POTENTIAL THREATS TO WATER QUALITY AT SUMIDA FARM CAUSED BY WASTEWATER, URBANIZATION, AND CLIMATE VARIABILITY

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I certify that I have read this thesis and that, in my 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 my parents, grandparents, brother, and all of my family, friends, and mentors who supported me throughout my time in the GES program.

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ABSTRACT

Wastewater infrastructure on the island of Oʻahu is expected to become increasingly vulnerable due to climate change, potentially increasing risks to agricultural areas surrounded by urbanized land by causing wastewater overflow in subsurface and surface runoff. Sumida Farm is the largest watercress producer on the island of Oʻahu and is located in a highly urbanized area in ʻAiea, Oʻahu. The farm is subject to stressors from the surrounding urban setting including runoff that can potentially contain wastewater. By analyzing the water at the farm for the presence of two pharmaceutical substances, caffeine and carbamazepine, that are consumed and excreted by humans and are thus commonly used as wastewater tracers, this project focuses on assessing the presence and temporal dynamics of wastewater runoff to the farm and in the watershed. Results were analyzed under various detection limits to determine, with increased confidence, whether caffeine and carbamazepine were present or absent in each sample. Overall, results indicate that caffeine and carbamazepine are rare or nonexistent at Sumida Farm. Due to the minimal presence of caffeine and carbamazepine at the farm and few precipitation events before sample collection dates, it could not be definitively determined whether there was a correlation between precipitation, caffeine, and carbamazepine during the study period. The results of this project will contribute to further understanding of potential threats to local agriculture and food sustainability on Oʻahu while also providing farm managers an overview of the current status of water quality at the farm, which may change in the future due to climate change.

Keywords: Agriculture, Wastewater, Local Food Sustainability, Climate Change

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CHAPTER 1. INTRODUCTION

1.1 Overview

Sumida Farm has been in operation as a watercress farm since 1928 and serves as an important agricultural oasis within an urban setting. The goal of this project is to assess whether water quality is linked spatially to the wastewater infrastructure lines running adjacent to Sumida Farm and if signs of wastewater leakage are found, if the leaks are linked temporally to rain events that could facilitate the flow of pollutants onto the farm. This study will help to characterize baseline water quality at Sumida Farm as climate change begins to lead to increasingly frequent and intense rainstorms and sea level rise, both of which are known to compromise wastewater infrastructure by causing overflowing and leakage into the environment (Spirandelli et al., 2018, Wastewater discharge advisories: https://eha-cloud.doh.hawaii.gov/cwb/#!/landing). Since agricultural areas surrounded by urbanized land will face higher risks from wastewater overflows in the subsurface and in surface runoff, this study may provide a better idea of how local farms may be impacted by urbanization now and in the future.

1.2 Local Agriculture

Prior to western contact, each of the Hawaiian Islands were split into numerous *moku*, or districts. Each *moku* contains *ahupuaʻa*, which are smaller, self-sustaining land divisions that often stretch from the mountains to the ocean (Sterling, 1993). The island of Oʻahu is split into six *moku*, Koʻolauloa, Koʻolaupoko, Waialua, Waiʻanae, Kona, and ʻEwa, each of which contains a multitude of *ahupuaʻa,* as shown in Figure 1 (Sterling, 1993). Sumida Farm is located in the *ahupua‘a* of Kalauao (Figure 1) which is in the *moku* of ‘Ewa. Water from many of the *ahupua‘a* in ‘Ewa, including Kalauao, flows into Pearl Harbor, traditionally known as Pu‘uloa.

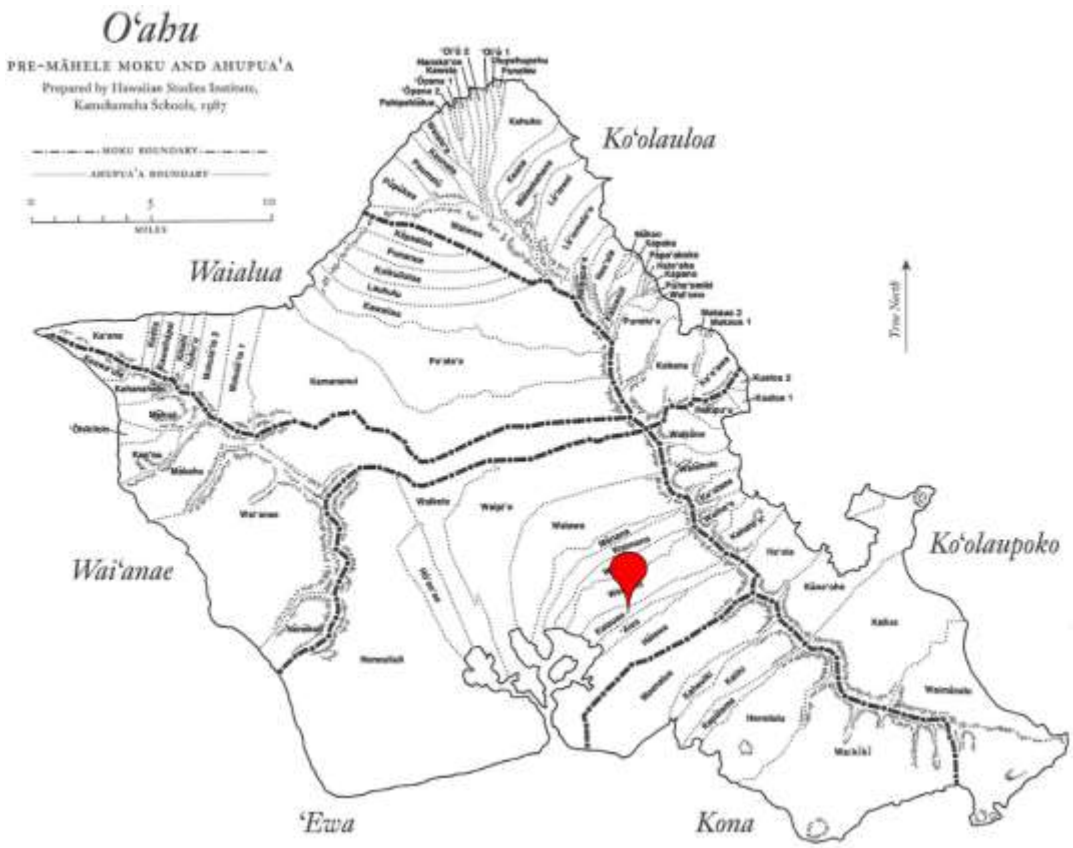

Figure 1. Map of O‘ahu *moku* and *ahupua‘a*. Kalauao *ahupua‘a* is highlighted by the red pin. Sourced from Hawaiian Studies Institute, Kamehameha Schools (1987).

Prior to western contact, highly productive *lo‘i kalo* (taro fields) made up the majority of wetland agriculture in Hawai‘i (Ladefoged et al., 2009). In the Kalauao area, both *lo‘i kalo* and *loko i‘a* relied on the Kalauao watershed system and freshwater springs to provide freshwater that would nourish taro and provide essential nutrient mixing in the brackish waters of the coastal *loko i‘a* that were located near the outflow of the watershed (Engels, n.d.). In the 100 years following western arrival in Hawai‘i in 1778, western influence contributed to the urbanization of the Honolulu area while society in Hawaiʻi began to change. As the population on Oʻahu increased, urbanization and large-scale agriculture of crops such as sugarcane and pineapple spread throughout the island in the late 19th and early 20th centuries (Oki, 1998). In the midst of these expansions, the Sumida watercress farm was established by Makiyo and Moriichi Sumida in 1928, drawing water from the Kalauao spring which has provided water for wetland crop cultivation in Kalauao for centuries ("Sumida Farm Inc., 2023). Figure 2 shows the location of Sumida Farm on the island of Oʻahu.

Figure 2. Map of Sumida Farm on the island of Oʻahu. Sumida Farm is located at the starred location. Adapted from ArcGIS Online (2023).

Throughout the late 20th and early 21st centuries, land use on Oʻahu has shifted away from large-scale agriculture towards urban use, with urban areas on Oʻahu increasing from 88.8 mi^2 to 148.8 mi^2 between 1968 and 1993 (Oliver, 1995). Since

1928, the landscape of Kalauao has also become highly urbanized, with Sumida Farm being one of the last green agricultural spaces in the area. Today, the fourth generation of the Sumida family watercress farmers continue to own and manage Sumida Farm, which produces around 70% of the watercress in Hawaiʻi, making the farm the largest watercress producer in the state (“Iconic Watercress Farm Aids UH Sustainability Research”, 2020).

Buying local foods has become a popular trend in the United States and in Hawaiʻi as consumers seek food from trusted local sources over large corporations and pursue efforts to live more sustainably (Xu, 2015). Consuming locally-grown food is also a significant part of supporting regional economies and employment (Xu, 2015). In Hawaiʻi, approximately 92% of food comes from overseas imports while the supply of fresh produce in the islands would last no longer than ten days if imports were halted (Kent, 2015). Hawaiʻi’s current reliance on out-of-state food sources thus makes Hawaiʻi residents vulnerable to food shortages if imports were halted in the event of a disaster (Kent, 2015).

As such, increasing food self-sufficiency and sustainability in Hawaiʻi has become an important topic as both residents and the local government strive to protect local food sources. As the largest watercress producer in the islands and one of the few remaining watercress and local, multi-generational, family-owned farms, Sumida Farm is an important facet of the agricultural sector on Oʻahu. Understanding potential threats to Sumida Farm, including urbanization and climate change, thus contributes to the broader understanding of threats to Oʻahu’s agricultural sector and capabilities for food sustainability.

1.3 Community and Ecosystem Services

The importance of Sumida Farm goes beyond the watercress it produces. For four generations, the Sumida family has managed the wetland, allowing the farm to survive despite various challenges for nearly 100 years in a community that has otherwise changed due to urbanization (Engels et al., 2020). Thus, Sumida Farm provides historical and social value for local residents. Other community services that Sumida Farm provides are associated with the farm's role as a green space in an urban setting. Figure 3 illustrates the location of Sumida Farm amidst the Pearlridge Shopping Center, Pali Momi Medical Center, Kamehameha Highway, and the Honolulu Rail Transit System in the ʻAiea area of Oʻahu.

