The Effects of Ultraviolet Radiation on the Mold Population Density in the Soil

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Abstract:

The purpose of our research project was to see whether an increase in exposure to ultraviolet radiation decreases the mold population density. We hypothesized that a greater exposure to UV radiation would result in a corresponding decrease in the mold population. The independent variable in our experiment was exposure to UV radiation, and the dependent variable was the amount of mold in the soil. We controlled the independent variable by placing different covers over the ground, and thereby blocking varying amounts of UV radiation. Unfortunately, due to uncontrollable fluctuations in the overall UV levels, largely due to a change in weather, despite a visible trend following our hypothesis, we were unable to achieve results that were statistically significant. This experiment should be repeated for an extended amount of time in order to minimize variations caused by weather related changes in the UV index.

Introduction:

The dependence of molds on moisture and warmth led us to question what was causing the mold populations in Site Four to decrease. Molds, in the fungi kingdom, are primarily made up of water, like humans. They are generally found where there is minimal sunlight to dry them out and reduce that water percentage, but the wetlands of Site Four had statistically fewer molds than the much drier Riparian Flood Zone of Site One. Mold populations are similarly affected by temperature—thriving at 26.7°C. Excessive heat, however, evaporates their moisture, killing off large numbers of them. It might seem that the increased heat, due to increased exposure to the sun, at Site Four, was causing the decreased population, but a team of researchers had already conclusively proven that temperature was not the source of the mold shortages (Brockmeyer, *et al*, 2001).

After performing a general biota survey on three microclimates, we discovered many statistically significant differences between sites one, three, and four. There was a distinctive "hill-effect" between the three sites, with site three (a heavily forested area near the stream) at the top, site four (a wetland) a massive drop down, and with site one (a riparian flood zone) a smaller drop below that, incidentally mirroring the height of the sites. The levels of certain chemicals and populations of many microbes all followed the trend of the sites' altitudes, showing that there was clearly some major factor that was causing a drastic difference between the three sites (E.S.S.R.E., 2002). More interesting to us were the elements of the environment that defied this model. Out of all of these measured aspects of our microclimates, mold levels were the only one to defy the "hill-effect" completely. Contrary to logic, which would speculate that the most molds would

be in the wettest environment, the marshland at site four actually had the lowest level of molds, and in fact was statistically different from sites one and three. We decided to try to discover the cause behind this complete deviation from the remainder of the elements of these microclimates.

Initially we thought that the increased sun exposure in site four might dry out the moisture that the molds thrived on, but one look at the swampy, muddy site quickly disabused us of this notion. We came up with many possible causes of a mold population decline—lack of water, a moisture-evaporating heat wave, too much exposure to ultraviolet radiation—but we kept coming back to the fact that there was plenty of non-evaporating water in site four that should have lead to large groups of molds. Then we realized something else about sunlight—beyond the drying heat that it produced: Sunlight carries with it deadly ultraviolet rays—somewhat hazardous to humans—but killing many microorganisms, in particular, fungi. In the end we decided to test this theory—did exposure to ultraviolet radiation decrease the mold population of site four? We decided to place different covers on the ground to block varying amounts of ultraviolet radiation, wait a few days, and then test the soil under them for mold to determine if a correlation existed between the amount of radiation and the number of molds present in the soil.

Methods and Materials:

Once we decided to test our theory about ultraviolet light being the killer of molds in Site Four, we needed to decide how to limit the ultraviolet radiation. As earlier research had shown that a change in temperature was not the cause of the mold discrepancies, we thought that placing various covers on the ground to limit the exposure

to sunlight would effectively achieve our goals. After much thought we settled on black plastic (completely blocking UV rays), white, unpainted canvas (blocking most UV rays), and mesh (blocking only a few UV rays). Initially were going to leave bare ground exposed as our negative control, but doing so could introduce differences, besides exposure to ultraviolet light, making our experiment uncontrolled. For that reason, we decided to use commercial food wrap (blocking no UV rays) as our negative control. As our positive control, we decided to clear a patch of ground in site one and cover it with commercial food wrap, increasing the amount of UV light reaching the ground, and test for a decrease in the mold population. This would help to ascertain if our hypothesis was universally valid or if, even if our experiment worked, was exclusive to Site Four.

