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Central MA Nematode Resilience

Devin G. Cunningham
Worcester Polytechnic Institute

Resilience of Central Massachusetts Nematodes

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Devin Cunningham

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1. Abstract

Soil nematodes have a key role in the environment, contributing greatly to soil nutrient turnover and occupying positions of primary and intermediate consumers in the food web of the soil ecosystem (Bongers and Ferris, 1999). The distribution of varying soil nematodes is known to be influenced by the qualities of the soil that composes their habitat. Specifically, a soil sample's proximity to a pollution source is known to potentially be correlated with variances in the sampled nematode population when compared to similar samples varying distances away from the source. Trends have been noted within species, such as differences in nematode sex ratios with exposure to pollutants (Pen-Mouratov et al., 2008). There have also been variations noted in similar locations of the types of nematodes present in more or less polluted areas, with some species being more scarce in polluted areas (Pen-Mouratov et al., 2010).

To explore the presence and qualities of nematodes in areas of Worcester, Massachusetts, sites with industrial history were determined and soil samples collected from these sites. Nematode presence was detected in all soil samples, with three samples producing nematodes that were successfully cultured. The cultured nematodes were grown on agar plates of varying pH values, to investigate their resilience and the impact of the pH on their growth rates. It was found that the nematodes generally grew best at a pH closest to the pH of the soil they came from, indicating that the nematodes present at these sites are well adapted to, and have possibly even evolved in response to, the acidic soil of Worcester.

2. Background

2.1 What are Nematodes

Nematodes are unsegmented multicellular invertebrate organisms found in freshwater, saltwater, and terrestrial environments. As well as being diverse in the environments they inhabit, nematodes can also be free living or parasitic. Free living nematodes can survive by consuming decaying plant matter, fecal matter, fungi, decaying organisms, and living organisms such as bacteria (Platt, 1994). Parasitic nematodes have immense diversity as well, parasitizing varying plants and animals, from ants, livestock, and pets, to humans (Yanoviak et al., 2008).

Soil nematodes in particular are immensely diverse, in both distribution and the number of known unique species. Samples taken in Antarctica have yielded fewer than ten unique

species of nematode, while pastures, prairies and rainforests have been known to yield up to hundreds of unique soil nematode species in a single ecosystem in a single day of soil collection. As a result of this immense diversity, along with the uneven distribution of nematodes among differing terrestrial environments, estimates as to the total number of terrestrial nematode species vary greatly (Ettema, 1998). With similar variances noted in aquatic nematodes, along with the general lack of exploration of aquatic environments such as deep ocean areas, the overall number of nematode species is often questioned and estimated, without a confident answer.

2.2. The Importance of Nematodes

Nematodes are valuable organisms to study and understand due to their potential as a model organism, their direct impact on humans, and the information that can be gathered based on their presence within a terrestrial ecosystem.

2.2.1 Nematodes as a Model Organism: C. Elegans

A key species of soil nematode is *C. elegans*. This nematode is utilized as a model organism for numerous studies, including those on neurobiology, developmental biology, and evolution, and the processes involved in some human diseases. This species is suitable for its role as a model organism due to many desirable traits. It is easy to observe with both a dissecting microscope or a compound or confocal microscope, depending on whether one wishes to view the organism as a whole or its individual cells, and its transparent body allows for observation of many anatomical features and processes in vivo, without any need for sacrifice and dissection. It also has a rapid life cycle, allowing for observation over several generations, and can be frozen and thawed as needed. *C. elegans* has both a hermaphroditic and male form, which can be utilized for different studies and purposes. For instance, utilizing only the hermaphroditic nematodes can ensure that a mutated gene is passed down (Corsi, Wightman, and Chalfie, 2015).

While C. elegans has many intrinsic traits that make it suitable as a model organism, work done by researchers has been essential to understanding this nematode. Due to complete genome sequencing and annotation, any mutations that develop can easily be identified, and genes can be introduced to modify the organism for studies. A complete cell lineage has been developed by tracking every cell in the organism from fertilization to maturity, and all neurons of

the hermaphrodite and male forms have been mapped as well. This has made C. elegans invaluable in developmental and neurobiological studies (Corsi, Wightman, and Chalfie, 2015).

2.2.2 Nematodes and Humans

Aside from their use in studies, a key way that nematodes interact with humans is through parasitism. It is estimated that worldwide, there are 3.5 billion cases of gastrointestinal nematode infections, and the prevalence of these infections has remained unchanged in over 50 years (Chan, 1997). The majority of these cases are asymptomatic, with the cases that result in symptoms not being directly fatal. The main species that infect humans are commonly known as roundworms, hookworms, pinworms, and whipworms.

A majority of cases of nematode infections occur in developing countries and areas of poverty. A key factor to transmission is frequent close contact between individuals, and poor personal hygiene. Individuals living in warmer environments are more prone to infection as well. A majority of infected people are children, who often have yet to develop proper personal hygiene. The main symptoms of infection include anemia, malnutrition, and secondary infections (Stepek et al., 2006).

Parasitic nematode infections in livestock can indirectly impact humans as well. Infections cause the same symptoms in livestock as in humans, with malnutrition being of particular concern in animals raised to be utilized for their meat. It is estimated that the costs of parasitic nematode infections in sheep and cattle exceed tens of billions of dollars annually worldwide (Roeber et al., 2013).

Nematodes can also parasitize or feed on plants, leading to a large impact on the agricultural industry. It is estimated that they lead to a yield loss of 12.3%, approximately valued at 157 billion dollars, annually (Singh, 2015).

2.2.3 Nematodes and Soil Quality

Free living soil nematodes are considered an inexpensive and useful indicator of soil quality. This is due to their abundance in most environments, diverse feeding habits, and simple sampling procedures. Numerous relationships between nematode populations and an environment’s level of pollution and human disturbance have been noted, and in general, differences can be noted between the nematode populations of human impacted and relatively untouched areas that are geographically close.

While patterns between pollution, general human impact, and soil quality have been noted, interpretation of these patterns requires more research in order to develop strong tools of determining ecosystem viability through nematode populations that can be applied to multiple different ecosystems and maintain validity (Porazinska, 1999).

It has been noted that different environments created by human activity tend to have different concentrations of nematode types. For instance, it was found that in livestock grazing areas, plant parasites were at exceptionally high concentrations, while bacterivores and omnivores were less numerous in samples collected closer to places such as power plants, but most numerous in areas with less industrial activity, such as recreational parks. Fungivores have been noted to be the least impacted by human activity, and also the least sensitive to pollutants, with bacterivores being the most sensitive to pollutants (Pen-Mouratov et al., 2010).

With nematodes occupying positions of primary and intermediate consumers, their maintained diversity can be key to the health of the soil ecosystem (Bongers and Ferris, 1999). Another key trend that has been observed is that soil nematode species diversity tends to be greater in soil ecosystems exposed to less stress, with a common stressor being pollutants. When the ecosystem is under stress, there tends to be a shift to dominance by an opportunistic species. As this species becomes more dominant of the ecosystem, species diversity decreases (Odum, 1985).

The USDA cites nematodes as a useful indicator of changes in soil quality due to this well-documented trend. Nematode populations are relatively stable as the temperature and moisture content of an area changes, so shifts towards less species diversity can be an important indicator of a reduction in soil quality, or the presence of contamination. For this purpose, the number of species is sometimes evaluated, or the distribution of nematodes present in a sample among trophic groups (Ingham, n.d.).