Figure 3. Aerial view of Sumida Farm and surrounding features. Sourced from Esri ArcGIS Online (2023).

Green spaces in urban areas provide benefits for the mental health, social interaction, and sense of community for urban residents (Irvine et al., 2010). Green spaces have also been found to positively contribute to personal identity and sense of place (Irvine et al., 2010). Sumida Farm also provides educational value to local schools and communities by hosting school field trips and educating community members about urban agriculture and food systems (Engels et al).

In addition to providing community value, Sumida Farm provides ecological benefits for the Kalauao watershed. The majority of the water used to cultivate the watercress at Sumida Farm comes from the Kalauao spring. After the water flows through each watercress plot, water returns to the watershed through two outflow points. Previous studies have shown that Sumida Farm acts similar to a wetland ecosystem in that it removes bioreactive nitrogen as water passes through the farm, thus providing nutrient retention services and preserving water quality for the surrounding watershed (Engels et al., 2020). In the middle of a highly urbanized area, Sumida Farm is also able to provide habitat for fish species, such as guppies, and a multitude of wetland birds, some of which, including the ‘ae‘o (black-necked stilt) are native to Hawai‘i.

1.4 Micropollutants and Wastewater Tracers

Micropollutants are organic chemicals that typically enter water systems from anthropogenic and natural sources. Anthropogenic micropollutants include synthetic chemicals from pharmaceuticals, industrial chemicals, pesticides, and other sources and are able to persist in the environment for varying lengths of time (Luo et al., 2014). In urban areas similar to the ‘Aiea area that surrounds Sumida Farm, domestic sewage, industries, and hospitals are the main sources of micropollutants into sewer systems once these substances are excreted or improperly disposed (Bavumiragira and Yin, 2022).

Due to the ability of pharmaceuticals to resist degradation to some extent, most wastewater treatment plants are not able to fully remove micropollutants during wastewater treatment processes. Thus, pharmaceutical micropollutants are often able to enter the environment via runoff or by passing through wastewater treatment facilities (Luo et al., 2014). Once in the environment, some pharmaceutical micropollutants can potentially bioaccumulate (accumulate in the tissue) in aquatic organisms and cause negative effects to ecosystems (Luo et al., 2014). Although pharmaceutical substances are formulated to provide health benefits to human patients, aquatic organisms in systems affected by wastewater discharge can undergo physiological changes after being exposed to pharmaceutical substances either episodically or throughout their life cycles (Liu et al., 2015). Figure 4 shows the proximity of main sewer lines (red), which can become sources of wastewater discharge if ruptured, to Sumida Farm.

Figure 4. Map of sewer lines in the ʻAiea area surrounding Sumida Farm. Main sewer lines are shown in red while Sumida Farm is outlined in black. Farm plots from which samples were collected are highlighted within the farm boundaries. Adapted from City and County of Honolulu,, Honolulu Land Information System (HoLIS).

In addition to being spatially approximate to Sumida Farm, wastewater assets and facilities, including sewer lines, are expected to become increasingly vulnerable in the coming decades due to climate change (Spirandelli et al., 2018). Climate change is expected to cause sea level rise and increased heavy rainfall in Hawaiʻi which can cause disruptions to wastewater assets by increasing the risk of flooding, saltwater intrusion, groundwater inundation, corrosion, and overflow and spillage in sewage systems (Spirandelli et al., 2018). Ruptures to wastewater assets can lead to the contamination of streams and coastal waters and could also facilitate an influx of micropollutants into the environment. Figure 5, adapted from Spirandelli et al (2018), depicts the sewer mains, laterals, and on-site sewage disposal systems (OSDS) in urban Honolulu that are projected to become vulnerable if sea levels rise by 3.2 feet due to climate change.

Figure 5. Map of vulnerable wastewater infrastructure in urban Honolulu under a 3.2 increase in sea level. Sewage assets include on-site disposal systems (OSDS) and sewer lines which are color coded according to the Asset Vulnerability Index (ASI). ASI values indicate the following risk levels: 0.00-0.99: Low vulnerability; 1.00-1.99: Medium vulnerability; 2.00-2.99: High vulnerability; 3.00-4.00: Very high vulnerability. Wastewater assets nearest to Sumida Farm fall within the range of low to high vulnerability. Adapted from Spirandelli et al. (2018).

This project focuses on two pharmaceutical micropollutants from anthropogenic sources that are commonly used as wastewater tracers; caffeine and carbamazepine. Carbamazepine is an indicator of waste solely from human sources since the substance is not found naturally in the environment. Caffeine is produced in plants that grow in Hawaii (e.g. coffee) but occurs only at specific locations that lie outside of the study site and watershed, so it will be a unique human wastewater tracer in this setting. Caffeine is a substance found in coffee, tea, chocolate, energy drinks, and medication and is commonly consumed and excreted by humans (Potera, 2012). Caffeine was selected as a wastewater tracer in this experiment since it is a substance that is widely consumed by humans and is typically correlated with the presence of human fecal coliforms when found in aquatic environments (Potera, 2012). The mean half-life of caffeine in aquatic systems is around 1.5 days but can vary slightly depending on the type of degradation that the water undergoes during micropollutant removal processes (Lam et al., 2004). Thus, the presence of caffeine in an aquatic environment would likely suggest that caffeine (and wastewater) have been released into the system within a recent time frame.

Carbamazepine is a chemical compound found in anticonvulsant and mood-stabilizing medications that are used to treat conditions such as epilepsy and bipolar disorder (Hai et al., 2018). Though not as commonly consumed as caffeine, carbamazepine is one of the most commonly detected pharmaceutical substances in the environment and it is poorly removed in wastewater treatment processes due to its highly persistent nature, thus making this substance a suitable tracer for wastewater (Hai et al., 2018). Carbamazepine has a mean half-life of around 82 days in water depending on the degree to which the water is treated (Lam et al., 2004). In river sediments, carbamazepine can have a much longer half-life of around 328 days (Bavumiragira and Yin, 2022). Carbamazepine naturally degrades over time in the environment through processes such as sorption to sediments, photolysis, aerobic microbial degradation, and dilution (Hai et al., 2018). However, the natural degradation methods of carbamazepine are poor (Pal et al., 2010). Carbamazepine has a half-life of 84 to 2,100 hours when degraded under photolysis (degradation under sunlight) and 3,000 to 5,600 hours through biodegradation in the environment due to its stable nature (Pal et al., 2010). The half-life of carbamazepine in different wastewater treatment plants (WWTP) may vary from the environmental half-life depending on mechanisms used to treat wastewater at different WWTP. In general, carbamazepine is not well-removed in most WWTP and most WWTP have an average removal efficiency below 10% for carbamazepine (Murl, 2016). Table 1 summarizes the basic characteristics of caffeine (CAF) and carbamazepine (CBZ).

Table 1. Basic characteristics of caffeine (CAF) and carbamazepine (CBZ). Data sourced from the National Center for Biotechnology Information (2023); Lam et al. (2014); Potera (2012); and Hai et al. (2018).

Substance	Solubility (mg/mL)	Half-life in Water (d)*	Avg. Daily Dose (mg)	Usage
CAF	10-50	~1.5	300	Nervous-system stimulant
CBZ	0.0177	~82	800	Anticonvulsant

*Half-life varies based on environment and water treatment applications

Though the persistence of carbamazepine in the environment can vary based on water treatment processes and environmental conditions, carbamazepine typically persists much longer than caffeine in the environment. Due to this prolonged persistence, the presence of carbamazepine in aquatic systems could indicate the presence of wastewater that was released into the system up to a few months in the past. Comparing caffeine and carbamazepine abundance can thus provide a better understanding of when wastewater release events may have occurred.

Although caffeine has a relatively short half-life in aquatic environments, continuous releases of caffeine and carbamazepine into the environment via continuous sewage leaks or dumping can cause prolonged exposure for aquatic organisms (Pal et al., 2010). Some research has suggested that hydrophobic pharmaceutical substances, such as carbamazepine, can potentially bioaccumulate in aquatic organisms by entering and accumulating in lipid portions of the organisms, though more research is needed to determine the effects of individual pharmaceutical substances on aquatic organisms and ecosystems (Liu et al., 2015). There is little data on the uptake of pharmaceutical substances in plants (especially watercress) and transport through food chains (Carvalho et al., 2014). However, some research has shown that carbamazepine can be absorbed into plants including cucumber, cabbage, and peas when the plants are grown in soils with high concentrations of carbamazepine (higher concentrations than are typically found in contaminated water). Some research has also shown that caffeine can be removed from aquatic systems through uptake in plants such as duckweed (Carvalho et al., 2014). Although there is little research on the ability of watercress to absorb carbamazepine and caffeine, one study observed that watercress absorbed two different pharmaceutical substances, sulfamethoxazole and ketoconazole, when the substances were present in concentrations ranging from 5-10 µg/kg in soils, again these are much higher levels than expected from wastewater leaks (Chitescu et al., 2012).

1.5 Hydrology

Sumida Farm is located in the Pearl Harbor region of the central Oʻahu groundwater flow system which lies between the Koʻolau and Waiʻanae mountain ranges (Oki, 1998). Specifically, Sumida Farm and the surrounding Pearl Harbor area lies within the southern Oʻahu groundwater sub-area, which has a southern border bounded by low-permeability coastal caprock that inhibits the discharge of fresh groundwater and the intrusion of saltwater on O‘ahu’s southern border (Oki, 2005). Groundwater within the southern O‘ahu groundwater area is recharged by rainfall and irrigation from the surrounding groundwater regions (Oki, 2005). Major sources of groundwater discharge from the southern O‘ahu groundwater area include withdrawals from pumped wells, discharge via onshore springs, and discharge into the ocean through the caprock on O‘ahu’s southern border (Oki, 2005).