Before we started clearing patches of ground in sites one and four, we readied our covers and the flags that we would use to mark them. We began by cutting four 15 x 15 centimeter squares of each of our cover materials (commercial food wrap, mesh, canvas, and hard plastic) and four additional squares of the commercial food wrap for the positive control. Realizing that the covers would wash or blow away if simply placed on the ground, we decided to secure the covers to the ground with flags at each of their corners. Doing so would provide an added bonus by allowing us to quickly locate our plots. We cut a small hole in each corner of each cover so that we could easily place the flags through the covers (especially those like the plastic that required something sharp to pierce them. We then labeled 80 flags (four per cover that we cut out) with the site number, quadrat number, and the type of cover used.

Our preparations finished, we went to site one, a site with a normal mold count, to set up our positive control. We chose a place in the woods that clearly received sunlight

so that we could be sure the molds in the soil would be exposed to larger amounts of UV radiation than they usually received. Next we cleared, by hand, a square half-meter of ground so that bare soil was exposed. We placed four squares of the commercial plastic food wrap on the ground (one the northwest corner of the plot, one in the northeast, one in the southeast, and one in the southwest), and secured the corners of each with the corresponding flags. We then went to site four, where we set up our negative control and our experimental samples. In each quadrat of site four we cleared a square half-meter of ground so that bare soil was exposed, removing or bending back all plants and clearing away the leaf litter layer. After we had cleared the ground, we placed one commercial plastic food wrap square (our negative control), one black wire mesh square, one unpainted canvas square, and one hard black plastic square on top of the soil in each cleared plot. As in site one, we secured the squares' corners with the corresponding flags.

The next day, Day 2 of the experiment, we labeled 35 plastic bags with the site, quadrat, and type of cover used. We labeled only one bag for the covers in quadrat one and for the northwest sample in site one, but two for all of the other covers. We went out and collected one 10-centimenter soil core sample from under the center of each cover in quadrat one of site four and the northwest cover in site one.

We then performed serial dilutions to the 10^{-4} power on each of the samples. After we had finished serial dilutions we plated the 10^{-2}, 10^{-3}, and 10^{-4} dilutions on Easygel™[1] plates (petri dishes with special solution that provide the food and space microbes need to grow). On days five, six, and seven, we took two samples from under

[1] This method was developed by the Micrology Laboratories, LLC. Contact them at www.micrologylabs.com for additional information.

each cover of the rest of the quadrats. After counting the number of mold colonies on the plate we computed the amount of molds in a cubic centimeter of soil using the following formula:

# of colonies $\times 10^4 \times 10^{|\text{dilution of that sample}|}$ = # of mold per cm^3 for that sample

We computed the number of molds for the 10^{-2}, 10^{-3}, and 10^{-4} dilution levels and averaged the results to get the number of molds present in that specific sample on that given day.

We used the same formula to calculate the number of molds in a cubic centimeter that we had used for the samples from quadrat one and averaged them together in the same way.[2] We waited for day eight to count the number of molds growing on the plates from quadrat three and the southeast plot. Finally, we analyzed our data through T-Tests and graphical analysis.

Results:

Because of time constraints, we were only able to let some sets of plates sit for two days, so we had two sets of comparable data—the mold counts after two days from Quadrats 2, 3, and 4, and the mold counts after three days from Quadrats 1, 2, and 4. Table 1 shows the number of molds per cubic centimeter of soil counted after being plated for two days in Quadrats 2,3, and 4. Although it can clearly be seen that the average mold population per cubic centimeter increases as the exposure to Ultraviolet Radiation decreases from Clingwrap to Hard Plastic, when we performed t-tests on the comparable data, we discovered absolutely no statistically significant difference between

[2] # of colonies $\times 10^4 \times 10^{|\text{dilution of that sample}|}$ = # of mold per cm^3 for that sample

any of the coverings for any of the data sets. This means that the null hypothesis is confirmed and the slight increases are due just to random chance. For the average number of molds per cubic centimeter of soil in Quadrats 1, 2, and 4 counted after being plated for three days (see Table 2), the mold populations varied randomly. Additional t-testing showed that there was no statistically significant difference between the mold populations under the covers either.

