, which lasted a duration of two weeks, 132 samples total were collected to measure CEC inventories. 66 were collected at the time CEC concentrates were added to filtered samples, 0 hours, and then again after two weeks, 336 hours. Using the specific compound Abraxis test kits, initial and final concentrations were measured. In caffeine treatment samples, Figure 13, most sites from Kahalu'u and 'Āhuimanu had initial concentrations that went over the maximum detection limit, 5 ng/mL. Then when

comparing to samples taken at the 336 hour mark, all sites within Kahalu‘u and ‘Āhuimanu streams had concentrations notably reduced to levels near the below detection limit. In contrast, endpoint samples from sites in Kāne‘ohe Bay did not show a measurable decline in caffeine.

As for SMX treatment samples Figure 13 illustrates that at all sites initial and final concentrations are both still above the maximum detection limit. Then with the unamended control remaining constant as well, this suggests that the CEC is not breaking down on its own. Finally, for glyphosate concentrations, there is a slight decrease in concentrations from the beginning to the end of the observation period within all sites. Though it is more noticeable in the ‘Āhuimanu streambed and channelized sections. As for the compound degrading on its own, due to possible error the control was not able to be used so it unclear whether glyphosate may have been degrading without microbe presence during the observation period.

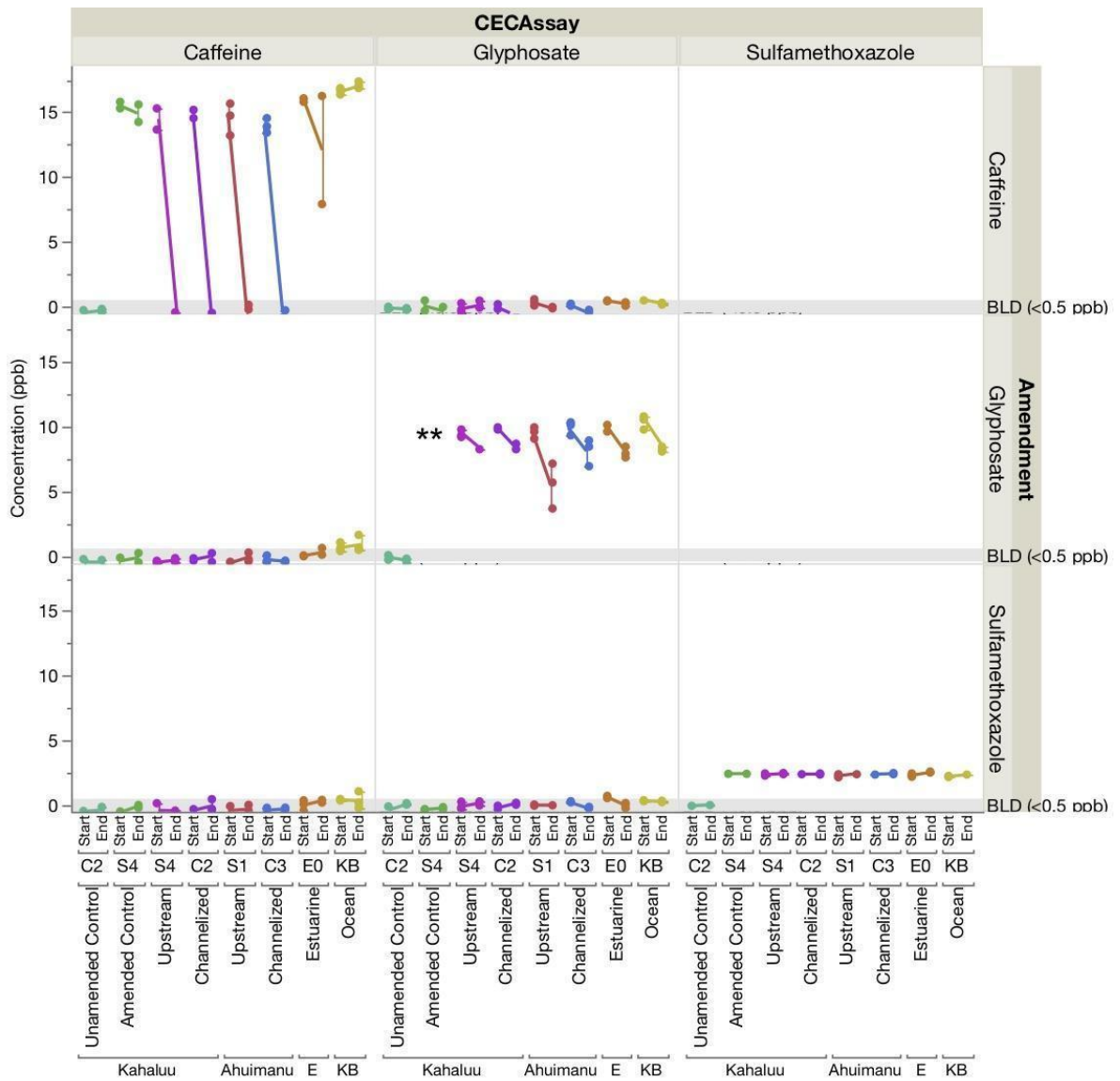