Springs within the Pearl Harbor and Pu‘uloa area have provided fresh water for wetland crop cultivation both before and after Western contact on O‘ahu. There have been five major spring complexes identified in the Pearl Harbor area, including the Waiau, Waimano, Waiawa, and Waikele springs (Oki, 2005). The fifth major spring, the Kalauao spring complex, is a major freshwater source for Sumida Farm, which relies on spring flow for 100% of its water input (Engels et al., 2020). Since 1880, four of the major spring complexes in the Pearl Harbor area have all declined in flow by around 50% (Engels et al., 2020). Increasing urbanization and groundwater pumping in the Pearl Harbor area are thought to contribute to the decrease in spring flow and groundwater resources (Engels, n.d.). A number of springs at Sumida Farm discharge through a thin layer of caprock along a break in slope on the farm’s upslope perimeter, providing water that is diverted to flow through the watercress plots (Engels et al., 2020). However, the hydrogeological structure of the aquifer beneath Sumida Farm is not well understood.

Sumida Farm is located in a watershed sometimes referred to as the Halawa-Waimalu stream watershed, which lies between the Waiawa and Moanalua stream watersheds. There are a number of perennial streams which flow year-round located within the same watershed and adjacent to Sumida Farm. Figure 6 shows the watershed boundaries and perennial streams of southern O‘ahu while figure 7 shows the Halawa-Waimalu stream watershed and Sumida Farm’s location within the watershed.

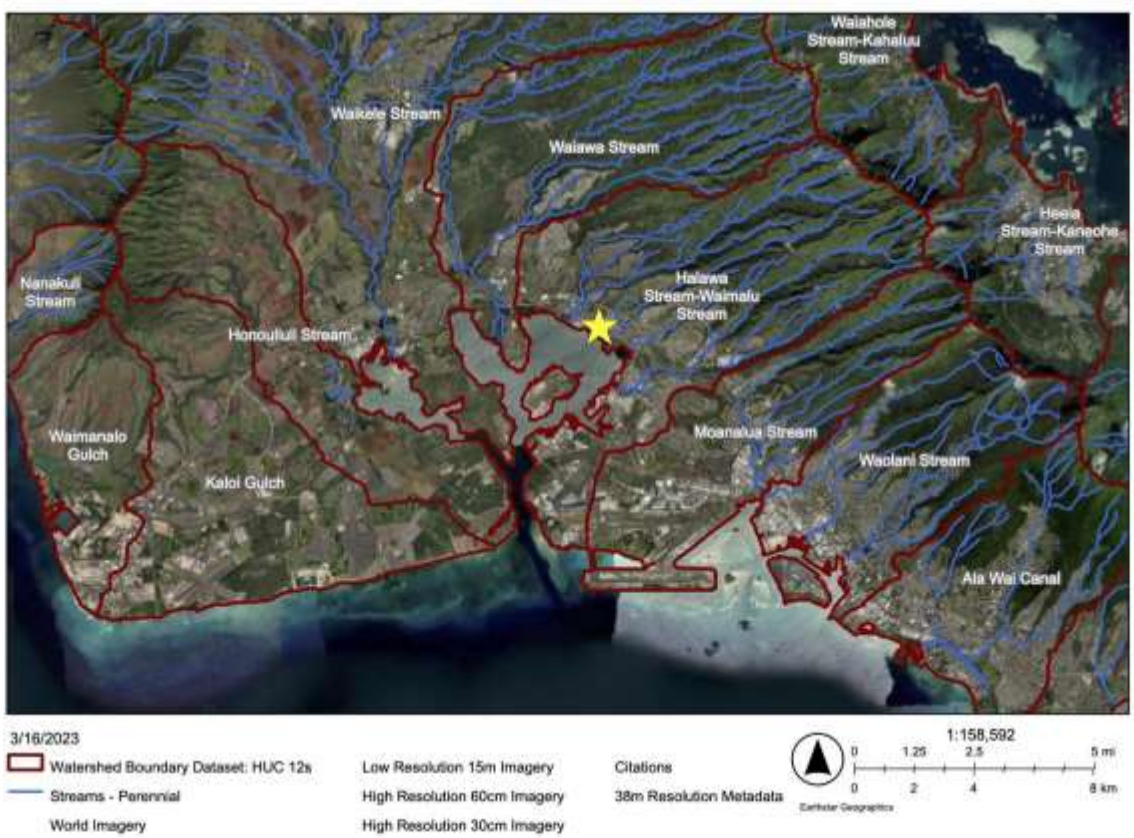

Figure 6. Map of southern O‘ahu watersheds and perennial streams. Watershed boundaries are shown in red and streams are indicated by blue lines. Sumida Farm is located in the starred location within the Halawa-Waimalu stream watershed. Adapted from the City and County of Honolulu Land Information System (HoLIS) and Esri Watershed Boundary Dataset (HUC 12s).

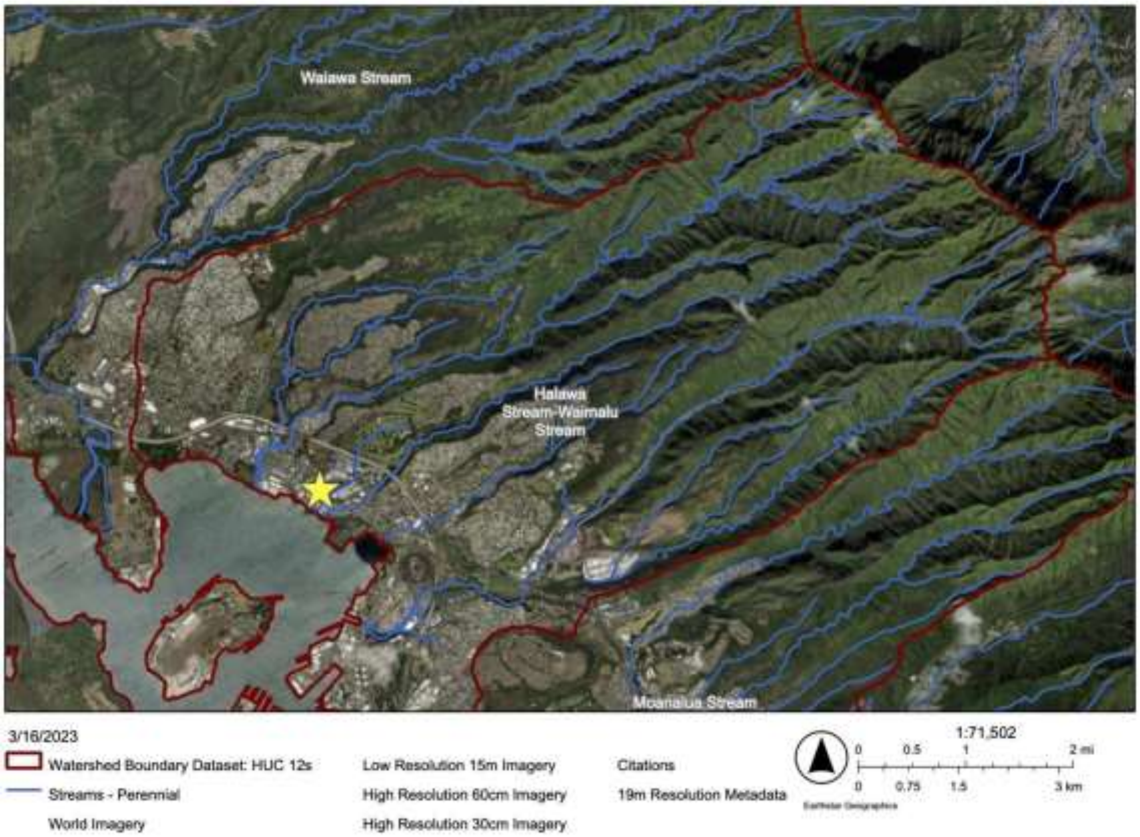

Figure 7. Map of Halawa-Waimalu watershed and perennial streams. Watershed boundaries are shown in red and streams are indicated by blue lines. Sumida Farm is located at the starred location. Adapted from the City and County of Honolulu Land Information System (HoLIS) and Esri Watershed Boundary Dataset (HUC 12s).

Since watercress is an aquatic or semi-aquatic herb, plots at Sumida Farm each contain about 0.5 to 1 inch of water that flows through each plot and is eventually returned to the watershed through two outflow points. Thus, assessing the freshwater at Sumida Farm for pharmaceutical wastewater tracers can also help to provide a better understanding of the water quality and the potential for contaminated water to flow into areas downstream of Sumida Farm in the Halawa-Waimalu watershed.

1.6 Prior Research

In the past few decades, farm managers at Sumida Farm have expressed concerns with decreasing crop yields (Engels et al., 2020). Data collected by farm managers revealed that between 1994 and 1998, monthly watercress yields were around 1,600 bundles. Decreases in monthly watercress yield at Sumida Farm were observed over time, with the monthly average yield decreasing to around 1,100 bundles between 2015 and 2019 (Engels et al., 2020). Other concerns at Sumida Farm involve the effects of the surrounding urbanization on water quality and crop yield at the farm.

Engels et al. (2020) assessed multiple parameters thought to be affecting crop yield at Sumida Farm including threats from pests, changes in temperature, and changes in farm water quality due to increased salinity (associated with saltwater intrusion and/or sea level rise), changing nutrient content, and pollution from the surrounding urban area. To assess the correlation of these environmental parameters to crop yield, hand-written records kept by farm managers since 1994 were digitized and compared to available data on air temperature, groundwater pumping, precipitation, and the Oceanic Niño Index (ONI) from 1994 to 2019. In addition, Engels et al. collected water samples from six major springs at Sumida Farm in September 2018 and February 2019 to allow for comparison between the fall dry period and spring wet period. Water samples were then analyzed for the presence of pesticides and pharmaceutical wastewater tracers (ethynylestradiol, caffeine, and carbamazepine).

Engels et al. (2020) found that high minimum and average temperatures had negative effects on watercress yield while precipitation had a weak negative relationship with watercress yield that was mostly driven by outlier events in precipitation data, likely because Sumida Farm primarily receives water from freshwater springs rather than sources directly from rainfall. Decreases in the Oceanic Niño Index, which is a collection of data on 3-month average temperature anomalies from the National Oceanic and Atmospheric Administration (NOAA), was also correlated with a slight decrease in watercress harvest yield.

