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Monitoring Local Soil Toxicity by *Daphnia magna* Viability

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Summary

***Daphnia magna*, a freshwater crustacean, can be used to test for soil toxicity in viability bioassays. In the current study, we collected soil samples with various concentrations of toxins from six locations in the Greater Boston area, and measured the viability of *D. magna* after exposure over time. Samples were placed in petri dishes containing the same amount of soil and different volumes of spring water. *D. magna* in samples from the dumpster at the Sri Maha Lakshmi Temple and from Dennison Manufacturing Company died at an unusually high rate, possibly indicating the presence of higher concentrations of toxins relative to other locations. A linear regression analysis of our data suggested that the soil samples at the Sri Maha Lakshmi Temple and Dennison Manufacturing Company contained toxins inhibiting *D. magna* survival, resulting in an unusually high death rate.**

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Introduction

Decreasing soil toxicity can increase the biodiversity of an ecosystem, and can increase the survivability of organisms that are beneficial to the local environment (1). One method of testing for the presence of toxic compounds in a soil sample is a bioassay (2,3). In a bioassay, a living organism serves as a detector for toxins (4). Bioassays for viability record how many organisms are present when the experiment starts, at different time points during the experiment, and at the end of experiment. *D. magna* was chosen because of an experiment performed at Moscow State University, where researchers successfully used *D. magna* as their organism for the bioassay (5). From this data, researchers can determine whether the rate at which these organisms died was normal, or whether factors such as toxins or a lack of nutrients in the soil samples caused the organisms to die at an unusually fast rate (6). Bioassays are extremely helpful to researchers, as they indicate whether a particular soil sample is toxic to potentially beneficial organisms (7).

In this experiment, we used *D. magna*, a freshwater crustacean, to test toxin levels in different samples of soil. The bioassay was used to monitor water toxicity and to see if toxins located in certain streams and ponds were toxic to aquatic animals (8). To monitor soil toxicity, we placed *D. magna* in different soil samples collected from the Greater Boston Area. The samples contained the same amount of dry soil but varying amounts of spring water. By recording the number of viable *D. magna* in the bioassay at different times, we analyzed which soil samples caused the organisms to die at an abnormally high rate. This bioassay was aimed to determine whether any of the suspected soil samples contained harmful toxins to organisms in that environment.

Results

We quantified the toxic effects of our soil samples by estimating the T50, or half-maximal response, of each sample on its *D. magna* colony (9). The T50 describes the amount of time it took for half of the *D. magna* in each sample to remain viable. The T50 of the control sample was 80 hours. In our experimental samples, we observed T50s of 60 hours for the E.L. Harvey sample, 20 hours for the dumpster sample, 60 hours for the Tennessee Gas Pipeline sample, 64 hours for the Wilson Street sample, 24 hours for the Dennison Manufacturing sample, and 50 hours for the Sri Maha Lakshmi Temple. The shortest T50s were observed for samples from the dumpster and Dennison Manufacturing (**Figure 1**). Generally, survivability decreased as a function of soil concentration across all locations.

We next measured the viability of *D. magna* by counting the number of organisms that remained alive

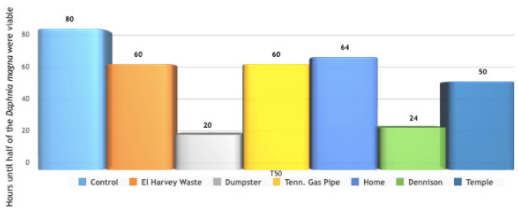

Figure 1. T50 of Various Locations. The T50 indicates the number of hours it took for the 50% (n=10) of the *Daphnia magna* to remain surviving.