Figure 13: Changes in CEC Concentration from Start (0 hours) to End (336 hours) of the observation period. Each CEC assay, except sulfamethoxazole measured concentrations in all samples not just those with the corresponding CEC amendment. Sample from site S4 in the glyphosate treatment did not receive a corresponding amendment due to error.

3.2.2: Flow Cytometry growth curves

The use of flow cytometry measurement on individual timepoint

samples to observe changes in cell concentration over the course of two weeks resulted in growth curves for controls (Figure 14) and each treatment scenario (Figure 15). Concentrations of bacterial cells (cells/ul) within control scenarios, where no amount of CEC was added to samples, shows both channelized sites (C2 & C3) having noticeably steeper curves and higher maxima than other sites (Figure 14). Kahalu‘u estuary site also exhibited an initial steep growth curve but then experienced a decline after around 50 hours and then concentrations closer to streambed sites were recorded for the rest of the period. As for the other sites, streambed sites S1 & S4 and the Kāne‘ohe Bay site mostly display similar growth curves, tending to reach a peak concentration in the range of 100-200 cells/μl between 100-200 hours before declining and reaching a stable population.

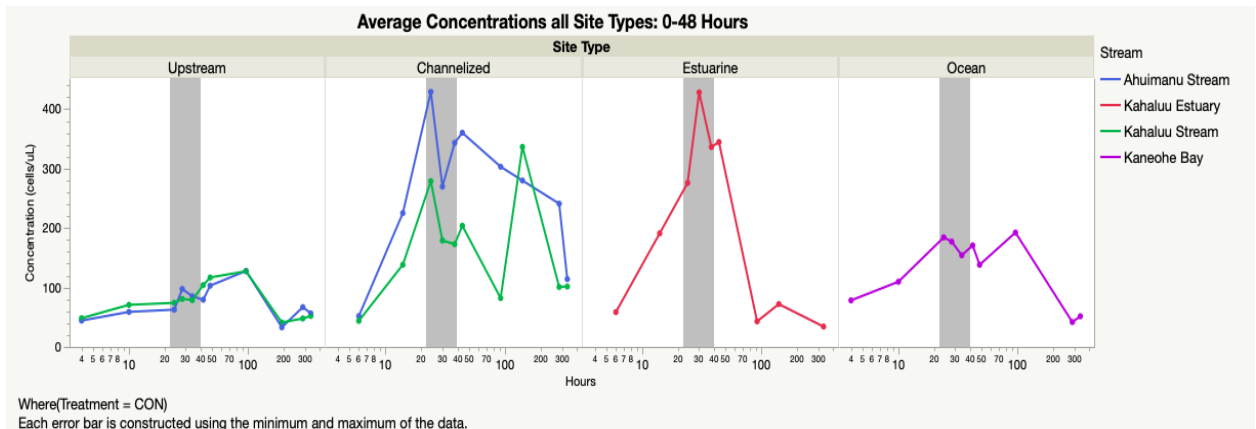


Figure 14: Time-series (0-336 hours) of cell concentrations (cells/uL) per site type (Upstream, Channelized, Estuarine, and Ocean) in unamended control samples.

Comparisons of growth dynamics among CEC amendment treatments showed no clear differences in growth associated with any CEC that differed from the unamended controls (Figure 15).