All of the pharmaceutical wastewater tracers ethynylestradiol, caffeine, and carbamazepine) measured in springs feeding the wetland by Engels et al. (2020), including carbamazepine and caffeine, were found to be below detection limits. These results confirmed that the springs are of excellent water quality, not affected by, and are hydrogeologically isolated from the urban stressors.

Building on those findings, this study focuses on water quality over a larger spatial scale beyond the springs, sampling at other surface water bodies across the farm, and collecting water samples monthly on a longer timeframe (11 months).

CHAPTER 2. METHODS

2.1 Study Site

Sumida Farm receives the majority of its water from multiple freshwater springs along the northeastern *ma uka* section of the farm, which is adjacent to the Pearlridge Shopping Center parking lot. Sumida Farm covers around 11 acres and is divided into 111 plots that are labeled by letter and number coordinates, as shown in Figure 8. After flowing through the watercress plots, the water from Sumida Farm is returned to the watershed via two outflow points.

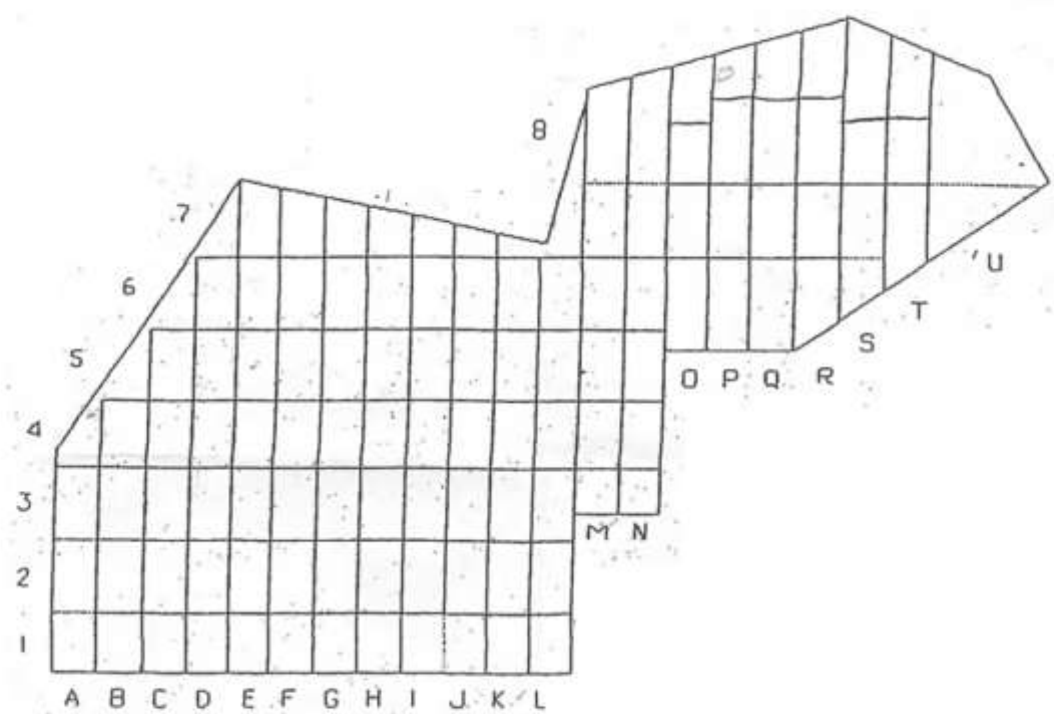

Figure 8. Drawing of coordinate system for watercress plots at Sumida Farm.

2.2 Data Collection

Monthly water samples were collected from Sumida Farm at ten different plots for 11 months from January to November 2022. The sample plots were selected based on recommendations from farm managers based on surrounding land-use. Overall, plots on the *ma uka* section of the farm were of most interest to farm managers due to the upslope location and proximity to uphill sewage lines, so the majority of the sample plots were evenly distributed across the *ma uka* area. Specific areas of the farm that were sampled, including column L and the northeast section of the farm, were chosen due to concerns with decreasing crop yield and other observed issues in these areas. The *ma kai* section of the farm was of least concern for farm managers and thus only one sample plot was designated in the *ma kai* area (plot A1). A map of the plots from which samples were collected is illustrated in figure 9.

Figure 9. Map of sampling locations at Sumida Farm. Adapted from Esri ArcGIS Online (2023).

Water samples were collected and stored in 40-mL amber vials using a peristaltic pump, pushpoint sampler, and 0.45 μm capsule filter (AquaPrep groundwater sampling capsule with supor membrane). Each sample was refrigerated between 4°C – 8 °C and stored until analysis at the Coastal Geochemistry and Hydrology Lab at University of Hawaiʻi at Mānoa.

To determine whether precipitation has a role in facilitating the flow of caffeine and carbamazepine onto the farm via runoff, precipitation data for the study period were assembled from the National Oceanic and Atmospheric Administration (NOAA) Record of Climatological Observations station at the Honolulu International Airport (USW00022521). This station is located approximately four miles southeast of Sumida Farm and is the closest weather station to the study site.

In addition to collecting water samples, temperature and specific conductance were also recorded at each plot at the time of sample collection. Specific conductance is used to measure the amount of dissolved ions in water and the ability of the water to conduct electric currents (Deletic, 1998). Temperature and specific conductance data were observed using a ProQuatro multiparameter water quality meter manufactured by Yellow Springs Instruments (YSI) Inc. Variations in temperature and specific conductance in a body of water can sometimes indicate that runoff and/or pollution has recently entered the water and caused the temperature and specific conductance to fluctuate as dissolved ions are added to the system (Deletic, 1998). Therefore, comparing positive detections of caffeine and carbamazepine against data on temperature and specific conductance may help to illustrate whether wastewater or runoff was discharged into the farm water and was correlated with positive caffeine or carbamazepine concentrations.

2.3 Data Analysis

All of the water samples were analyzed in two batches at the Coastal Geochemistry and Hydrology Lab at the University of Hawaiʻi at Mānoa. Samples from December 2021 to March 2022 were analyzed in April 2022, while the second batch of samples from April to November of 2022 were analyzed at the end of November 2022. Both rounds of samples were analyzed for caffeine and carbamazepine using Enzyme-Linked Immunosorbent Assay (ELISA) kits purchased from Abraxis, LLC (product numbers 515575 and 515585).

During analysis, samples were assessed for caffeine and carbamazepine using separate microtiter plates for each substance. Each plate received the prescribed volume of the water samples (or standards), antibody solution (50 µL), and enzyme conjugate solution (50 µL) according to the ELISA assay procedures before being covered with parafilm and incubated for 45 minutes (caffeine) or 90 minutes (carbamazepine). After the prescribed incubation period, each well was emptied before being washed four times with a wash solution with blotting in between each wash. Each plate then received 100 µL of color solution using a multichannel pipette before being incubated for 30 minutes. The color intensity of each well was inversely proportional to the concentration of the selected substance in the sample. A stop solution was then added to the plates. Within 15 minutes, each plate was analyzed for absorbance using a microplate ELISA photometer at 450 nm.

All standards were analyzed in duplicate. During the first round of sample analysis in May 2022, 19.2% of the samples for each caffeine and carbamazepine kit were analyzed in duplicates. In the second round of sample analysis in November 2022, 20.6% (caffeine kit) and 16.2% (carbamazepine kit) of the samples were analyzed in duplicates. In order to determine the confidence in the results generated by the ELISA method, standard curves were created to analyze the data. The B/B_0 for each sample and standard was calculated by dividing the mean absorbance value for each sample (B) by the 0 Standard mean absorbance (B_0). Standard curves were then created for the two rounds of sample analysis for each substance by graphing B/B_0 vs. the corresponding caffeine or carbamazepine concentration on the horizontal logarithmic axis. Concentrations of caffeine/carbamazepine in the samples were then compared to the standard curve and the test kit sensitivity.

CHAPTER 3. RESULTS

3.1 Software-Generated CAF and CBZ Concentrations

Out of 122 total samples collected from ten plots over the course of 11 months, 25 samples (20.5%) contained caffeine at levels above the manufacturer-reported ELISA kit sensitivity while three samples (2.5%) contained carbamazepine at levels above the manufacturer-reported kit sensitivity. There were two samples (1.6%) out of 122 total that contained both caffeine and carbamazepine. Upon inspection of the derived standard curves, it became clear that the caffeine kits did not perform as expected, with the standards showing a great scatter and varying from the concentrations reported by the manufacturer. We concluded that the manufacturer reported kit detection limit may not hold true and detection limits had to be re-evaluated. Many of the so-called positive results needed to be re-evaluated and the calculations used to re-evaluate the initial results are discussed in section 3.2 of the Results. Table 2 summarizes manufacturer-reported sensitivities, calculated sensitivities, and standard recovery based on 90% of the sample absorbance to blank absorbance ratio (B/B_0) for each of the caffeine (CAF) and carbamazepine (CBZ) ELISA kits.

Table 2. Performance of ELISA kits. Calculated sensitivity was calculated as 90% of B/B_0.

ELISA Kit*	Manufacturer-reported sensitivity (ng/mL)	Calculated Sensitivity (ng/mL)	Standard Recovery (%)
CAF 1	0.150	0.517	98.5±37.8**
CAF 2	0.150	0.035	198.1±307.2
CBZ 1	0.021	0.006	101.0±51.0
CBZ 2	0.021	0.016	99.1±23.3

*CAF 1 and CBZ 1 were kits used to analyze samples from January through April 2022 and CAF 2 and CBZ 2 were kits used to analyze samples from May through November 2022.
**Calculated after eliminating the two worst-performing standards (0.5 ppb and 1 ppb). Including the worst-performing standards, standard recovery was 63.346.2.

Results from the ELISA sample analysis including absorbances and software-derived concentrations for caffeine (CAF) and carbamazepine (CBZ) for all samples and standards are summarized in Table 3.

Table 3. Results of caffeine and carbamazepine sample analysis. Out(LR) indicate absorbances above the 0 standard range. Color coding indicates: gray: below manufacture reported detection limit (ManDL), ManDL<yellow< 90%B/B0, ManDL<blue>90%B/B0 and ManDL<green<50%B/B_0. There were six to seven standards for each kit that were analyzed in duplicates and are listed as CAFStd or CBZStd. NA indicates that data was not applicable. > = concentration above the kits maximum detection limit.