2.3 Worcester's Development

After serving as a center for American revolutionary activity in the 1770s, Worcester, Massachusetts developed a manufacturing-based economy in the early 1800s (Worcester Historical Museum, 2013). Large manufacturing companies such as the Washburn and Moen company and Wyman-Gordon company later developed, and secured Worcester's status as an industrial city. When the Worcester and Boston Railroad was opened in 1835, the city became a transportation center, and the economy continued to grow and strengthen (Ricciardi and Mahoney, 2013).

However, after World War II, with cheaper overseas alternatives for manufacturing, Worcester began to decline as its numerous factories and processing plants became obsolete. Currently, there are revitalization plans in place for Worcester, focusing on the downtown area, which aim to improve residential quality of life and increase the number of tourist destinations (The City of Worcester, 2019). As a result, Worcester is currently a unique mix of more natural areas, areas of past industrial development, areas of current industrial use, and areas currently being rebuilt and repurposed with new construction.

For this study, six locations of historical and industrial significance in Worcester were selected. Brief histories of these sites are presented in Appendix A.

3. Methods

3.1 Soil Sample Collection

Soil samples were collected from sites in Worcester of industrial significance. These sites were selected with the aid of humanities professor Joseph Cullon. The sites chosen were Oread Castle Park, the Lofts, Coes Pond, Hadwen Arboretum, Saint Gobain's Abrasives, and Institute Park.

At each site, soil was located and collected into a plastic bag, to allow for ease of collection of a large volume of soil at once. Most samples were collected by digging at least 2 inches deep. The precise coordinates of where each sample was obtained were recorded, along with the soil temperature and how deep into the ground was dug to obtain the sample. These details are presented in Appendix B. Samples were stored at room temperature.

3.2 Agar Plates

Nematode growth media (NGM) agar plates were produced utilizing the composition and protocol outlined by Stiernagle for *C. elegans* maintenance. While the ingredient ratios were held consistent, smaller batches of media were generally produced as needed, rather than a liter of media at a time.

Later plates were produced with the addition of amphotericin B, and cycloheximide at concentrations of 10 micrograms per mL to discourage fungal growth. To produce plates of varying pH, the standard protocol was followed until the media was to be autoclaved. Prior to autoclaving, a pH probe was inserted into the media, and acid or base added dropwise until the

desired pH was obtained. It was thought that ingredients added after autoclaving would have minimal impact on the pH of the resulting media, and thus the pH values obtained at this time would be consistent throughout the production and use of these plates. The target pH values were 5, 5.5, 6, 6.5, and 7.

3.3 Isolation on an Agar Culture Plate

To isolate the nematodes from the soil samples, a modified version of the plating method was utilized (Barrière and Félix, 2006). Soil was placed in a conical tube, and water added at a ratio of 1mL of water for every 5mL of the tube filled with soil. The moistened soil was scooped and placed around the edges of the agar plates, with any remainder placed on one half of another plate. For the initial exploration of nematode presence, two plates were left as-is, with two having a drop of OP50 liquid culture placed in the center of the soil ring. The setup of these plates can be observed in Figure 1.

OP50 was selected as a food source for omnivorous or bacterivorous nematodes due to its relatively slow growth that would not obscure the plate. It was grown in a liquid culture after transfer from the edge of a colony grown on an agar plate utilizing a wire inoculating loop. The liquid culture was stored in a shaking incubator to allow for the OP50 density to increase, and then later refrigerated. New liquid cultures were produced as needed.

While it is described by Barrière and Félix that nematodes begin to leave the soil within minutes, the plates were initially monitored every other day for a period of ten days using a dissection microscope. Most plates did not begin to show signs of nematode activity until several days after the initial plating, justifying this extension to the observation period.

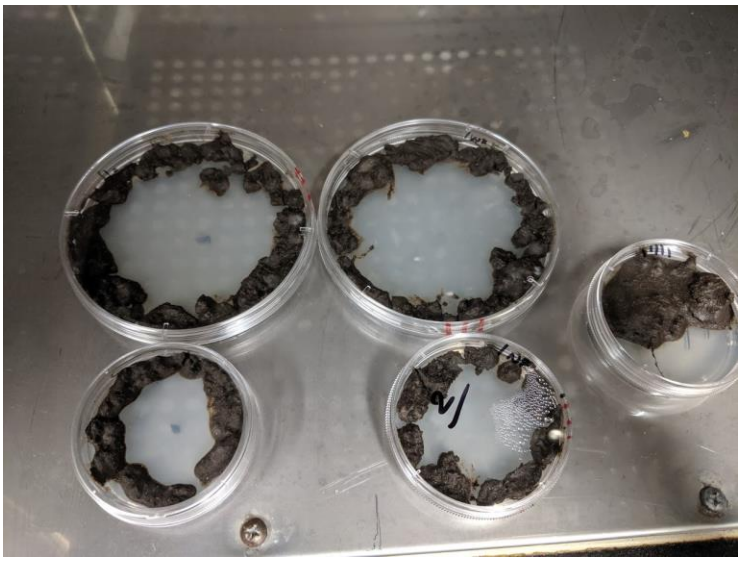

Figure 1. Initial setup for isolation utilizing agar culture plates. The marks at the center of the two left plates demonstrate the placement of the OP50 drops.

This method was later repeated with slight adjustments to collect nematodes for transfer to plates without soil. Two plates were produced for each sample, with OP50 drops on each. As nematodes were observed, they were transferred to new plates that had been spotted with OP50 at least 24 hours prior. These plates were then used to maintain nematode populations. Nematodes were initially grown at room temperature, with the plates being refrigerated after a sizable population was produced, but before all of the OP50 was consumed. Additional plates of nematodes were produced by picking several individuals from a populated plate and moving them to a new OP50 spotted plate. In this way, populations were maintained for further research.

3.4 Soil pH Testing

The pH of each soil sample was determined by filling a 15mL conical tube to the 2mL mark with soil, then filling to 10mL with distilled water. Each tube was then inverted several times, vortexed for 30 seconds, and inverted again. At this stage, each soil sample was thoroughly mixed into the water, with no dry soil remaining in the bottom of the conical tube. The samples were allowed to sit still for 5 minutes, then vortexed again. Each sample was then individually placed into a beaker, to hold it upright and collect any displaced water when the pH probe was inserted. The pH probe was left in the sample without movement until the pH value

displayed remained consistent, after which the pH for the sample was recorded, the probe thoroughly rinsed, and the next sample evaluated.

3.5 pH Tolerance Testing

To assess the pH tolerance of the nematodes, the plates of varying pH values produced were spotted with OP50 48 hours prior to plating nematodes. After this time period passed, 3 plates of each pH value were allotted to each nematode sample, with 10 of the isolated nematodes placed on each plate. After placement, each nematode was briefly observed for movement, to ensure that each had survived the transfer. The plates were stored in a dark area at room temperature for the duration of observation.

Every 24 hours, the nematodes on each plate were manually counted under a dissection microscope. This daily count was conducted for a total of 10 days. All visible nematodes of any stage of life were counted, and distinctions were not made between younger and older nematodes.

4. Results and Discussion

4.1 Sample Collection

Samples were given a number designation based on the order in which the sites were visited and samples collected. Therefore, samples 1, 2, 3, 4, 5, and 6 correspond to Oread Castle Park, the Lofts, Coes Pond, Hadwen Arboretum, Saint Gobain's Abrasives, and Institute Park respectively.

A map of the distribution of these sites was generated using Google Maps, and is presented as Figure 2.