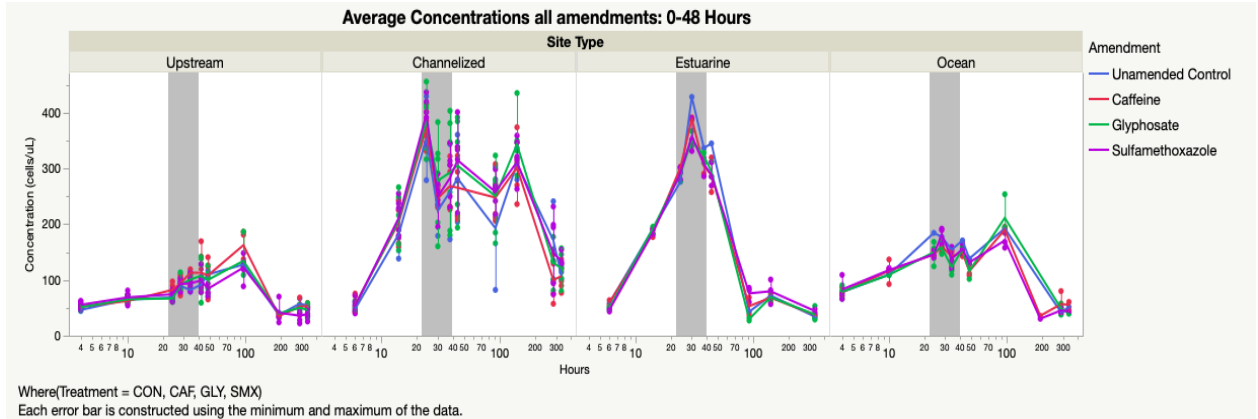


Figure 15: Time-series (0-336 hours) of cell concentrations (cell/uL) per treatment scenario (unamended control, caffeine, glyphosate, and sulfamethoxazole).

To explore whether growth differed within the first 2d of the experiment when bacteria were in their logarithmic growth phase, we analyzed the mean cell concentrations from 24-36h across treatments. No significant differences were found from controls, but there was a slight indication of enrichment in cell abundance in the Caffeine treatments in the Upstream sites in both streams (Figure 16).

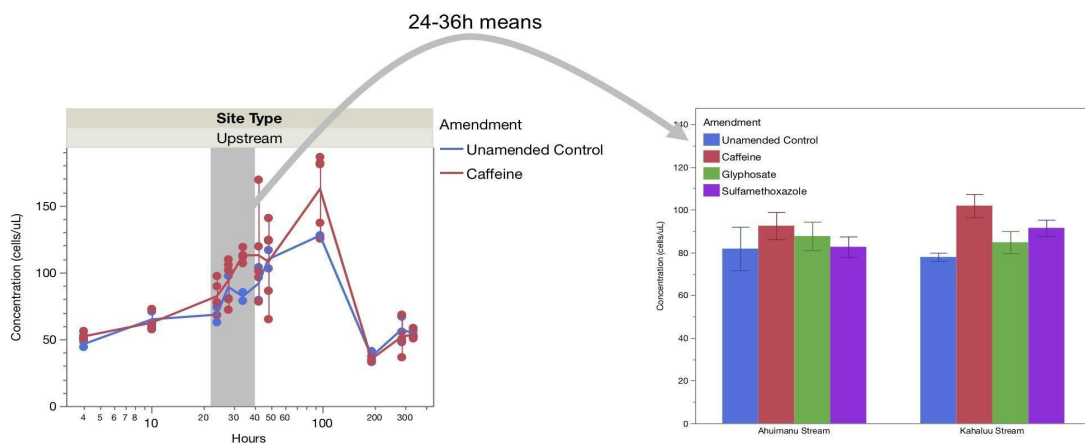


Figure 16: Comparison of caffeine and control treatment growth curves (24-36 hours).

CHAPTER 4:

DISCUSSION

4.1.1: Average CEC inventories & corresponding growth curves

Sites selected from the Summer 2021 survey for flow cytometry analysis exhibited higher average CEC concentration for one or more contaminants along with measurable radon concentrations indicating groundwater input. These characteristics made the sites applicable for further study into whether CEC sourced from groundwater has influence on microbial community growth and if stream environments could be an additional factor to this. Through further analysis of data collected from flow cytometry and measuring CEC inventories over time multiple observations were made on whether CEC were affecting microbial community growth as well as pointing out areas that illustrated the notable differences between control and certain treatment conditions.