Plot	Date Sampled*	CAF Concentration** (ppb)	CAF Mean Absorbance	CBZ Concentration** (ppb)	CBZ Mean Absorbance
H7	1/26/22	2.252	1.185	Out(LR)	1.304
K3	1/26/22	Out(LR)	1.4545	0.003	1.0555
L6	1/26/22	Out(LR)	1.379	0.006	0.969
U8	1/26/22	Out(LR)	1.39	0.012	0.9325
H5	2/23/22	2.147	1.262	Out(LR)	1.119
K3	2/23/22	Out(LR)	1.429	0.001	1.1205
P7	2/23/22	2.242	1.197	Out(LR)	1.071
R8	2/23/22	Out(LR)	1.307	0.001	1.025
R8	3/30/22	Out(LR)	1.349	0.014	0.907
A1	4/27/22	Out(LR)	1.431	0.002	1.01
D5	4/27/22	Out(LR)	1.354	0.004	0.996
F7	4/27/22	2.075	1.279	Out(LR)	1.104
H5	4/27/22	Out(LR)	1.387	0.001	1.031
K3	4/27/22	2.088	1.277	0.043	0.779
L6	4/27/22	2.16	1.257	Out(LR)	1.242
M8	4/27/22	1.1	1.279	0.027	0.844
P7	4/27/22	Out(LR)	1.302	0.02	0.877
R8	4/27/22	2.201	1.234	0.02	0.873
U8	4/27/22	2.182	1.246	Out(LR)	1.191
A1	5/28/22	0.065	1.048	Out(LR)	1.655
K3	5/28/22	0.04	1.0315	Out(LR)	1.505
L6	5/28/22	0.66	0.793	Out(LR)	1.492
M8	5/28/22	0.113	0.955	Out(LR)	1.492
U8	5/28/22	Out(LR)	1.1905	0.005	1.1115

D5	6/25/22	0.019	1.022	Out(LR)	1.581
H7	6/25/22	1.195	0.697	Out(LR)	1.481
A1	7/30/22	0.763	0.8045	Out(LR)	1.5855
L6	7/30/22	0.001	1.059	Out(LR)	1.5425
M8	7/30/22	0.052	0.991	Out(LR)	1.516
P7	7/30/22	2.039	0.582	Out(LR)	1.411
H5	9/10/22	0.175	0.927	Out(LR)	1.361
H7	9/10/22	2.727	0.505	Out(LR)	1.568
R8	9/10/22	Out(LR)	1.16	0.035	0.927
U8	9/10/22	0.002	1.052	Out(LR)	1.185
A1	10/3/22	0.834	0.7625	Out(LR)	1.423
H7	10/3/22	Out(LR)	1.114	0.01	1.069
M8	10/3/22	0.001	1.055	Out(LR)	1.647
P7	10/3/22	1.266	0.686	Out(LR)	1.585
R8	10/3/22	0.163	0.932	Out(LR)	1.64
D5	10/31/22	0.263	0.895	Out(LR)	1.22
H5	10/31/22	0.012	1.031	Out(LR)	1.329
H7	10/31/22	2.304	0.551	Out(LR)	1.398
L4	10/31/22	1.227	0.692	Out(LR)	1.431
L6	10/31/22	0.107	0.988	Out(LR)	1.327
M8	10/31/22	0.002	1.051	Out(LR)	1.218
A1	11/21/22	0.553	0.852	Out(LR)	1.512
D5	11/21/22	0.158	0.934	Out(LR)	1.531
M8	11/21/22	0.492	0.831	Out(LR)	1.552
P7	11/21/22	0.355	0.867	Out(LR)	1.452
CAFStd1	5/24/22	0.000	1.327	NA	NA
CAFStd1	5/24/22	0.000	1.327	NA	NA
CAFStd2	5/24/22	2.201	1.233	NA	NA
CAFStd2	5/24/22	2.201	1.233	NA	NA
CAFStd3	5/24/22	1.076	1.288	NA	NA
CAFStd3	5/24/22	1.076	1.288	NA	NA
CAFStd4	5/24/22	1.044	1.306	NA	NA
CAFStd4	5/24/22	1.044	1.306	NA	NA
CAFStd5	5/24/22	2.501	0.602	NA	NA
CAFStd5	5/24/22	2.501	0.602	NA	NA
CAFStd6	5/24/22	3.008	0.385	NA	NA
CAFStd6	5/24/22	3.008	0.385	NA	NA
CBZStd1	5/24/22	NA	NA	0.000	1.048
CBZStd1	5/24/22	NA	NA	0.000	1.048
CBZStd2	5/24/22	NA	NA	0.018	0.888
CBZStd2	5/24/22	NA	NA	0.018	0.888
CBZStd3	5/24/22	NA	NA	0.074	0.694
CBZStd3	5/24/22	NA	NA	0.074	0.694

CBZStd4	5/24/22	NA	NA	0.088	0.665
CBZStd4	5/24/22	NA	NA	0.088	0.665
CBZStd5	5/24/22	NA	NA	0.237	0.480
CBZStd5	5/24/22	NA	NA	0.237	0.480
CBZStd6	5/24/22	NA	NA	0.535	0.327
CBZStd6	5/24/22	NA	NA	0.535	0.327
CBZStd7	5/24/22	NA	NA	2.258	0.119
CBZStd7	5/24/22	NA	NA	2.258	0.119
CAFStd1	11/29/22	0.000	1.126	NA	NA
CAFStd1	11/29/22	0.000	1.126	NA	NA
CAFStd2	11/29/22	1.152	0.722	NA	NA
CAFStd2	11/29/22	1.152	0.722	NA	NA
CAFStd3	11/29/22	0.439	0.88	NA	NA
CAFStd3	11/29/22	0.439	0.88	NA	NA
CAFStd4	11/29/22	0.311	0.881	NA	NA
CAFStd4	11/29/22	0.311	0.881	NA	NA
CAFStd5	11/29/22	2.874	0.490	NA	NA
CAFStd5	11/29/22	2.874	0.490	NA	NA
CAFStd6	11/29/22	4.847	0.285	NA	NA
CAFStd6	11/29/22	4.847	0.285	NA	NA
CBZStd1	11/29/22	NA	NA	0.001	1.1475
CBZStd1	11/29/22	NA	NA	0.001	1.1475
CBZStd2	11/29/22	NA	NA	0.021	1.005
CBZStd2	11/29/22	NA	NA	0.021	1.005
CBZStd3	11/29/22	NA	NA	0.054	0.8545
CBZStd3	11/29/22	NA	NA	0.054	0.8545
CBZStd4	11/29/22	NA	NA	0.114	0.692
CBZStd4	11/29/22	NA	NA	0.114	0.692
CBZStd5	11/29/22	NA	NA	0.243	0.501
CBZStd5	11/29/22	NA	NA	0.243	0.501
CBZStd6	11/29/22	NA	NA	0.47	0.3535
CBZStd6	11/29/22	NA	NA	0.47	0.3535
CBZStd7	11/29/22	NA	NA	>	0.117
CBZStd7	11/29/22	NA	NA	>	0.117

*Dates for standards are the dates on which the standard was analyzed in the lab
**Concentration results are valid based on the manufacturer's reported minimum detection limits, but results varied based on test kit performance, as discussed in the Discussion section.

3.2 Analysis of Software-Generated Results

Initial results from ELISA sample analysis reported in Table 3 were software-derived results that reported all concentrations above the manufacturer-reported minimum detection limits of 0.150 ppb for caffeine (CAF) and 0.021 ppb carbamazepine (CBZ) (and a few concentrations slightly below the minimum detection limit, highlighted in gray). The software used to report the results listed in Table 3 uses a 4-parameter commercial ELISA evaluation program. Manufacturer-derived detection limits are from 90% B/Bo established in standardized laboratory settings. More appropriate kit- and laboratory-specific detection limits that account for local laboratory conditions (e.g. temperature), user laboratory practices (reproducibility of pipetting), and other variables, can be derived from the standard 0 (blank) measurements for each kit. We derived these kit-specific detection limits as 90% B/Bo (Eurofins Abraxis Inc., 2021a; Eurofins Abraxis Inc., 2021b). Another way to report presence/absence for ELISA kits is to consider a very conservative approach of 50% B/Bo also called an I-50 (Eurofins Abraxis Inc., 2021a; Eurofins Abraxis Inc., 2021b). I-50 is the concentration of CAF or CBZ that shows 50% less color absorbance than the 0 Standard (which is the same as 50% B/B_0) (Neogen Corporation, 2018). By comparing the sample absorbances of CAF and CBZ against the manufacturer-reported minimum detection limit, kit specific 90% B/B_0, and I-50, the results can be interpreted for varying levels of confidence in how much the sample absorbance differs from background or standard 0 absorbances. Greater difference from background, or standard 0, means that it is more likely that the sample contains a true positive concentration of CAF or CBZ at greater confidence.

Concentrations reported in Table 3 (with the exception of values highlighted in gray) represent all of the samples with CAF and CBZ concentrations above the manufacturer-reported minimum detection limit, which in this case is the least conservative approach used for data analysis but also the least appropriate since we did not have standardized laboratory conditions. Sample concentrations were then plotted against a standard curve for concentration vs. B/B_0 (Figure 10) and compared to kit specific 90% B/B_0 and I-50, which filtered out the samples based on two different levels of confidence with I-50 being the most conservative confidence measurement used for sample analysis. Figure 10 illustrates CAF and CBZ concentrations vs. B/B_0 for standards and samples for each of the CAF and CBZ kits used in two rounds of sample analysis. Figure 10 also illustrates the cutoff levels for the manufacturer-reported sensitivity, 90% B/B_0, and I-50 for each kit.