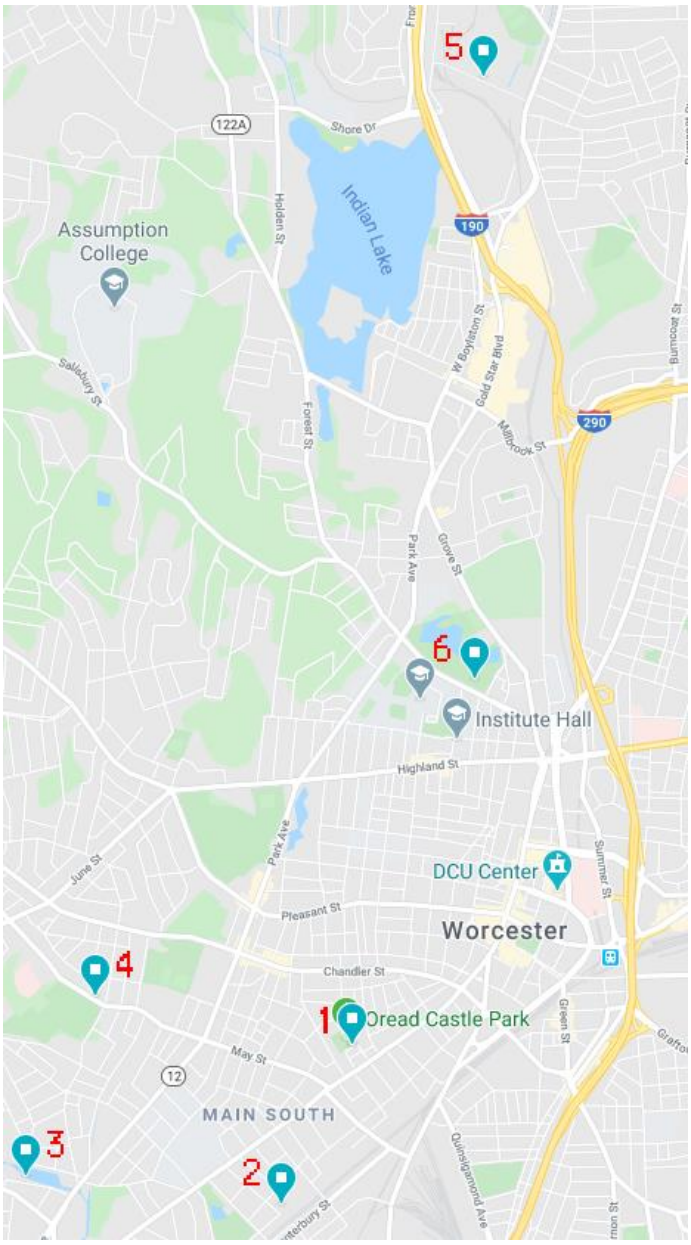

Figure 2. Map of sites of soil sample collection for samples 1 through 6. Teal pins with white squares mark each site.

The coordinates of each collection site and conditions under which the samples were collected are presented in Appendix B. All samples were collected on September 21st, 2019. A key observation is that a majority of the soil samples were very dry, which is not ideal for nematodes, as they generally prefer moist conditions.

4.2 Isolation

For the initial isolation on agar plates, the nematodes were counted every other day, with there being 5 plates for each soil sample. The raw data of these counts is presented in Appendix C. The total number of nematodes counted from each sample over time is presented in Figure 3.

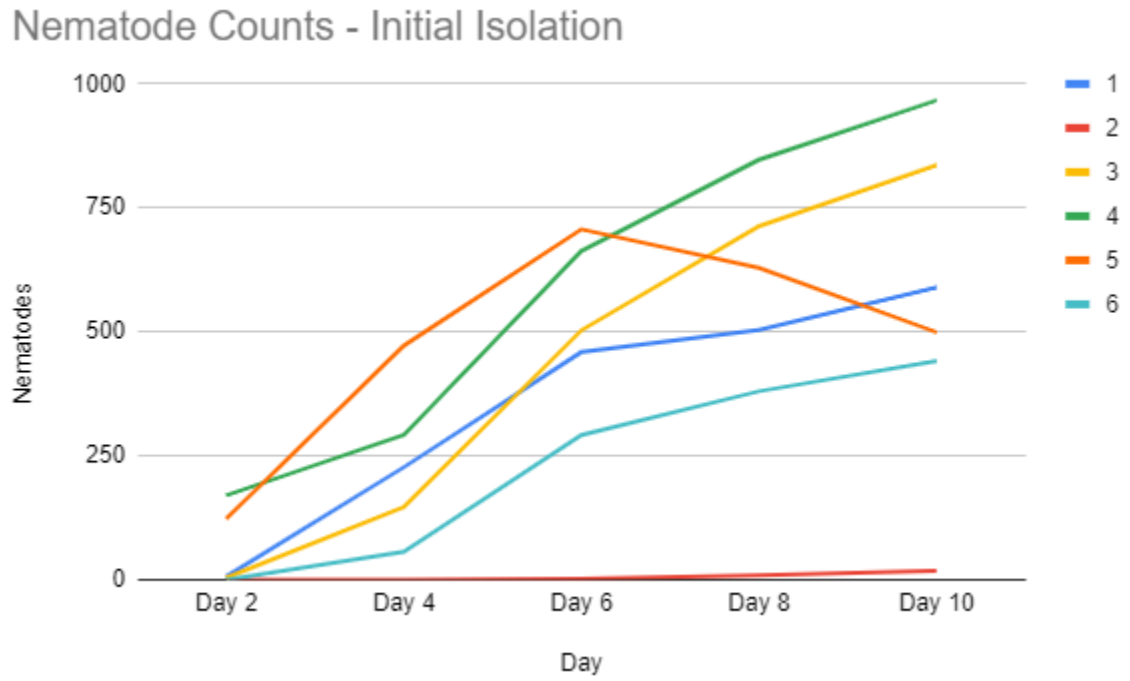

Figure 3 Nematode counts from initial plate isolation

Nematodes were ultimately found in every soil sample, although for some individual plates or samples overall, they took several days to begin to emerge. While overall nematode counts were fairly high for most samples, some samples had individual plates that never had any nematodes emerge. There were no significant differences between nematode counts on plates that were spotted with OP50 and plates that were not, although the larger plates generally yielded more nematodes. The lack of difference in populations given OP50 or no bacteria indicates that it is likely that the nematodes were surviving by consuming materials present in the soil samples, rather than the provided bacteria.

While the data obtained from this procedure is a useful indicator of nematode presence in each sample, it cannot be used for quantitative analysis of nematode population growth or initial nematode populations. While a similar volume of each soil sample was utilized, due to the varying composition of each soil sample, it is unlikely that the amount of air between the soil

particles was the same, leading to varying amounts of soil used. This would lead to inaccurate conclusions, should numbers of nematodes be compared between samples. Soil mass could have been measured instead of volume, should this analysis have been desired, but was determined to be unnecessary for this stage of the experiment.

When the isolation on agar plates procedure was repeated, nematodes were picked from the plates and moved to plates without any soil that had been spotted with OP50. The goal of this was to produce a consistent population of nematodes for each sample, to be utilized in further experiments. Despite several attempts being made to produce plates of each nematode, only nematodes from samples 1, 4, and 5 were able to be maintained on plates without soil.