4.1.2: Finding presence of CEC before and after observation period

Samples collected at the beginning and end of the observation period (0 & 336 hours) were measured to observe if microbial communities within samples were consuming any of the CEC. After observing potential error in the measurement of standard curves within the CEC kits, absorbance values from the spectrophotometer were used to calculate concentrations through linear regression. These calculated concentrations were then used to demonstrate potential changes in CEC inventory within samples.

4.1.3: CEC continued presence within Glyphosate and Sulfamethoxazole treatments

This method allowed the following observations on CEC inventories to be made. First, all samples analyzed for glyphosate and sulfamethoxazole that had these

CEC enrichments added to them showed the continued presence of these CECs through their concentrations at the end of the observation period. This would suggest that these contaminants are not being broken down by microbial communities. There was no evidence of the breakdown of Caffeine or SMX in the absence of microbial inocula as evidenced by the Amended Controls. While there was a mistake made in preparing the Amended Controls for glyphosate there was a relatively little breakdown in some of the upstream sites, further indicating that there was no significant natural decomposition in the absence of microbial inocula.

As for samples within the caffeine treatments, concentrations between initial and final readings was observed in multiple sites, implying potential microbial consumption. All sites within Kahalu‘u and ‘Āhuimanu streams indicated the absence of caffeine from the beginning to the end of the observation period with most replicates supporting this. While sites in the coastal areas beyond the stream system (E0 & KB), displayed a continued presence of caffeine though there was some evidence of consumption within the Kahalu‘u estuary. This could support the hypothesis that environmental factors in part determine if the present microbial community is likely to degrade a certain pollutant.

4.1.4: Analysis of growth curve peaks under CEC treatments

Within the upstream sites (Figure 14) both streams exhibit cell concentrations much lower than their channelized counterparts. These samples in both ‘Āhuimanu and Kahalu‘u streams have concentrations barely above 100 cells/uL at their peak, while growth curves in channelized and estuarine sections have curves that reach peaks of 400 cells/uL or higher. Although channelized has higher cell concentrations paired with the absence of caffeine at the end of the observation period, when comparing caffeine growth curves to unamended controls notable growth was not observed in this area

(Figure 15). Meanwhile, when further analyzing upstream growth curves, there is evidence of notable density growth between the caffeine treatment and the controls (Figure 16). This may support the hypothesis that the environmental conditions of the stream may influence what types of microbes are supported there. Which could mean that certain environmental conditions support microbes that can break down caffeine as an energy source and grow their population within the microbial community better. It is further supported with the positive attenuation values for caffeine calculated from the Summer 2021 survey of the natural streambed portions for both streams. A positive relative attenuation value proposing that an area of the stream is likely to not accumulate caffeine and even likely to have present concentrations be reduced.

Kahalu‘u upstream sites show a more clear relationship between microbe growth and CEC presence than others, yet these sites also have the lowest densities out of all with the highest concentration barely reaching 120 cells/ul while the channelized and estuary samples average at >200 cells/ul. This could be from an environmental influence as inland, natural streambed stream sites receive less sunlight from a larger riparian area providing more shade along with less nutrient input as runoff collects faster in their channelized counterparts. These factors and more may have an influence on the reduced cell densities these sites have. It may also affect the types of microbes able to grow within their communities and could determine whether microbes with the ability to degrade certain CEC are able to be present in an environment.

The absence of caffeine was found at the end of the observation period in all stream system samples. While significant cell density growth was not observed in CEC treatment scenarios, further analysis in Figure 21 Kahalu‘u stream seems to show a noticeable difference in concentration between control and caffeine treatment scenarios. This may also support one of the hypothesis stated in the introduction that

OSDS density may have an effect on microbial communities. Only samples from the upstream Kahalu‘u stream sites showed a noticeable difference between control and treatment scenarios and the OSDS density illustrated in Figure 2 shows that the Kahalu‘u sections has a notably higher OSDS density than the surveyed ‘Āhuimanu channelized section. Since at least two of these contaminants, caffeine & SMX, can be sourced from OSDS unit leeching (McKenzie, Trista, et al., 2019) then observing higher concentrations under treatment scenarios in the Kahalu‘u sites alone can encourage the idea of microbial communities near areas of high OSDS density being influenced by the input of OSDS units to groundwater discharge. It was also observed in the Summer 2021 survey that the channelized section of Kahalu‘u has higher average radon concentrations than the channelized ‘Āhuimanu section (Figure 15). Radon concentrations have been observed to correlate with groundwater discharge (McKenzie, Trista, et al., 2019), then it may suggest that the Kahalu‘u channelized section is more likely to receive pollutants from groundwater discharge. Though it is not supported whether the microbial community in this section is able to grow off these pollutants. However, further research could be conducted to observe whether it is affecting microbial metabolism.

