Determining whether pH impacts bacterial composition in soils found in various San Franciscan microclimates

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Introduction:

San Francisco is home to significant topographical and geographical diversity. This phenomenon results in many microclimates – local changes to atmospheric conditions found throughout the city. Though microclimates play a significant role in air temperature and humidity, research has shown that they can impact smaller components of nature including the makeup and composition of soil (1) – a complex mixture of sediment, plants, microbes, and other debris. While it's known that bacteria and fungi play a critical role in soil nutrient availability, very little is known about what species are found and what roles they play. In terms of microclimates, very little is known how they impact bacterial composition and activity. More specifically, it's unclear how microclimates affect soil acidity levels and whether acidity impacts the diversity and the population of bacteria found in a given soil sample(2,3). As acidity is known to slow bacterial growth in the laboratory (4, 5), the hypothesis for this project is that soil collected from sites with lower pHs will have less overall microbial population but more diversity. Increased acidity will stunt the growth of most bacteria, allowing for greater resource availability for other bacteria to use. This is hypothesized to result in more biodiversity but less population. By studying this, we can use this information to plan where specific plants would have the most success in growing.

Methods:

Sample Collection: Soil samples were individually collected from three locations in the SF Bay Area and placed into sterile plastic bags. The first sample was collected at Golden Gate Park at the coordinates 37.772113851997375, -122.4867492322314 at 12/6/23 at 3:36 pm PST with a temperature of 61 degrees Fahrenheit. The second sample was collected at Dolores Park at the coordinates 37.760170961030475, -122.42703660621126 12/6/23 at 3:50 pm PST with a temperature of 61 degrees Fahrenheit. The third sample was collected in the presidio at the coordinates 37.80204343736357, -122.46153947362056 12/6/23 at 4:23 pm with a temperature of 58 degrees Fahrenheit.

Soil Processing and Microscopy: About 5 oz. of soil per site was diluted with approximately 100mL of 1x Phosphate Buffered Saline (PBS) in separate containers; the mixture was mixed with a mixing rod. One drop of the mixture was added to a microscope slide and allowed to air dry. A bacterial staining kit (Gram stain) was used to stain the slide. Once stained, the slide was put under a microscope to visualize the sample. Differences in shape, size, color, and formation were recorded and compared amongst the different soil samples.

Cell Count Analysis: 1 mL of the mixture (described above) was added to 9 mL PBS, resulting in a 1/10 dilution of the starting sample. This was done five more times so that the final concentration was 1/1000000 of the starting amount. After dilution, one drop of the mixture was added to a petri dish filled with nutrient agar and spread around with a Q-tip. The lid was then taped shut on the petri dish and the entire dish was flipped upside down for incubation at room temperature for 5 days. After 5 days, the size,

shape, color and count of the colonies was recorded.

Figure 1: Map of collection sites

Results

To determine the bacteria diversity across San Francisco and test whether there was a correlation between soil pH and bacterial makeup, soil was collected from three sites across the city (as shown in **Figure 1**). Site 1(the westernmost site) was chosen because of its proximity to multiple static bodies of water; it is located in Golden Gate Park. Site 2 (the northernmost site) was chosen because of its proximity to the ocean; it is located in the Presidio region of San Francisco. Finally Site 3 (the southernmost site) was chosen because of its isolation from large bodies of water; it is located in Dolores park. Images of these samples are shown in **Figure 2.**

Figure 2: Images depicting soil collected from each site

As shown in **Figure #3**, out of the three soil collection sites, Golden Gate Park soil had more bacterial counts compared to the other two locations. All colonies were uniform in shape and color with no major differences between the samples. Similarly, there were no major changes in pH between the samples (data not shown).

Finally, in terms of morphologies seen under the microscope **(Figure #4)**, in the Golden Gate Park sample, significant changes to the saturation of the colors were observed along with more bacterial counts.