We then performed a chi-squared test between mold populations in Site 4 during the course of our experiment (Loya, K. *et al*, 2002) and the mold populations previous to our experiment from the general biota survey that we had performed. Achieving a chi-squared value of approximately 252,000,000 in comparison to the expected chi-squared value of 23.685, it was blatantly clear that something in the environment besides the amount of ultraviolet radiation that the soil was receiving changed during the course of our experiment.

We then t-tested the overall changes between the days that the samples were taken. Between Day Two and Day Five, there was t-test value of 2.05 in comparison to the t-alpha value for 90% surety of 1.729 and a t-alpha value for 95% surety of 2.093. This means that we are at least 90% sure and almost 95% sure that there was a statistically significant difference in the overall populations between Day Two and Day Five. Between Day Five and Day Six, there was a t-test value of 2.514 in comparison to the t-alpha value for 95% surety of 2.145*, which means that there is over a 95% surety that there is statistically significant difference in the overall mold populations between Day Five and Day Six.

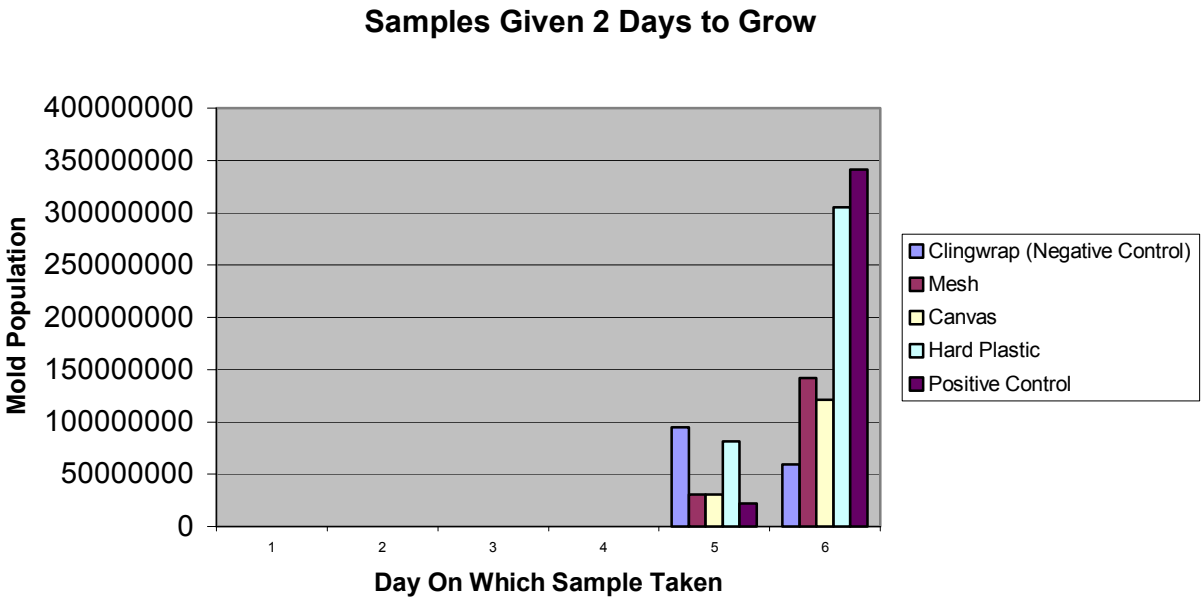
Table 1: Mold Counts Per Cm^3 of soil

	Clingwrap (Negative Control)	Mesh	Canvas	Hard Plastic	Positive Control
Quadrat 1 (NW)	38,666,667	38,666,667	71,000,000	14,000,000	45,000,000
Quadrat 2 (NE)					
Sample 1	109,000,000	55,000,000	7,000,000	36,000,000	67,000,000
Sample 2	79,000,000	174,666,667	38,666,667	105,666,667	17,000,000
Quadrat 4 (SW)					
Sample 1	128,333,333	54,500,000	98,000,000	55,000,000	43,666,667
Sample 2	711,000,000	76,666,667	38,500,000	266,333,333	18,000,000
Average	213,200,000	79,900,000	50,633,333	95,400,000	38,133,333