It was thought that sample 2 nematodes, obtained from the driest sample, may not be well-adapted to the moist environment of the agar plates, or may not be a culturable species of nematode. Their presence was barely detected during the initial isolation procedure, indicating a low population to start, and it is a possibility that new nematodes were not being produced, but instead that the observed increase in nematodes over time was a result of nematodes leaving the soil in search of food.

Samples 3 and 6, however, yielded a significantly greater number of nematodes over time compared to sample 2, but the nematodes failed to survive and reproduce on the plates without soil. Both of these soil samples were observed to contain decomposing plant material, which had been transferred to the initial isolation plates. Therefore, it was thought that possibly, the nematodes had been consuming this plant matter on the isolation plates with soil, and when transferred to plates with only OP50, the nematodes starved without plant material to consume. It is also possible that the nematodes had consumed a different food source, such as fungus, that was not present on the OP50 plates.

Nematodes from samples 1, 4, and 5, however, survived and reproduced on the OP50 spotted plates, indicating that they were bacterivores or omnivores that were capable of eating this food source. Since consistent populations of these nematodes were produced, they were later utilized for the pH resilience procedure.

4.3 Soil pH

The pH of each soil sample was measured and is presented in Table 1.

Table 1. pH values of each soil sample.

Sample	pH
1	5.89
2	5.92
3	6.29
4	5.50
5	5.90
6	5.27

Each soil had an acidic pH, ranging from 5.27 to 6.29, with the average pH being 5.795. This data was used to determine the pH values to be tested on the nematodes. Since the nematodes from each sample were presumably adapted to acidic pH environments, the pH range to initially be tested was 5-7. The acidic nature of each soil could possibly be due to soil acidification brought on by pollution from Worcester's industrial sites.

To determine if there was a correlation between the pH of the source soil and the number of nematodes retrieved, the two were compared in Figure 4. The data utilized for nematode count was the day 10 data from the initial plate isolation, with each data point representing the total nematode count from each site plotted against the soil's pH.

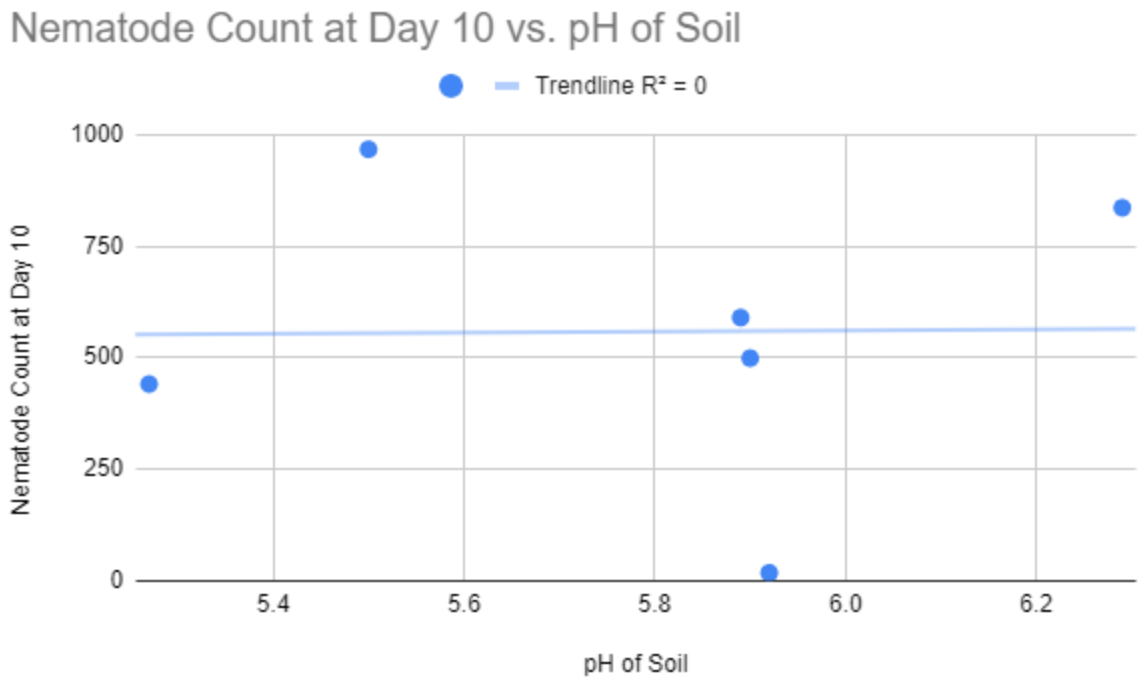

Figure 4 Nematode counts versus pH of the source soil

With a R^2 value of 0 and no clear trend, no relationship between the soil sample's pH and the number of nematodes counted was observed.

4.4 pH Impact on Growth

For each sample that yielded a consistent nematode culture, a starting population of 10 nematodes was placed on a plate of each pH tested, with 3 replicates at each pH. Each day, the nematodes in each plate were counted. The raw data obtained by these counts is presented in Appendix D. The averages of the data are presented in Appendix E and were utilized to produce figures. Figures 5 through 7 display the average nematode count per plate over time for each sample.

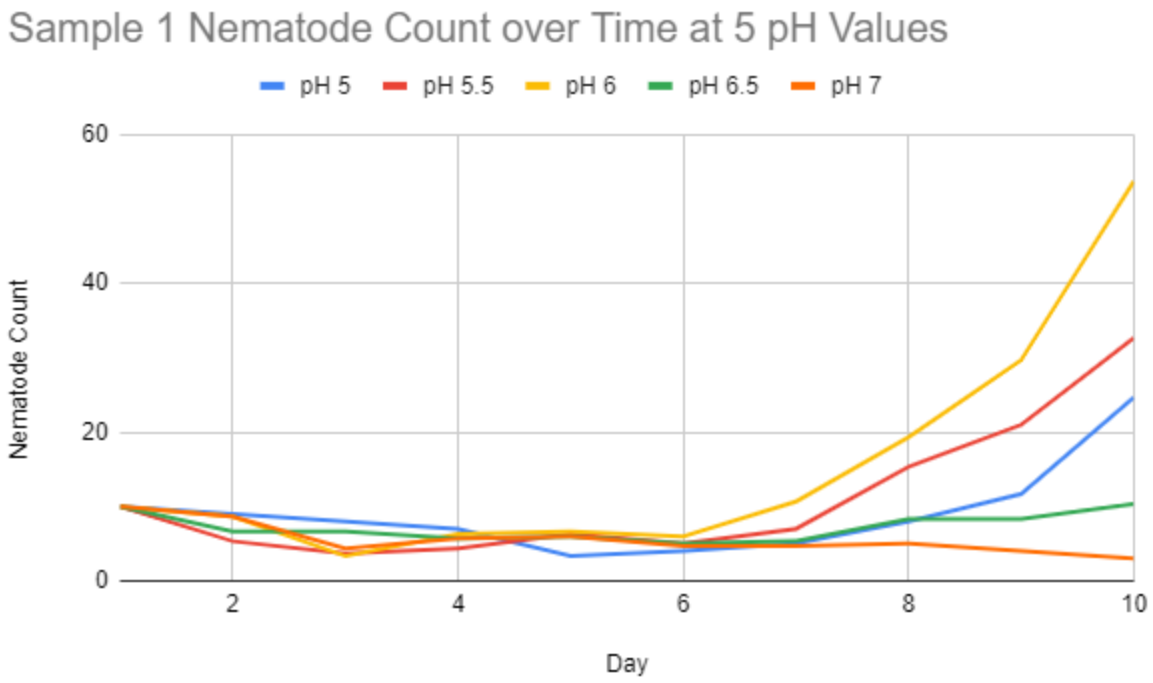

Figure 5. Nematode count over time for the sample 1 nematodes.