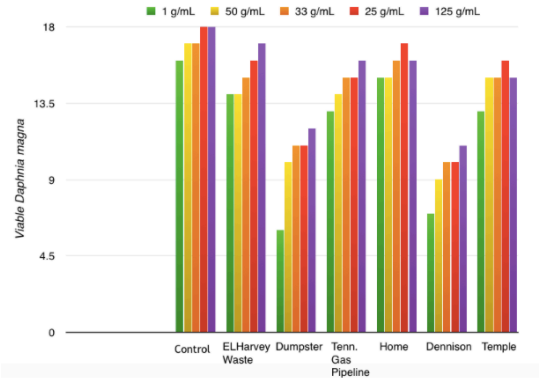

Figure 2. Viable *Daphnia magna* after 24 Hours. The number of surviving *D. magna* across seven locations including control, for five different concentrations, in grams of soil per mL of water. Initial Dapnia *N*=20.

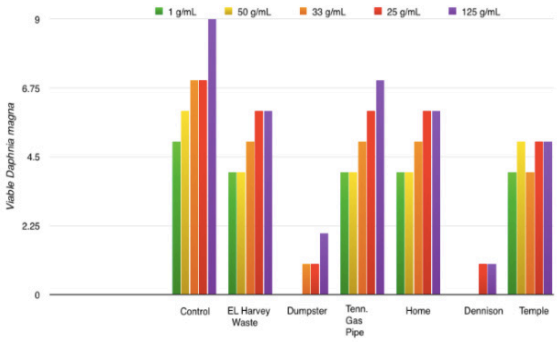

Figure 3. Viable *Daphnia magna* after 96 Hours. The number of surviving *D. magna* across seven locations, including control, for five different concentrations, in grams of soil per mL of water. No bar per concentration indicates that all *D. magna* died.

after 24 hours of exposure to various soil concentrations (**Figure 2**). Colored horizontal lines show the different concentrations of the soil samples from each location. After 24 hours, the samples from the dumpster and Dennison Manufacturing produced a higher death rate relative to the other soil samples. The 25 g/ml concentration is representative of this result. At this concentration, the average number of viable *D. magna* at 24 hours was 14.7 across all samples, while the Dennison and dumpster samples had only 10 and 11 surviving, respectively (**Figure 2**). After 96 hours, samples from the Dumpster and from Dennison Manufacturing retained higher death rates than the other locations (**Figure 3**). The average viability at this time interval was 4.5, while the Dennison and dumpster samples had only 1 surviving organism each, (**Figure 3**). At both the 24 and 96 hour time points, these two locations had approximately 20-25% fewer *D. magna* per sample compared to other soil samples.

This negative correlation between the amount of *D. magna* and time shows that the rate of *D. magna* decrease varies between different soil samples (**Figure 4**). Based on the slope of the linear regression analysis, *D. magna* in the sample from the dumpster and Dennison Manufacturing were dying at a much faster rate than all the other samples. Overall, the results were statistically significant among different locations and suggest significant differences as seen at different concentrations. The results were verified to be significant as a Chi-Square analysis was conducted and the *p*-value for both samples was 0.02.

Discussion

The purpose of this experiment was to determine whether any of the six soil samples collected contained toxic compounds lethal to a representative organism, *D. magna*. Recording how many of the crustaceans were viable before and after the experiment revealed two general trends. First, *D. magna* in higher concentrations of soil and water died off much more quickly than the *D. magna* in lower concentrations. Second, samples from Dennison Manufacturing and the dumpster were more lethal than other samples. Once these two trends were found, we calculated the soil concentration at which only half of the *D. magna* colony remained viable. For the control, this was 80 hours, but for the two suspected toxic samples this was less than 24 hours. After the T50 concentrations were determined, a linear regression analysis was conducted to analyze the rate at which each sample of *D. magna* was dying. The data suggested that both the Dennison and dumpster samples had a much higher death rate than the other samples. Finally, in order to determine if the results were strictly due to chance or if there were some environmental factors affecting the *D. magna*'s viability, a Chi-Square Analysis was conducted. The analysis rejected the null hypothesis for the two suspected samples, which meant that these results were likely not due to chance, but rather some toxic compound in the soil samples that the *D. magna* could not survive.