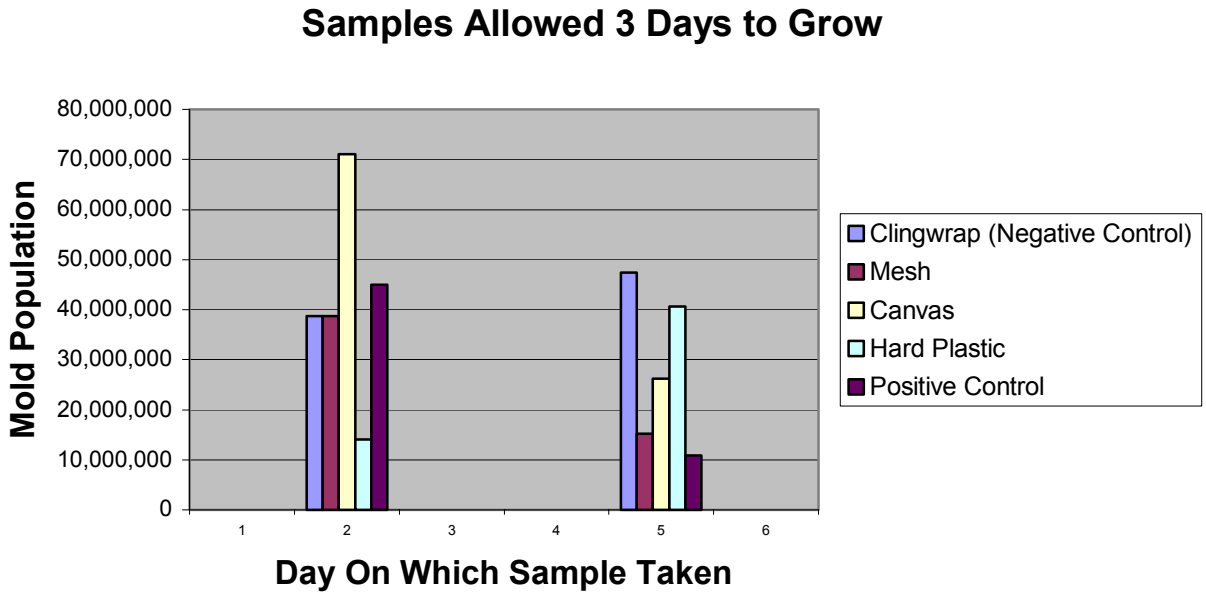
Table 2: Mold Counts Per Cm^3 of soil

	Clingwrap (Negative Control)	Mesh	Canvas	Hard Plastic	Positive Control
Quadrat 2 (NE)					
Sample 1	25,000,000	4,000,000	37,333,333	38,000,000	17,000,000
Sample 2	76,333,333	12,000,000	6,500,000	37,333,333	8,500,000
Quadrat 3 (SE)					
Sample 1	77,666,667	200,333,333	234,333,333	191,000,000	665,000,000
Sample 2	41,000,000	83,000,000	8,000,000	419,666,667	17,000,000
Quadrat 4 (SW)					
Sample 1	8,500,000	7,000,000	3,000,000	8,000,000	10,000,000
Sample 2	79,666,667	38,000,000	14,000,000	79,000,000	8,000,000
Average	51,361,111.17	57,388,888.83	61,361,111.11	128,833,333.33	120,916,666.67