Figure 5 reveals that for the sample 1 nematodes, obtained from a soil sample with a pH of 5.89, the nematodes grew best at a pH of 6, followed by 5.5. This would be as expected, since a pH of 6 is the closest to the pH of the soil that the nematodes were isolated from. The most acidic pH tested, 5, was the third best, with the two more neutral pH values of 6.5 and 7 leading to minimal growth. This indicates that the nematodes may be more capable of adapting to more acidic environments than neutral ones.

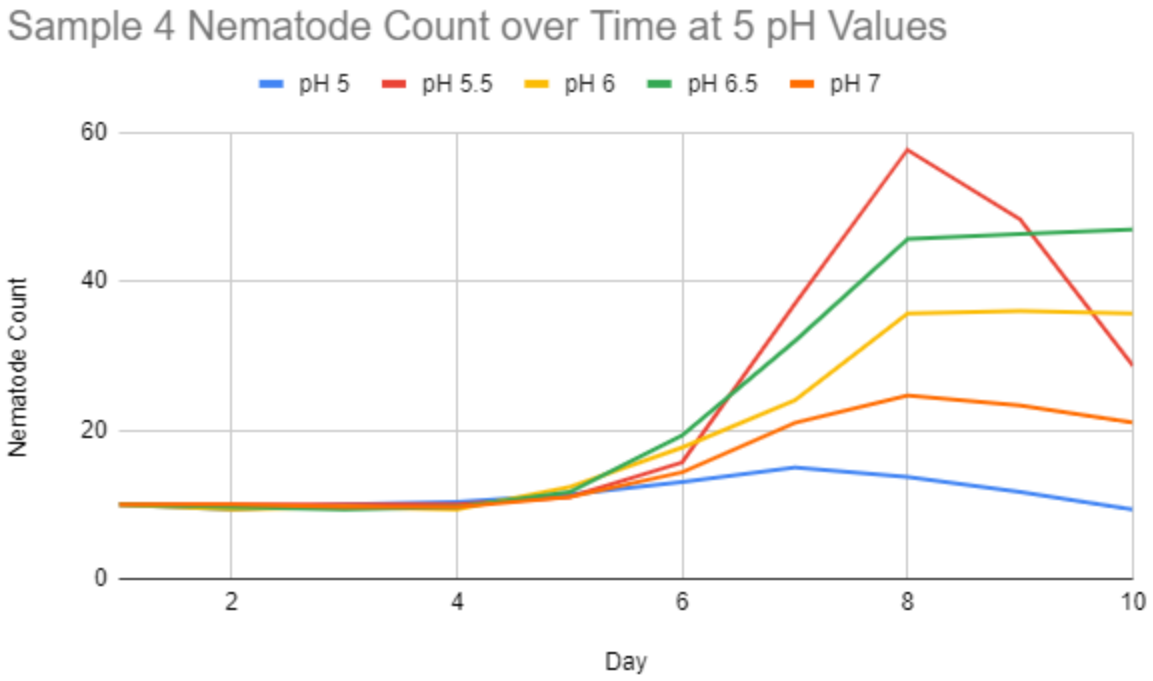

Figure 6. Nematode count over time for the sample 4 nematodes.

Figure 6 shows a similar stagnancy of the nematode population at all pH values for the first 4 days, with increases occurring for several days afterwards. However, most nematode populations experienced a minor decline in their numbers at about day 8, with the sharpest increase and resulting drop occurring with the nematodes kept at a pH of 5.5. The nematodes from this sample were observed to enter and remain within the OP50 lawn, while other nematodes would spend time outside of the bacterial lawn, potentially dragging the bacteria to new areas to grow with less interruption or competition for nutrients than in the central lawn. It is a possibility that in the later stages of the experiment, the bacteria had been consumed and the nematodes starved. However, examining the first 7 days of the data, it appears that the nematodes grew best at a pH of 5.5. The soil that these nematodes were isolated from had a measured pH of 5.50, indicating that these nematodes are likely well adapted to their environment. Interestingly, the nematode population grew more at a pH of 6.5 than 6. It is a possibility, however, that this is due to random chance, as the sample size was relatively small with only three trials conducted for each pH. In this instance, the nematode population grew more rapidly at pH values above the soil pH, and the population grew the least at the pH of 5, indicating that the nematodes are well-suited to an environment with a pH of 5.5, but cannot withstand more acidic conditions.

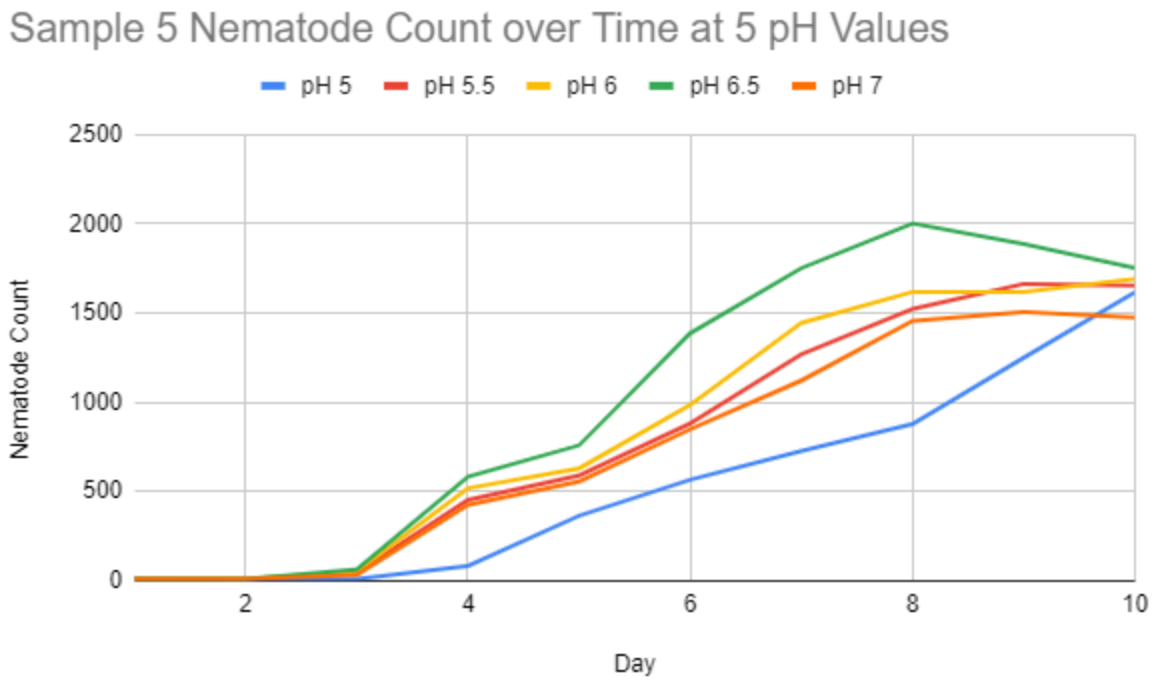

Figure 7. Nematode count over time for the sample 5 nematodes.