Figure 3: Graph of the colony counts from each site

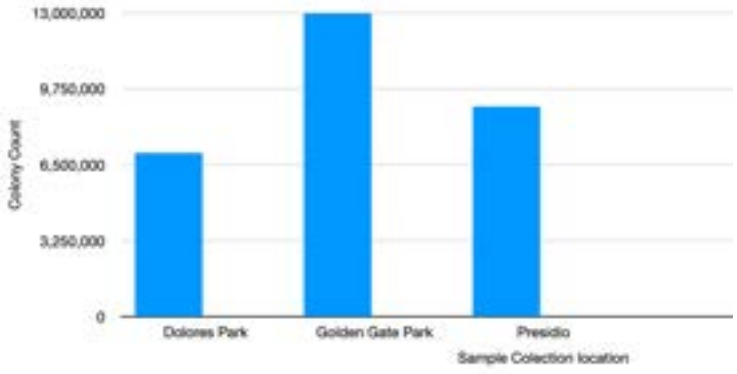
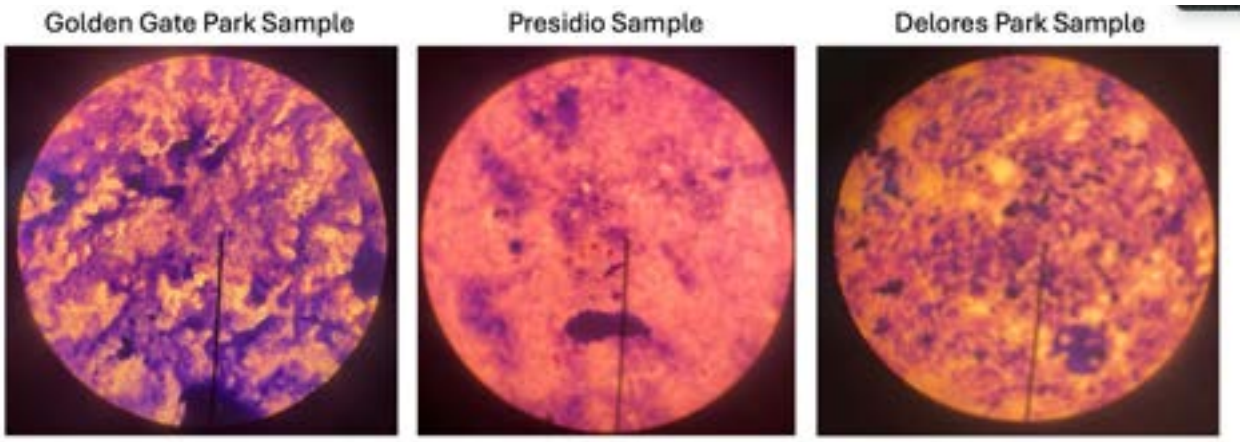


Figure 4: Microscopy images of Gram stained soil sample



Conclusion / Discussion

The location chosen for this experiment vastly changed the results. The first example of this was seen in the Golden Gate Park collection site; the Golden Gate Park sample had the most amount of bacterial activity out of all the samples with approximately 13,000,000 colonies. This may be due to its proximity to multiple still bodies of water. Correspondingly, the Dolores Park sample, which is nowhere near any body of water, had the lowest number of bacterial colonies (7,000,000). The Presidio sample had an inbetween result; while the Presidio is relatively close to the ocean, there were no major standing bodies of water near it. Interestingly, this sample had only 9,000,000 colonies – in between the other two collection sites.

Across all samples, there were no major changes to pH, suggesting that this part of the original hypothesis wasn't a major driver in terms of differences in bacterial diversity. Nevertheless, diverse population differences were still seen. As the presence of water (or the proximity to standing bodies of water) differed between each sample site, it's reasonable to think this factor is a major contributor and should be studied further.

The results from these experiments can contribute to the scientific community in a multitude of ways. First, we found that bacterial populations may change depending on proximity to water; this means that the colonial bacterial population increases when closer to water and decreases when farther. Though this study didn't test this, these results could be because most bodies of water contain not only nutrients but can help bacteria spread to the surrounding areas. Secondly, from this study, we found that bacterial populations aren't solely dependent on pH levels in the soil. Lastly, some challenges with this study included determining what locations to sample, the method as to how soil should be collected, and how materials can be cheaply & easily obtained. These challenges can be avoided by planning in advance the specific coordinates that soil will be collected from as well as doing a great deal of research on materials before making anything permanent.

Citations:

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