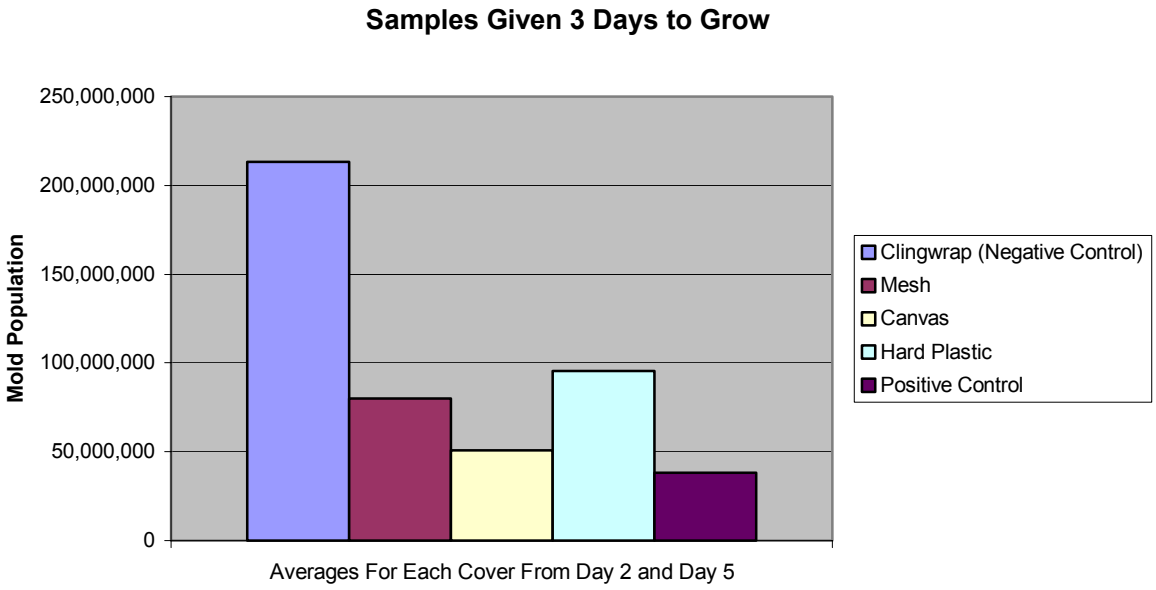
Graph 1:



Graph 2:



Graph 3:



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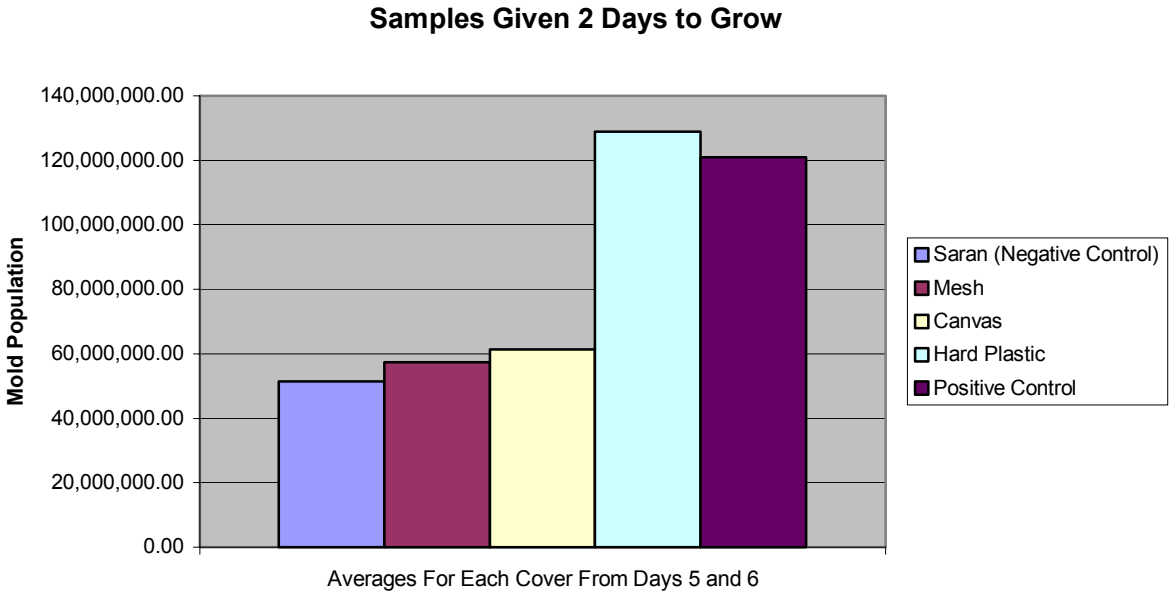