An alternative possibility is that the soil samples that induced higher levels of lethality did so as because they lacked adequate nutrients required by the *D. magna* for survival (10). While the organisms were fed every 24 hours, it cannot be ruled out that the bacteria and yeast which they were fed were insufficient in providing the complete nutrition that *D. magna* would find in their natural habitat. Not enough is known about the nutritive and metabolic requirements of these organisms to be certain that the food supplied met their needs completely. However, in the control sample with uncontaminated water and food sources, *D. magna* was able to remain

viable during the course of the experiment; suggesting their viability was not affected due to lack of nutrients.

Additionally, an ecotoxicity test could be conducted to determine the exact substance in the two toxic samples that caused the *D. magna* colony to die at such an accelerated rate. This test would measure how each of the chemicals and matter that is in the soil will be able to isolate particular substances that caused the *Daphnia magna* to perish at such an accelerated rate (11). The Massachusetts Department of Environmental Protection (DEP) has posted several articles about establishments that have been fined for violating their rules of conduct. Some of these regulations include having a certain toxicity in local ponds and streams, getting inspections done routinely from the Massachusetts DEP , and sharing results with the department. Further data could be obtained by testing soil samples from places such as Duro Textiles LLC, Economic Enviro Techs Inc., and A. K. S. Recycling to evaluate whether they have fixed their processes to comply with Massachusetts DEP recommendations.

Methods

Samples for this experiment were collected from six different locations using a pair of latex gloves, a trowel, and Ziploc bags. The samples were from E.L. Harvey Waste Management (Westborough, MA), Dennison Manufacturing (Framingham, MA), Tennessee Gas Pipeline (Hopkinton, MA), Wilson Street (Hopkinton, MA), and two samples from the Maha Lakshmi Temple (Ashland, MA). These locations have received complaints from the state about their soil toxicity and how they are conducting their regulatory affairs. Each sample weighed 10 grams and was collected three inches below the ground. Five grams of soil sample was added to a petri dish and mixed with the spring water, to yield soil concentrations of 125 g/mL, 50 g/mL, 33 g/mL, 25 g/mL, and 1 g/mL. Visible soil sediment formed at the bottom of each petri dish. There were 35 different petri dishes across the six samples of soil, five different concentrations of soil per water volume. Once the *D. magna* arrived from Carolina Biological from Burlington, North Carolina, 20 of them were placed into each petri dish. The crustaceans had to be kept at room temperature and could not be refrigerated due to them being cold-blooded. They were fed samples of bacteria and yeast every 24 hours. Every 12 hours, the viability of the *D. magna* was measured through the use of a microscope. The *D. magna* displayed a bright orange color when living, but upon death became a dull white. Additionally, when the crustaceans perished, they floated to the top of the water. The data was taken eight times during the course of four days. In order to test for the statistical

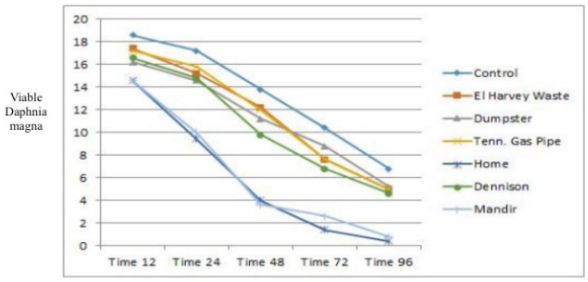

Figure 4. Linear Regression Analysis of Viability. Analysis for all seven locations at 33 g/mL during the allotted time period.

significance, a Chi-square analysis was conducted The expected value of the experiment came from the control group at each time and concentration while the observed value was derived from each sample. The Chi-square regression formula was used to determine a p-value for each sample. Additionally, a linear regression analysis was performed to by graphing each the viable *D. magna* was performed by graphing the viable D. magna at a 33 g/mL soil concentration and analyzing the rate at which they perished during the 96 hour time period.

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