Finally, observing growth curve peaks in both nearshore sites (Kahalu‘u Estuary & Kaneohe Bay once again supports that CEC addition is most likely not utilized by the microbial community present in these sites. Both E0 & KB samples all showed the continued presence of all CEC from the beginning to the end of the observation period, implicating that these samples are not degrading CEC compounds. Then by observing the concentrations of their growth curve peaks it is shown that control scenarios are either at the same concentration or greater than the treatment scenarios. This information suggests that the added CEC concentrations were not

utilized as an energy source that led to increased growth. It may also suggest that sites accumulate these contaminants, especially when glyphosate and sulfamethoxazole have been found to have continued presence in most samples throughout the stream system. Average CEC concentrations were not recorded at these sites during the Summer 2021 survey but studying them in the future could contribute observations that would help determine whether these pollutants are able to pass through the Kahalu‘u-‘Āhuimanu stream system and into the connecting coastal environment.

CHAPTER 5

CONCLUSION:

Through this study, more information on the prevalence of CEC within the Kahalu‘u-‘Āhuimanu stream system was uncovered along with potential factors for the removal of these pollutants. The present radon and CEC concentrations within multiple sites in channelized and natural streambed sections continue to support the theory of CEC transport through groundwater discharge within the stream system and towards coastal environments (McKenzie, Trista, et al., 2019). These findings then encouraged further study into the effect of selected CEC, caffeine, sulfamethoxazole, & glyphosate on microbial community growth.

Overall data from measuring CEC concentrations and from flow cytometry only show caffeine concentrations being potentially degraded through primarily headwater areas. Another site within Kahalu‘u channelized section may also show the potential of breaking down caffeine concentrations though this would need to be supported further with viable control treatments as with all other treatments in this study. Meanwhile, glyphosate and sulfamethoxazole showed continued presence in samples beyond the observation period based on absorbance values, indicating that significant consumption of these CEC by microorganisms is unlikely. Though individual samples within glyphosate treatment show a noticeable loss in concentration between initial and final samples. This could suggest potential degradation, especially in sites C2, S1, & S4 whose cell concentrations are notably higher under glyphosate treatment scenarios. Perhaps further examination of newer samples with a greater amount of control scenarios could provide more secure insights into the relationship between glyphosate concentrations and microbial community growth. Sulfamethoxazole may be most likely out of all CEC studied here to not be utilized by

microbes present in samples. As concentrations are shown not to decrease below maximum detection level between initial and final readings for almost all samples. As well as continued presence being observed through absorbance values. Yet, continued examination of SMX within certain sites (S4, S1, & C2) could provide useful information as flow cytometry data from those sites under SMX treatment displayed cell concentrations noticeably higher than control scenarios.

It is imperative to understand if pollutants from human activity are likely to transport through streams into coastal areas, especially when CEC are transported through not only surface runoff but through submarine groundwater discharge as well (McKenzie, Trista, et al., 2019). The areas observed to potentially reduce some CEC concentration in this study were within non-channelized, inland portions. These areas may be more likely to reduce pollutant concentration due to their environmental characteristics encouraging bioremediation processes within streams. This could be a cause for concern though, due to the channelized section of the stream system being the portion that then transports stream waters directly to the coast. If CEC are released from areas near the channelized portion then it may be less likely to degrade before entering the coasts. Then based from the data collected here, coastal sites were unlikely to degrade CEC as well. The allocation of CEC in coastal environments may lead to the increase of environmental threats connected CEC presence (EPA, 2015 & Hernando et al., 2006) to organisms residing there. Continued research on these questions along with influences on surface runoff and submarine groundwater could be vital in understanding future threats to coastal ecosystem health.

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