It is of note that the scale of figure 7 is immensely greater than that of the previous two figures. While the nematode counts for samples 1 and 4 only once exceeded 50, populations achieved by the nematodes from sample 5 reached as high as just over 2000 nematodes on a plate. For these nematodes, in most instances, stagnancy in population growth was noticed at about day 8, with some population declines occurring in most conditions after that point. This is very likely due to a lack of remaining bacteria, as the nematode populations grew very large and consumed mass amounts of bacteria, forming dense clumps in areas where any remained. For these nematodes, the ideal pH value seemed to be 6.5, with similar intermediate growth noted at pH values of 5.5, 6, and 7, with the least growth by day 8 occurring at a pH of 5. The soil that these nematodes were extracted from had a pH of 5.90. In this instance, the ideal conditions for the nematodes from this sample seem to differ from that of the soil that they were found in. While the nematodes grew well at a pH of 6, the closest tested pH to that of the soil sample, they grew more rapidly at a pH of 6.5.

Given the rapid growth of the sample 5 nematodes at all pH values, it is likely that they are an opportunistic species. Generally, when an ecosystem is stressed, the community structure shifts towards dominance by an opportunistic species, and this trend is noted in nematodes (Odum, 1985). It is a possibility that pollution from the nearby abrasive plant has

placed stress on the organisms present in the soil and could have led to the dominance of the fast growing, resilient nematodes found in sample 5.

5. Conclusions

Ultimately, through the research conducted, it appears that the city of Worcester, Massachusetts has a widespread soil nematode population, with nematodes detected in all sites tested, from parks to industrial plants. Generally, the soil of Worcester tends to be acidic, possibly a result of pollution from the city's years of industrial activity. However, the nematodes present in the soil seem to be well-suited to this acidic environment, generally growing at the most rapid rates in agar plates with similar pH values to that of their soil of origin.

This could potentially be evidence of natural selection in the soil nematodes of Worcester. It is a possibility that as the soil underwent acidification, the nematodes better suited to surviving and reproducing in these conditions had a fitness advantage, outcompeting nematodes that could not reproduce as quickly in the pH of their environment. With nematodes maturing and reproducing relatively quickly compared to many other organisms, it is not unreasonable to think that natural selection could have impacted the population in this way since the early 1800s, when Worcester began to develop a large number of manufacturing and industrial facilities and pollution increased.

6. Further Work

Due to measures enacted by the state of Massachusetts and WPI to prevent the spread of COVID-19, research was abruptly cut off as the WPI campus was closed. In this section, additional experimental procedures are proposed that could have been explored, should research have continued.

6.1 Sample 5 Nematodes: Pollution and Sex

While three of the samples yielded stable, culturable nematode populations, the nematodes of sample 5 were of particular interest. They had the greatest rate of reproduction, and appeared to be androdioecious, with hermaphrodites and males observed. Figure 8 depicts some of these sample 5 nematodes.

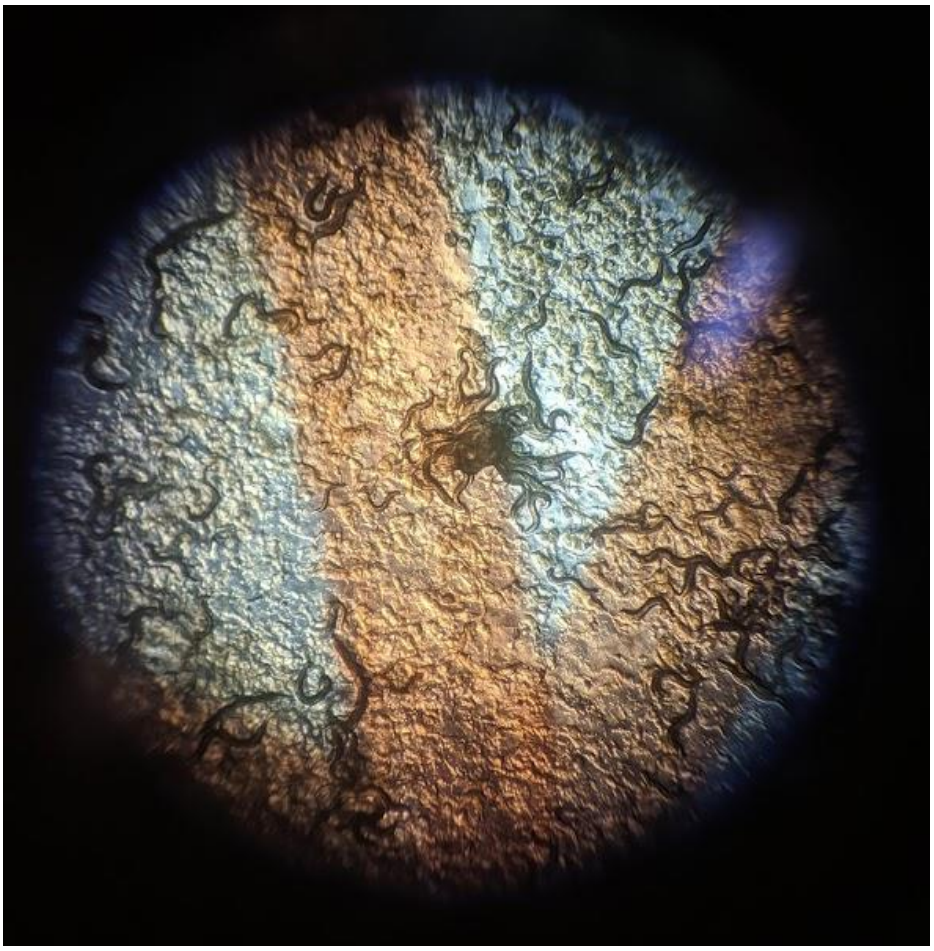

Figure 8. Sample 5 nematodes under dissection microscope.

The nematodes from this sample, particularly the hermaphrodites, were observed to be unusually large compared to the other soil nematodes, with individuals easily visible to the naked eye. Figure 9 shows how the nematodes appear without any magnification.

Figure 9. Sample 5 nematodes under no magnification. One nematode has been circled, and a few others are visible on the outskirts of the OP50 lawn.

An additional study that could be of interest would be to produce agar plates with varying levels of pollution and evaluate the sex ratio of the sample 5 nematodes on these plates over time. The soil samples could be tested for pollutant presence and concentration, and this data could be utilized to determine what concentrations to plate and test, as was done with the pH. Common soil pollutants that could be tested include heavy metals, such as arsenic and chromium and polycyclic aromatic hydrocarbons (Lemos et al., 2018). Since the sample 5 nematodes came from soil surrounding an abrasive plant, it could be of interest to test fine particles of abrasives produced by Saint-Gobain, such as aluminum oxides, silicone carbide, and ceramic grits.

A prior study conducted by Pen-Mouratov, Shukurov, and Steinberger in 2008 also evaluated the sex ratio of nematodes at varying distances from a pollution source. At the pollution source, the ratio of males:females:juveniles was 9:23:1, while at a farther sample, it was 1:2:3. Given these results and the assumption that hermaphroditic nematodes would be comparable to females, if an experiment was conducted with the sample 5 nematodes, it would be expected that at low pollutant concentrations, the sex ratio would favor juveniles, with males

being the least common, while at higher pollutant concentrations, hermaphrodites would be significantly more common.

6.2 Nematode Growth and Heavy Metals

Similar to the pH experiment conducted and the pollution experiment described previously, nematodes from each sample could be plated on agar plates containing varying levels of heavy metals. The soil could first be tested to determine what metals are present and their concentrations, and this data utilized to choose which metals to test and at what concentrations, but nematode tolerance of novel pollutants could be explored as well. Nematodes could initially be plated on agar plates with increasing amounts of heavy metals, to determine at what concentration the nematodes quickly die. For heavy metal concentrations below this lethal dose, nematodes could be plated on varying concentrations and their population growth evaluated daily to determine the extent to which the nematodes are impacted by these metals.