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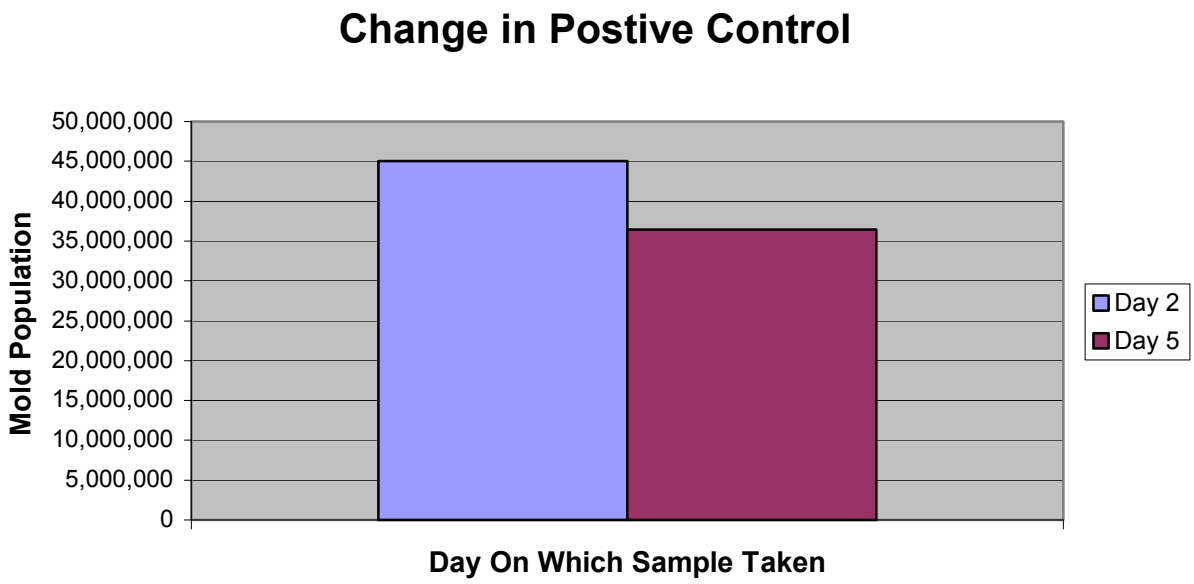
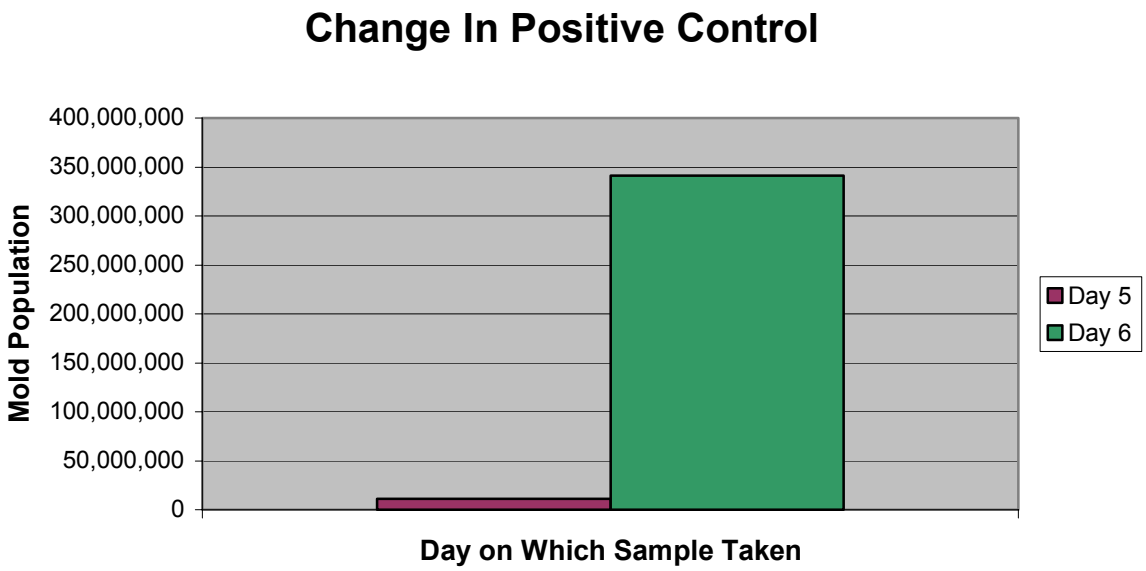