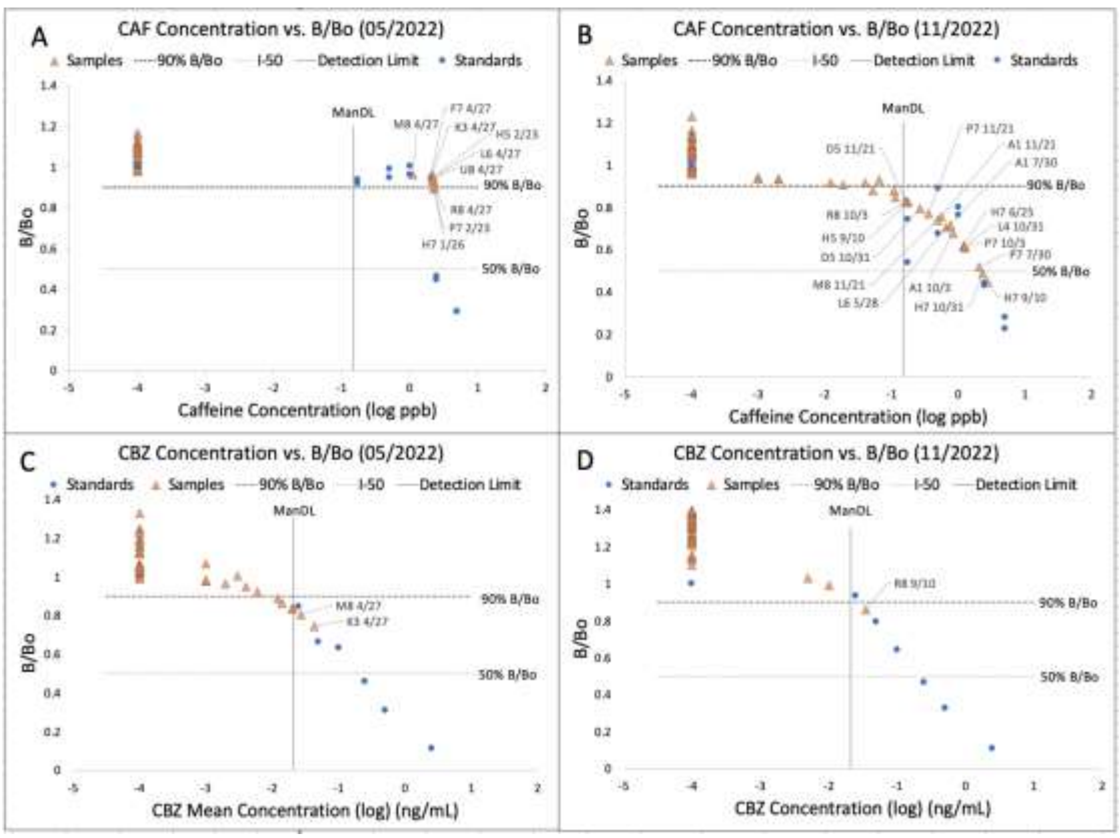

Figure 10. CAF and CBZ concentrations vs. B/B_0 for standards and samples. A: May caffeine analysis of samples from January through April 2022. B: November caffeine analysis of samples from May through November 2022. C: May carbamazepine analysis of samples collected from January through April 2022. D: November carbamazepine analysis of samples from January through April 2022. Manufacturers detection limit is indicated as ManDL. All samples above the manufacturer-reported minimum detection limit are labeled by plot and sample collection date. *log(0.0001) was used as an arbitrary concentration instead of log(0). Samples with a concentration of -4 thus represent samples with zero CAF or CBZ concentration.

Histograms were created to visualize the distribution of the sample absorbances for each test kit (Figure 11). Figure 11 also shows the absorbances for 90% B/B_0 and 50% B/B_0 (I-50) compared to each histogram.

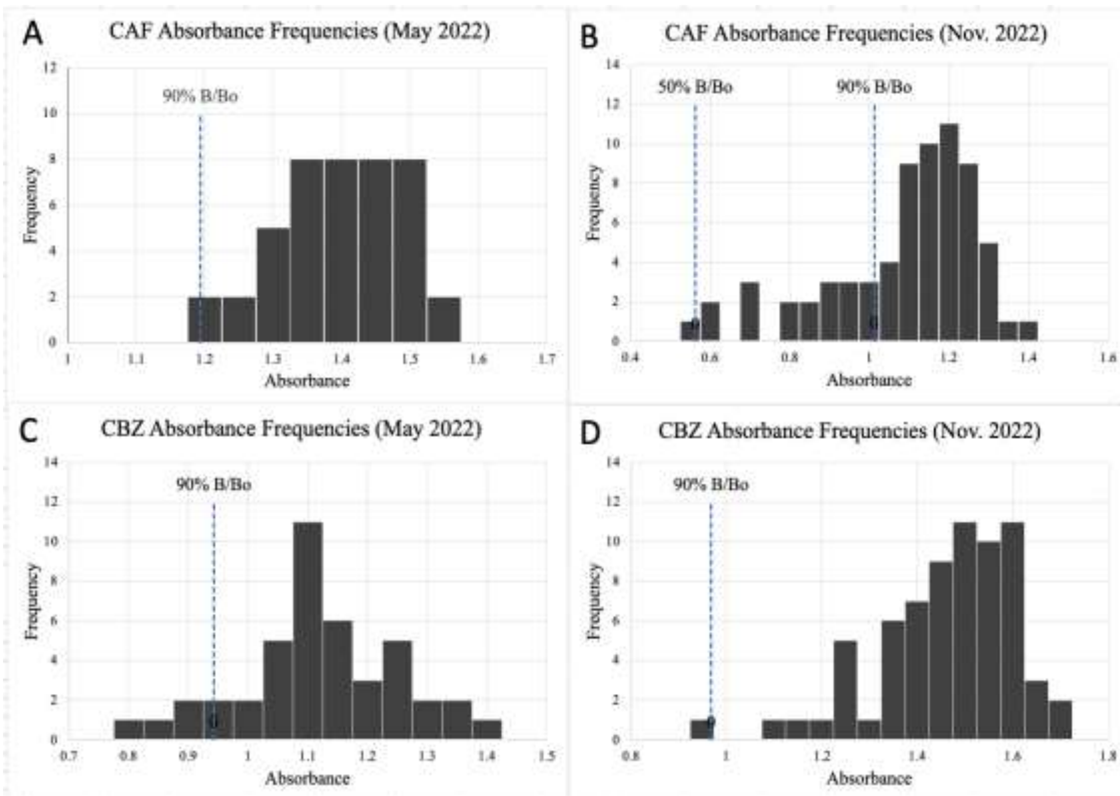

Figure 11. Distribution histograms for all CAF and CBZ kits. A: May caffeine analysis of samples from January through April 2022. B: November caffeine analysis of samples from May through November 2022. C: May carbamazepine analysis of samples from January through April 2022. D: November carbamazepine analysis of samples from January through April 2022. Blue dotted lines indicate absorbances matching 90% B/B_0 and 50% B/B_0 (I-50). Missing lines for 50% B/B_0 indicate that the absorbance corresponding to 50% B/B_0 was below the minimum absorbance visible within the figure.

Standard recoveries and standard curves for the carbamazepine kits indicated reproducible and highly effective kit performance. The caffeine kits did not perform at the same level. The caffeine standards analyzed in duplicates (plotted in blue in figure 10) did not align well within duplicates nor along the expected standard curve. In particular, standards 3 and 4 (0.5 ppb and 1.0 ppb) of the first round of caffeine analysis and standards 2, 3, and 4 (0.175 ppb, 0.5 ppb, and 1.0 ppb) in the second round of caffeine analysis performed poorly and did not reflect the manufacturer's-reported concentrations. The poor performance of standards in the caffeine kit may have been due to manufacturer or user error (e.g. improper storage that led to degradation of standards or inaccurate pipetting of standards into the kit wells). Due to the relatively poor performance of these standards in the caffeine kits, it is difficult to determine the true accuracy of the resulting sample caffeine concentrations below 1 ppb.

While initial results reported that 25 out of 122 samples contained caffeine, the comparison of samples to the kit specific 90% B/B_0 cutoff indicated that 15 samples (12.3%) out of 122 had positive levels of caffeine, while the I-50 cutoff resulted in only two samples (1.6%) with positive concentrations of caffeine. Initial results for carbamazepine indicated that three out of 122 samples contained carbamazepine and the comparison of the samples to the 90% B/B_0 cutoff affirmed that the same three samples (2.5%) contained carbamazepine, while there were zero samples with carbamazepine concentrations that were also below the I-50 cutoff. Thus, using the manufacturer-reported minimum detection limit indicates that all of the results in Table 3 are correct, but the amount of samples with a positive concentration of CAF or CBZ decreases when using kit specific 90% B/B_0 or I-50 as detection limits. Table 4 summarizes the number of samples with positive concentrations of CAF or CBZ using the various cutoff limits.

Table 4. Number of samples with positive concentrations under various detection limits. CAF=caffeine and CBZ=carbamazepine.

Substance	Positives Using Manuf. Detection Limit	Positives Using 90% B/B_0	Positives Using I-50
CAF	25	15	2
CBZ	3	3	0

All samples with CAF or CBZ concentrations above the manufacturer's minimum detection limit and B/B_0 below 90% were highlighted in yellow in table 3 in the Results section. Samples with CAF or CBZ concentration above the manufacturer's minimum detection limit and below I-50 were highlighted in green in table 3. Values highlighted in blue in table 3 indicate that although the concentration was above the manufacturer's detection limit, B/B_0 was above 90%. Lastly, values highlighted in gray in table 3 indicate values that were reported by the ELISA software, but are below the manufacturer-reported detection limit.

The importance of determining which samples had detectable CAF and CBZ levels is crucial when deciding whether or not there is wastewater leakage into the farm. After filtering the results based on 90% B/B_0 and I-50, there were no samples that contained both caffeine and carbamazepine. When analyzing samples under 90% B/B_0, plots A1 and P7 were found to contain caffeine most frequently with three occurrences of caffeine at each of these plots during different sampling dates throughout the study period. Plots H7 and D5 each had two occurrences of caffeine throughout the study period, while plots L6, H5, R8, L4, and M8 each had one occurrence of caffeine presence throughout the study period. Plot U8 did not have any positive detections of caffeine throughout the study period. When analyzing samples under I-50, only plot H7 was found to have caffeine present with two occurrences on September 10, and October 31, 2022. Frequencies of detected caffeine presence under 90% B/B_0 throughout the study period are visualized in figure 12.