It would be expected that the sample 5 nematodes could tolerate the greatest amount of heavy metals, as they came from soil nearby an abrasive plant, and also displayed the greatest pH tolerance. Sample 4 nematodes, from Hadwen Aboretum, would be expected to be the least adaptable as this area has the least history of industrial activity.

6.3 Genetic Sequencing and Species Identification

While hypotheses were proposed regarding the diets of nematodes obtained, determining this without the aid of genetics can be difficult and unreliable. To identify the nematode species and learn more about them to further evaluate the data obtained, it would be immensely valuable to genetically sequence the nematodes from each sample and compare the results to the NCBI BLAST database.

There are many possible methods of extracting and purifying nematode DNA, and it has been shown that some methods are more suitable to nematodes from specific environments, while others produce good results in a wider range of conditions. Furthermore, some methods result in excess DNA concentration, leading to a need for dilution protocols. Of all the DNA extraction methods available, the one of choice would likely be physical disruption of the nematode sample, followed by purification with Purelink PCR Purification columns. This method produces a suitable concentration of template DNA and the greatest volume of PCR product of the methods tested. Wizard PCR Preps DNA purification systems and QIAquick PCR

Purification kits fail to produce a suitable volume of PCR product, while another kit, the Chargeswitch PCR purification kit, resulted in no detectable DNA recovery. Another method tested, phenol chloroform extraction, has been shown to lead to excess template DNA concentrations, requiring 10-fold dilution, and while this procedure requires this extra step, the resulting PCR product is less than that produced by the Purelink purification kit (Donn et al., 2008). Ultimately, the method of choice for DNA extraction and amplification would be physical disruption, followed by purification with a Purelink purification kit.

The most common DNA to sequence for nematodes is the 18S ribosomal gene. This could be extracted from the genomic DNA with primers, amplified with PCR, and then sequenced in a much more cost-efficient manner than sequencing the full genome (Foucher et al., 2004).

With the 18S gene obtained from the nematodes, it could then be sent out for sequencing. The genetic sequences returned could then be compared to a database such as NCBI BLAST, with the closest genetic match likely being the identity of the species. Knowing the nematode species, the diet and other details of the nematode, such as population doubling time or general sex ratio, could then be researched.

It would be expected that sample 1 and 4 nematodes are omnivores, as they survived and reproduced on plates with only OP50 but reproduced slowly. Sample 5 nematodes would likely be bacterivores, as they thrived with OP50 as their only food sources. Sample 2 nematodes are uncertain, as they were found in dry, sandy soil with no decaying vegetation, minimal fungal growth, and likely minimal animal presence, as the area was fenced in. However, these nematodes are likely not bacterivores, as they did not survive on plates with only OP50 as a food source. Sample 3 and 6 nematodes would be expected to be a species known to consume decaying plant matter, as they were found in soil samples with much plant matter present and seemed to starve when provided with OP50.

6.4 General Additions

In addition to the further research previously described, several smaller tasks could be conducted to further improve the study and would have been conducted had the lab not been closed. One such task is measuring the nematodes. While qualitative observations were made, it could be of interest to see if there is any trend between source soil pH and nematode size, or other factors such as survival rate when suddenly transferred to plates with different pH values.

Furthermore, it would be useful to have frozen nematode samples for future study. The nematodes could be frozen in a liquid or soft agar freezing solution, utilizing methods outlined by Dr. Stiernagle in *The Worm Book*. If the nematodes were frozen, they could be stored indefinitely in a freezer, which would be a less labor-intensive way of maintaining large nematode populations ready for use than keeping the nematodes on a large volume of OP50 spotted agar plates.

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Appendix A: Sample Collection Site Significance

Prior to becoming a city park, the land comprising Oread Castle Park was a women's college, Oread Collegiate Institute. The institute closed in 1881 and was demolished in the 1930s, with the land then being converted to a park (The City of Worcester, 2020).

The Lofts is an affordable apartment community that opened in September of 2015. The building was originally an abandoned mill building, built in the late 1800s, that had been severely neglected and was falling apart. The building was renovated, with many of its industrial features preserved, and converted to apartments mostly reserved for low-income families. In addition to renovating the dilapidated mill building, a nearby brownfield was remediated as a part of the project, improving the nearby environmental conditions (Eckelbecker, 2016).

The area around Coes Pond was formerly occupied by mills and other industrial facilities, such as the Coes Knife factory building. Now, the area consists of a playground, public beach where kayaks and paddle boats can be rented, multi-use field, and large park area with walking and biking trails (The City of Worcester, n.d.).

Hadwen Arboretum is an area of forested land owned by Clark University. The area is used by the university for study, and also has a network of walking trails. Within the arboretum

is also a community garden. The area has always been forested, and has never been the site of an industrial complex itself, despite being surrounded by the city (Hadwen Arboretum, n.d).

Saint-Gobain Abrasives, initially called Norton Abrasives, was founded in 1885, and initially manufactured ceramic grinding wheels. In 1990, Saint-Gobain, a French company, purchased the complex. Presently, the abrasive plant is still functioning, and produces a wide variety of abrasives are produced for many applications, such as the automotive industry, construction, and personal home use (Story of the Norton Company, n.d.).

The land for Institute Park was donated by Stephen Salisbury III in 1887, with the intent of it being used as an adjunct to the Worcester Free Institute of Industrial Science, now known as Worcester Polytechnic Institute, as well as a public park. The land was initially farmland and pasture, and after the development of paths, Salisbury had many structures developed, including a boathouse, bridge, tower, four gazebos, two Tremont columns, and several other structures, most of which have since been removed. The park underwent many significant alterations and events, such as having the pond drained in 1954 to allow for the construction of the causeway at Grove Street. Starting in 1970, efforts were taken to clean up pollution that had entered the park through drainage from industrial sites, overflow of the sewer system, and other sources (History of Institute Park, n.d.).

Appendix B: Soil Sample Data

Sample Number	Location	Coordinates	Soil Temperature (Degrees C)	Depth Dug (inches)	Notes
1	Oread Castle Park	42°15'23.8"N 71°48'46.2"W	28	3	Soil moderately moist. Collected under a tree, in a patch without grass.
2	The Lofts	42°14'54.5"N 71°49'04.0"W	28	Collected from surface	Very sandy and dry. Scraped off of the surface, minimal areas with soil not fenced off at this location.
3	Coes Pond	42°14'59.5"N 71°50'07.4"W	24	2.5	Moist, vegetation present in sample.
4	Hadwen Arboretum	42°15'32.7"N 71°49'50.1"W	24	1	Fairly dry, some dead vegetation in soil. Collection site fairly close to the road.
5	Saint-Gobain Abrasives	42°18'22.2"N 71°48'13.5"W	30	4.3	Very moist, collected from under a bench.
6	Institute Park	42°16'31.3"N 71°48'15.5"W	26	2.5	Fairly dry. Collected from under a tree. Moderate amount of grass and decomposing leaves in soil.