Graph 6:



Discussion:

The statistical significance of the t-test values between overall mold population densities on each day of the experiment gives a clear picture of why there was no statistically significant difference in population densities between the covers despite the fact that they allowed through different levels of ultraviolet radiation. For the week of the biota survey, the temperature was blisteringly hot with an incredibly high ultraviolet index, averaging 8.23 (NOAA, 2002) and as a consequence, we believe the extra UV radiation caused the low mold population density of the original survey. There was also only an average of 23.33% cloud cover (National Weather Service, 2002). However, from the time we started our individual experiment, the temperature decreased and the clouds rolled in, substantially decreasing the amount of ultraviolet radiation. The ultraviolet index was only 6.5 (NOAA, 2002) and there was 80% cloud cover (National Weather Service, 2002) during the first two days of our experiment. This caused a universal bloom in the mold population density. The decreased influence of ultraviolet light allowed other factors like nutrients to become important and to begin influencing the mold population density creating seemingly random variations in the mold population density. Not even the clingwrap samples were getting enough ultraviolet light to decrease their mold population density by any significant amount. However, as the week progressed, the sun reemerged, with 46.67% cloud cover (National Weather Service, 2002) and the ultraviolet index increased again, to an average of 7.2 (NOAA, 2002). The reinstatement of ultraviolet light as the dominant influencing factor is evidentially supported by the beginnings of a visible skewed distribution, with clingwrap having the lowest population and hard plastic having the highest population (See graphs 3 and 4).

The positive control similarly supported our hypothesis. Starting with a high average mold population of 45,000,000 because the dirt was almost completely covered by ivy, by the time we took the second sample, it had significantly dropped with an average of 10,875,000 after being plated for two days and 36,416,667 after being plated for three days. Despite the cloud cover at that time, there was still significantly more exposure to ultraviolet radiation with bare dirt than with dirt covered completely by English Ivy.

This line of reasoning led us to the conclusion that we have strong evidence supporting the hypothesis that the increased influence of ultraviolet light on exposed soil causes a subsequent decrease in the mold population density under that soil. Although, because of time constraints, we were unable to take additional samples which would have increased the difference between the covers to the extent that there would be statistical difference between the mold population density under each cover. If the ultraviolet index had been dramatically higher or lower, we believe that the actual experimental results would have been statistically significant between each of the covers. This would have allowed us to claim conclusive proof for our hypothesis. This being the case, for future research we would advise running the experiment trying to choose a time when the weather forecasted is either an extremely high ultraviolet index or at least fairly constant amount of cloud cover, and running the experiment for a longer time period.

Certain errors, other than the variations in the ultraviolet index, occurred that might have slightly altered our results. For the first day on which we took samples, we were unable to perfectly repeat our experiment. Taking our samples on different days from different quadrats provided replication, but the first day we did not repeat by taking more than one sample from each covering per day. The second day we tried to correct

that error—we took multiple samples from under each covering. When plating Quadrat 1, Canvas, 10^{-1} and 10^{-2}, the easygel solution was accidentally placed in normal petri dishes instead of easygel dishes, obviously causing it not to harden. When the mistake was realized, we poured the easygel from the sterile petri dishes into easygel plates. In addition, the saran wrap came off one of the squares (Quadrat 4, Saran). We were able to take a sample (# 1) from under the area that was still covered, but our other sample (# 2) came from the area that would have been covered if the saran wrap had not torn.

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