Figure 12. Frequencies of caffeine occurrence throughout the study period under 90% B/B_0. Dark brown plots had three occurrences with detected caffeine, light brown plots had two occurrences of detected caffeine, and white plots had one or less occurrences of detected caffeine throughout the study period. Map adapted from Esri ArcGIS Online (2023).

When results were filtered for carbamazepine based on kit specific 90% B/B_0, three samples had a positive concentration of carbamazepine. Plots K3, M8, and R8 each had one occurrence of carbamazepine throughout the study period. No plots had positive carbamazepine concentrations when results were filtered based on the I-50 cutoff limits.

These data indicate there are low levels of caffeine and carbamazepine present at Sumida Farm, if any at all. When data are filtered based on the most conservative B/B_0 limit of 50% (I-50), two samples out of 122 contained caffeine and zero samples contained carbamazepine.

3.3 Environmental Results

Weather data collected from the nearest NOAA weather station (USW00022521) to Sumida Farm are illustrated as daily precipitation measurements throughout the study period of January to November 2022 (Figure 13).

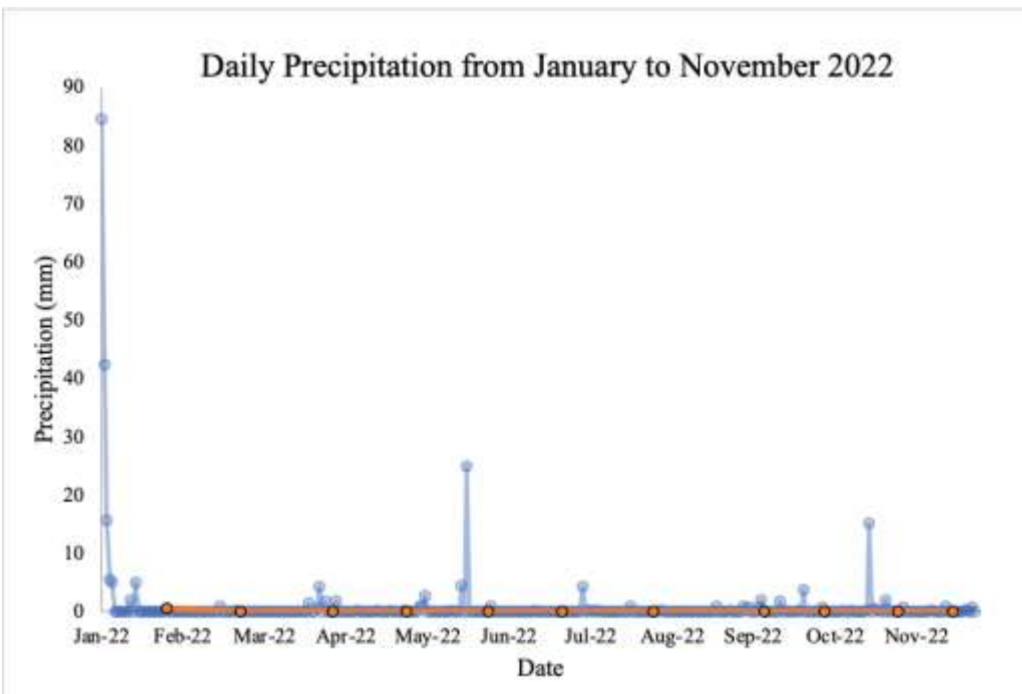

Figure 13. Daily precipitation data for January through November, 2022. Precipitation on sampling dates is indicated by orange points. Precipitation is reported in millimeters. Data sourced from the National Oceanic and Atmospheric Administration (NOAA) Record of Climatological Observations, Honolulu International Airport station (USW00022521), 2022.

Daily precipitation throughout the study period ranged from a minimum of 0.0 mm to a maximum of 84.6 mm. The average daily precipitation throughout the study period was about 0.76 mm. During the wet season in October to April, average daily precipitation was about 1.31 mm and during the dry season (April to October), average daily precipitation was about 0.30 mm. January 26, 2022 was the only sampling date on which rainfall occurred, with about 0.51 mm of rainfall on this date. However, rainfall occurred on the day before three sampling dates; April 27, September 10, and October 3, 2022. Rainfall was also recorded two days prior to the sampling date on September 10, 2022. Overall, the average daily precipitation that occurred on sampling dates and two days prior was about 0.13 mm. Daily precipitation characteristics throughout the study period are summarized in table 5.

Table 5. Daily precipitation characteristics throughout the study period. All values are reported in mm. Data derived from the National Oceanic and Atmospheric Administration (NOAA) Record of Climatological Observations, Honolulu International Airport station (USW00022521), 2022

Study Period Avg.	Study Period Min.	Study Period Max.	Wet Season Avg.*	Dry Season Avg.*	Avg. on Sampling Dates and Two Days Prior
0.76	0.00	84.58	1.31	0.30	0.13

*Wet season: October to April. Dry season: April to October

The average water temperature in all plots throughout the study period was approximately 22.94 °C while the average specific conductance in all plots throughout the study period was approximately 795.95 µs/cm. The average water temperature in the *ma uka* sampling plots (rows 6-8) was about 22.72°C while the average temperature in the *ma kai* sampling plots (rows 1-5) was slightly lower at about 22.69°C. Water temperature and specific conductance data collected at the time of sample collection are shown in Table 6.

Table 6. Temperature and specific conductance data for sampling dates and plots. NA indicates that data was lost and not available.

Date	Plot	Water Temperature (°C)	Specific Conductance (μs/cm)
1/26/22	A1	23.6	783
1/26/22	D5	22.0	804
1/26/22	H5	17.4	513
1/26/22	H7	22.0	526
1/26/22	K3	22.5	3,050
1/26/22	L6	21.8	593
1/26/22	M8	20.9	1,426
1/26/22	P7	22.8	1,400
1/26/22	R8	21.0	490
1/26/22	U8	22.7	305
2/23/22	A1	23.8	832
2/23/22	D5	22.7	766
2/23/22	H5	21.7	544
2/23/22	H7	22.9	572
2/23/22	K3	22.1	1,808
2/23/22	L6	21.7	397
2/23/22	M8	21.2	575
2/23/22	P7	22.8	1,457
2/23/22	R8	23.6	582
2/23/22	U8	24.8	48
3/30/22	A1	25.7	737
3/30/22	D5	22.4	783
3/30/22	H5	27.9	646
3/30/22	H7	25.2	523
3/30/22	H7S	23.2	676
3/30/22	K3	23.5	1,284
3/30/22	L6	22.3	458
3/30/22	M8S	20.3	489
3/30/22	M8	21.5	475
3/30/22	N5S	20.5	1,892
3/30/22	N5S	20.5	1,892
3/30/22	Q8	NA	NA
3/30/22	R8	22.4	636
4/27/22	A1	24.4	860
4/27/22	D5	21.1	926
4/27/22	F7	NA	NA
4/27/22	H5	22.3	771
4/27/22	K3	22.7	2,070
4/27/22	L6	21.3	528
4/27/22	M8	21.2	528
4/27/22	P7	24.7	658
4/27/22	R8	21.5	608
4/27/22	U8	23.9	586

5/28/22	A1	22.2	819
5/28/22	D5	23.0	937
5/28/22	H5	21.2	714
5/28/22	H7	21.9	600
5/28/22	K3	21.6	2,376
5/28/22	L6	21.7	549
5/28/22	M8	20.6	522
5/28/22	P7	21.7	1,392
5/28/22	R8	20.9	558
5/28/22	U8	22.0	538
6/25/22	A1	24.2	963
6/25/22	D5	22.1	1,075
6/25/22	H5	23.7	626
6/25/22	H7	21.7	565
6/25/22	K3	22.4	1,609
6/25/22	L6	21.3	413
6/25/22	M8	21.2	71
6/25/22	R8	24.0	458
6/25/22	U8	24.5	672
7/30/22	A1	23.7	836
7/30/22	D5	22.8	699
7/30/22	H5	23.7	934
7/30/22	H7	23.9	557
7/30/22	K3	23.5	2,513
7/30/22	L6	22.1	485
7/30/22	M8	22.1	449
7/30/22	P7	25.3	1,387
7/30/22	R8	25.6	556
7/30/22	U8	25.4	606
9/10/22	A1	24.9	563
9/10/22	D5	22.1	433
9/10/22	H5	21.3	1,081
9/10/22	H7	22.9	376
9/10/22	L4	25.3	3,160
9/10/22	L6	25.1	614
9/10/22	M8	23.4	1,153
9/10/22	P7	24.3	1,334
9/10/22	R8	21.8	607
9/10/22	U8	22.3	594
10/3/22	A1	25.7	820
10/3/22	D5	23.9	374
10/3/22	H5	27.0	892
10/3/22	H7	22.0	299
10/3/22	L4	23.6	1,016
10/3/22	L6	22.3	364
10/3/22	M8	21.6	771
10/3/22	P7	23.4	1,280
10/3/22	R8	27.0	644
10/3/22	U8	28.4	726

10/31/22	A1	25.6	863
10/31/22	D5	26.4	917
10/31/22	H5	25.8	647
10/31/22	H7	24.5	614
10/31/22	L4	23.9	886
10/31/22	L6	22.4	507
10/31/22	M8	21.5	548
10/31/22	P7	24.1	1,232
10/31/22	R8	22.7	576
10/31/22	U8	23.4	477
11/21/22	A1	24.4	745
11/21/22	D5	23.2	178
11/21/22	H5	22.0	642
11/21/22	H7	21.8	560
11/21/22	L4	22.9	1,437
11/21/22	L6	21.4	322
11/21/22	M8	21.3	97
11/21/22	P7	24.6	133
11/21/22	R8	22.3	407
11/21/22	U8	21.9	646

CHAPTER 4. DISCUSSION

4.1 Selected Substances

Due to increasing concerns about potential threats caused by wastewater infrastructure and urbanization near Sumida Farm in the midst of climate change, this study analyzed water at the farm for two pharmaceutical substances that are classified as contaminants of emerging concern (CEC) in the United States. Carbamazepine and caffeine were the two pharmaceutical wastewater tracers selected for this study due to their widespread use and differences in environmental persistence. Carbamazepine is one of the most common CEC's in the United States and is frequently identified in risk assessments and wastewater treatment plant (WWTP) sampling data due to its high potential for environmental impact (Murl, 2016). Although caffeine is also categorized as a CEC that is frequently detected in waters in the United States, it is considered to pose a low risk to the environment and is thus mainly used as a tracer for human activity (ie. wastewater) (Murl, 2016). Other alternative pharmaceutical CEC's, such as aciclovir, domperidone, and clonixin among others, are also frequently detected in aquatic systems in the United States but were not selected for this study because there is little knowledge on the impact of these contaminants in the environment (Murl, 2016).