Appendix C: Initial Nematode Counts

Sample Number	Treatment	Day 2	Day 4	Day 6	Day 8	Day 10	Observations
1	Small, No Bacteria	0	26	77	174	231	Many nematodes appeared still/unmoving at this final count.
1	Small, Bacteria	4	51	148	156	161	
1	Clump	0	0	0	0	0	
1	Large, No Bacteria	1	71	76			Day 8, large portions of the plate were obscured by mold. Some mobile nematodes were noticed, but an accurate count could not be obtained.
1	Large, Bacteria	1	79	158	174	198	
2	Small, No Bacteria	0	0	0	1	4	
2	Small, Bacteria	0	0	1	5	6	
2	Clump	0	0	0	0	0	
2	Large, No Bacteria	0	0	0	0	0	
2	Large, Bacteria	0	0	1	3	8	
3	Small, No Bacteria	1	31	59	128	192	
3	Small, Bacteria	1	14	62	146	174	
3	Clump	1	7				Day 6, was too obscured by mold growth.
3	Large, No Bacteria	0	39	194	208	207	
3	Large, Bacteria	1	56	188	231	263	
4	Small, No Bacteria	16	49	114	167	209	
4	Small, Bacteria	2	33	109	137	169	

4	Clump	3	16	97	163	171	
4	Large, No Bacteria	108	123	169	182	183	
4	Large, Bacteria	41	71	174	198	235	
5	Small, No Bacteria	15	34	75	89	92	
5	Small, Bacteria	29	54	69	74	77	
5	Clump	15	96	126	132	137	
5	Large, No Bacteria	39	157	173	132	84	
5	Large, Bacteria	25	131	264	202	109	
6	Small, No Bacteria	0	4				Day 6 Became very obscured with mold (growth up to plate lid)
6	Small, Bacteria	0	0	2	8	13	
6	Clump	0	0	0	0	3	
6	Large, No Bacteria	0	36	158	171	194	
6	Large, Bacteria	0	16	132	201	231	

Appendix D: pH Data

Sample 1										
Day	1	2	3	4	5	6	7	8	9	10
pH 5, 1	10	8	8	7	0	2	2	4	4	4
pH 5, 2	10	9	7	7	5	5	6	9	12	34
pH 5, 3	10	10	9	7	5	5	7	11	19	36

pH 5.5, 1	10	6	2	2	6	5	7	14	22	33
pH 5.5, 2	10	3	4	6	8	5	8	13	17	26
pH 5.5, 3	10	7	5	5	5	5	6	19	24	39
pH 6, 1	10	9	2	7	6	6	8	15	15	19
pH 6, 2	10	7	4	6	6	4	14	27	43	77
pH 6, 3	10	10	4	6	8	8	10	16	31	65
pH 6.5, 1	10	6	6	2	4	1	1	4	5	5
pH 6.5, 2	10	9	9	10	8	8	9	13	12	18
pH 6.5, 3	10	5	5	5	6	6	6	8	8	8
pH 7, 1	10	8	4	7	5	5	5	4	3	1
pH 7, 2	10	10	5	6	6	2	2	2	2	2
pH 7, 3	10	8	4	4	7	7	7	9	7	6
Sample 4										
Day	1	2	3	4	5	6	7	8	9	10
pH 5, 1	10	8	10	11	12	14	15	9	7	6
pH 5, 2	10	10	10	10	10	13	16	18	14	10
pH 5, 3	10	10	10	10	12	12	14	14	14	12
pH 5.5, 1	10	10	10	10	10	20	46	78	66	37
pH 5.5, 2	10	10	10	10	10	10	29	48	41	21
pH 5.5, 3	10	10	10	10	13	17	36	47	38	28
pH 6, 1	10	9	10	9	13	15	22	32	31	21

pH 6, 2	10	9	9	9	10	15	16	19	19	22
pH 6, 3	10	10	10	10	14	23	34	56	58	64
pH 6.5, 1	10	9	8	8	9	9	10	13	15	16
pH 6.5, 2	10	10	10	10	12	34	52	74	75	74
pH 6.5, 3	10	10	10	11	14	15	34	50	49	51
pH 7, 1	10	10	10	11	12	21	34	43	40	35
pH 7, 2	10	11	10	9	11	12	14	15	15	15
pH 7, 3	10	9	9	9	10	10	15	16	15	13
Sample 5										
Day	1	2	3	4	5	6	7	8	9	10
pH 5, 1	10	7	9	78	382	576	714	893	1157	1566
pH 5, 2	10	9	14	92	417	603	796	928	1235	1644
pH 5, 3	10	9	13	81	296	521	672	814	1358	1640
pH 5.5, 1	10	10	20	364	489	787	912	1171	1645	1783
pH 5.5, 2	10	10	52	481	595	864	1224	1423	1460	1521
pH 5.5, 3	10	10	66	513	684	993	1674	1977	1888	1661
pH 6, 1	10	9	51	594	714	1252	1679	1854	1764	1799
pH 6, 2	10	11	46	396	571	829	1275	1424	1506	1632
pH 6, 3	10	9	64	567	604	878	1382	1580	1588	1639
pH 6.5, 1	10	12	61	549	734	1269	1817	2105	1802	1694
pH 6.5, 2	10	9	49	509	699	1389	1637	1872	1890	1741

pH 6.5, 3	10	10	79	691	845	1507	1799	2027	1972	1823
pH 7, 1	10	10	22	418	570	810	1055	1362	1463	1501
pH 7, 2	10	11	30	390	512	792	1166	1517	1594	1442
pH 7, 3	1	10	46	469	586	947	1147	1491	1463	1479

Appendix E: pH Data Averages

Sample 1										
Day	1	2	3	4	5	6	7	8	9	10
pH 5	10	9	8	7	3.333333333	4	5	8	11.66666667	24.66666667
pH 5.5	10	5.333333333	3.666666667	4.333333333	6.333333333	5	7	15.33333333	21	32.66666667
pH 6	10	8.666666667	3.333333333	6.333333333	6.666666667	6	10.66666667	19.33333333	29.66666667	53.66666667
pH 6.5	10	6.666666667	6.666666667	5.666666667	6	5	5.333333333	8.333333333	8.333333333	10.33333333
pH 7	10	8.666666667	4.333333333	5.666666667	6	4.666666667	4.666666667	5	4	3
Sample 4										
Day	1	2	3	4	5	6	7	8	9	10
pH 5	10	9.333333333	10	10.33333333	11.33333333	13	15	13.66666667	11.66666667	9.333333333
pH 5.5	10	10	10	10	11	15.66666667	37	57.66666667	48.33333333	28.66666667
pH 6	10	9.333333333	9.666666667	9.333333333	12.33333333	17.66666667	24	35.66666667	36	35.66666667
pH 6.5	10	9.666666667	9.333333333	9.666666667	11.66666667	19.33333333	32	45.66666667	46.33333333	47
pH 7	10	10	9.666666667	9.666666667	11	14.33333333	21	24.66666667	23.33333333	21
Sample 5										
Day	1	2	3	4	5	6	7	8	9	10
pH 5	10	8.333333333	12	83.66666667	365	566.6666667	727.3333333	878.3333333	1250	1616.666667
pH 5.5	10	10	46	452.6666667	589.3333333	881.3333333	1270	1523.666667	1664.333333	1655
pH 6	10	9.666666667	53.66666667	519	629.6666667	986.3333333	1445.333333	1619.333333	1619.333333	1690

pH 6.5	10	10.3333 3333	63	583	759.333 3333	1388.33 3333	1751	2001.33 3333	1888	1752.66 6667
pH 7	10	10.3333 3333	32.6666 6667	425.666 6667	556	849.666 6667	1122.66 6667	1456.66 6667	1506.66 6667	1474