4.2 Pharmaceutical Results and Prior Research

Prior research at Sumida Farm found that pharmaceutical wastewater tracers were not present in the six major springs that provide water to the farm. Engels et al. (2020) tested the six major springs at Sumida Farm for three pharmaceutical wastewater tracers (ethynylestradiol, caffeine, and carbamazepine) in September 2018 and February 2019 to allow for comparison between the fall dry period and spring wet period. All of the pharmaceutical wastewater tracers were found to be below detection limits in the major springs (Engels et al., 2020). These results showed that the springs that feed Sumida Farm have excellent water quality and are hydrogeologically isolated from the surrounding urban stressors.

This study builds upon the prior research of pharmaceutical tracers at Sumida Farm by assessing current conditions and collecting water samples over a larger spatial scale and time period. Thus, the results of this study will indicate whether conditions have changed since 2019, if there are fluctuations in wastewater tracers at the farm throughout different times of the year, whether the fresh water springs at the farm continue to produce excellent water quality, and whether numerous plots at Sumida Farm may be subject to wastewater runoff-related stressors despite spring water quality being high.

Overall, this study affirmed that wastewater contamination is not a current threat to Sumida Farm and water quality at the farm remains excellent. Using the most conservative approach in this study (using I-50 as a detection limit), there were two positive detections of CAF throughout the study period and no detections of CBZ out of 122 total samples. Thus, similar to the six springs that were sampled by Engels et al., in 2018 and 2019, the sample plots at Sumida Farm also reflect excellent water quality with no indications of wastewater contamination based on these two wastewater tracers.

4.3 Environmental Results and Correlations

In order to narrow down the results to the samples with a higher likelihood of true positive concentrations of CAF or CBZ, only samples with a %B/B_0 below 90% were used in comparison to precipitation, water temperature, and specific conductance data. Since there were only two detections of caffeine and no detections of carbamazepine under I-50, the results from I-50 were not used to assess any correlation between CAF, CBZ, and environmental parameters.

Out of 11 total sample collection dates, four sampling dates (36.4%) experienced precipitation on the same day or within two days prior to sampling. During the study period, the average daily precipitation on all sampling dates and two days prior was about 0.13 mm. The average daily precipitation on or two days prior to positive caffeine detections (under 90% B/B_0) was about 0.17 mm, slightly higher than the average for all sampling dates combined. However, on or two days prior to positive carbamazepine detections, average daily precipitation was lower than average at 0.02 mm. Out of 15 total detections of caffeine at Sumida Farm under 90% B/B_0, 10 detections (66.7%) occurred during the wet season from October 1 to March 30 while 5 detections (33.3%) occurred during the dry season between April 1 and September 20. Overall, establishing a correlation between precipitation, CAF, and CBZ at Sumida Farm proved to be difficult since there are very few occurrences of CAF and CBZ at Sumida Farm and there were only four sampling dates that took place in the days following a precipitation event.

In addition to comparing positive detections of CAF and CBZ to precipitation, values were also compared to water temperature and specific conductance data since variations in temperature and specific conductance in a body of water can sometimes indicate that runoff and/or pollution has recently entered the water (Deletic, 1998). The average water temperature of all sample plots throughout the study period was approximately 23.0°C, while temperature within plots that contained caffeine (according to the 90% B/B_0 cutoff) was slightly higher at about 23.7°C and the average temperature of plots that contained carbamazepine was slightly lower at about 21.9°C, though this is based on very few detections of CAF and CBZ.

The average specific conductance (SPC) of all sample plots throughout the study period was approximately 813.7 µs/cm while average SPC in plots that contained caffeine (under 90% B/B_0) was slightly lower at about 709.6 µs/cm. In the plots with positive detections of carbamazepine under 90% B/B_0, average specific conductance was about 683.1 µs/cm.

Based on very limited data and the averages listed above, it initially seems that there is no correlation between caffeine, carbamazepine, and the target environmental parameters at the farm. However, it was difficult to determine any correlation between CAF and CBZ presence and environmental parameters such as precipitation, water temperature, and specific conductance because of the lack of CAF and CBZ presence on the farm under 90% and 50% B/B_0. Additionally, most of the precipitation events throughout the study period did not align with the sample collection dates, making it difficult to determine whether precipitation had an influence on the CAF and CBZ concentrations found in each sample.

Traditional correlation analyses were not conducted between the target environmental parameters and CAF and CBZ presence due to the low presence of CAF and CBZ at the farm. It was determined that a traditional analysis of the environmental parameters using such limited data would have resulted in a correlation that would not reflect the true nature of any correlations between CAF, CBZ, and environmental parameters at Sumida Farm and instead, more research should be done (perhaps over a longer time period) to determine whether CAF and CBZ presence at Sumida Farm is correlated with precipitation, water temperature, and specific conductance. Further research on the correlation of environmental parameters to pharmaceutical wastewater tracers at Sumida Farm, especially precipitation, will become increasingly important in the future as climate and weather patterns vary due to climate change.

4.4 Limitations

Despite the very scarce detection of CAF and CBZ at Sumida Farm in this study, the minimal presence of caffeine and carbamazepine may be attributed to a lack of widespread consumption of these substances in the surrounding area. For example, although caffeine is typically a widely consumed substance, carbamazepine is typically found in drugs that may not be widely consumed. Thus, even if there was wastewater present at Sumida Farm, carbamazepine may not be present simply because it was not in the wastewater to begin with. This study analyzed two pharmaceutical substances with varying widespread usage to compare the abundance of the two substances and reduce the probability that both pharmaceuticals would not be in the wastewater to begin with. In the future, the water at Sumida Farm could be tested for other common pharmaceutical wastewater tracers, such as sulfamethoxazole or ibuprofen, to verify and build upon the results in this study by further reducing the likelihood that the target wastewater tracers are not highly utilized in the surrounding area.

One factor that may have slightly influenced the overall absence of caffeine and carbamazepine at Sumida Farm is uptake by plants at the farm. Some research has shown that some aquatic plants such as duckweed, which is present at Sumida Farm, have the ability to absorb caffeine when continuously exposed to concentrations around 0.01 µg/mL in a nutrient medium, though it is unlikely that plants would be exposed to these growing conditions in the setting of Sumida Farm (Carvalho et al., 2014). There has been some research on the ability of some plants to absorb carbamazepine, but plants that can absorb carbamazepine generally only do so when grown in soils with carbamazepine concentrations much higher than typically found in water, so plant uptake of carbamazepine at Sumida Farm would be unlikely. Conditions at Sumida Farm, with free-flowing water through each plot, would differ from the growing conditions utilized in such studies. The water samples analyzed in this study provide a baseline for any recent discharges of caffeine or carbamazepine (or wastewater that contains these substances) that have not yet been absorbed or degraded under the environmental conditions at Sumida Farm.

The results of this study are useful for providing a baseline analysis of current caffeine and carbamazepine concentrations (and thus wastewater presence or lack thereof) at Sumida Farm. However, in the future as climate change continues to change the precipitation patterns and sea level in Hawaiʻi, wastewater assets adjacent to Sumida Farm may become compromised due to flooding and/or saltwater intrusion. For example, as indicated in Figure 4 of the Introduction, wastewater assets close to Sumida Farm are ranked as having low to high vulnerability under a 3.2-foot increase in sea level, which could lead to the corrosion of wastewater infrastructure and leaks that could increase the presence of wastewater and wastewater tracers at Sumida Farm under these conditions. Other changes such as increasing storm frequency and changes in rainfall patterns under climate change may also change the water quality at Sumida Farm as increased runoff and storm frequency can potentially facilitate the flow of wastewater-contaminated surface and subsurface runoff onto the farm. Since Sumida Farm receives the majority of its water from fresh water springs, it is important to monitor threats to the fresh water springs and identify sources of potential contamination. In the future, wastewater tracers can be continuously monitored at Sumida Farm to see if there are any changes to the wastewater presence at the farm as the effects of climate change become increasingly magnified, with the results of this study providing a baseline for current conditions at the farm in 2022.

CHAPTER 5. CONCLUSION

The results of this study indicate that wastewater is not likely to be an issue of large concern at Sumida Farm and within adjacent regions of the Kalauao watershed based on the analysis of two wastewater tracers; caffeine and carbamazepine. Due to the minimal presence of caffeine and carbamazepine at the farm, it was not possible to determine whether the presence of these substances is correlated with precipitation, and thus determining whether precipitation can facilitate the flow of these substances (and wastewater) onto the farm would require further research.

Since there seems to be little or no wastewater presence at Sumida Farm based on these tracers, issues observed by farm managers at Sumida Farm, such as decreasing crop yield and water quality in some plots, may be caused by other issues that are not related to wastewater contamination. In the future, research at Sumida Farm can be continued to further analyze the contribution of other factors to the concerns of farm managers, such as contamination from non-wastewater sources, increasing urbanization, changing climate and weather patterns, or other factors. The results of this study can also be expanded upon by testing for other pharmaceutical wastewater tracers, expanding the study period, or by planning sampling dates to better observe any correlation between wastewater tracers and precipitation. This study provides a useful baseline for current conditions at Sumida Farm as of 2022. With changing climate conditions, particularly sea level rise, that will impact nearby wastewater infrastructure that can cause changes to future water quality and wastewater presence at the farm, this study will allow for the comparison of current conditions to any changes found in future research.

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