Mold to the Rescue?

A Study of the Effects of Mold Density on Active Aluminum and Ferric Iron Levels in Soil

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Abstract

The levels of mold in an ecosystem are critical to the health of its organisms. If the molds suffer, so will the plants due to a build up of toxic metals, such as iron and aluminum, in the soil. We decided, following the E.S.S.R.E. Biota Survey, to compare the densities of mold against aluminum and iron across sites two, three, and four due to observed discrepancies. We tested these soil samples from the deviant sites simultaneously for mold density and iron and aluminum content. We saw, through T-testing, that there seems to be a significant relationship between each element of testing: mold is inversely related to aluminum and iron, and vice versa, that is, when one group's population goes up, the other group's population goes down. Therefore, our group concluded that the mold in an environment will have an effect on the concentrations of harmful iron and aluminum in its soils.

Introduction

The E.S.S.R.E. 2005 biota survey (E.S.S.R.E. 2005) took place in a wooded area (temperate deciduous forest) behind the Roland Park Country School in Baltimore, Maryland. In this survey, research groups observed unusually high Active Aluminum and Ferric Iron levels in the soil of some of the research sites. Aluminum reached levels of 125 ppm in some areas. Levels of aluminum this high can be toxic to plants, because excessive Active Al in the soil stunts root growth and inhibits the plant's ability to absorb calcium and phosphorus: essential nutrients. Calcium, in fact, regulates many cellular functions (*Elmhurst College* n.d.). Also, stunted root growth is a serious problem for plants, because it prevents the plant's roots from reaching more soil to obtain nutrients and water (Haynes 1992). We found the high iron levels (as high as 28.75 ppm) disconcerting as well, because oxidative stress, or increased levels of oxidants that degrade the plant's condition, is caused by the plant absorbing too much iron. Excess iron in plant tissues can cause chemical reactions which generate hydroxyl radicals. Due to their strong negative charge, these hydroxyl radicals can damage molecules essential to the proper functioning of the plant: lipids, DNA, and proteins (Connolly 2002).

Our group chose to investigate the unusual Fe and Al levels further. Through research, we found that "Decomposition of the organic matter produces organic acids which combine with iron and aluminum ions, thereby reducing their potential toxicity to plants" (Flannery 1999). The decomposition process involves organisms such as bacteria and mold that obtain nutrients such as carbon and nitrogen from the organic material that they are decomposing (*Texas A&M University* n.d.). In the decomposition process, certain organic acids are produced as an intermediate step between organic material and simpler inorganic

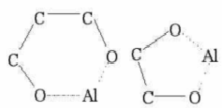

Figure 1

substances (*Nolan-ITU Pty Ltd.* 2002). These organic acids can bind to active aluminum in the manner shown in Figure 1,[1] thus rendering the aluminum harmless to plants. In a similar way, organic acids can help prevent ferric iron from harming plants.

In addition to the high active aluminum and ferric iron levels, we also observed unusually low densities of mold (as low as 1,667 per cc). These densities were found in a warm, damp area. Since mold typically thrives in such conditions, this measurement was quite unexpected. Furthermore, the Al levels in this area were much higher than Al levels in a second area which had much more mold. Contrary to what might be expected, this second area was drier and colder than the first. Our data seemed to show an inverse relationship between mold densities and Al levels. Though all of our data from the biota survey did not fit this pattern, we felt that enough corresponded to warrant further investigation.

In our experiment, we decided to evaluate the relationship between Fe levels and mold densities as well, since we had also observed unusual iron levels in our research area. We decided to test soil samples from various areas ranging in aluminum and iron levels from highest in the area to lowest, to determine whether unusually high aluminum and iron levels were accompanied by low densities of decomposers. Such results, coupled with our research on organic acids produced by decomposition, would indicate that inadequate densities of mold were causing aluminum and iron levels to be excessively high, because insufficient decomposer densities would result in low levels of organic acids, which neutralize toxic forms of active aluminum and ferric iron.

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[1] Figure 1 available online at http://www.plantstress.com/Articles/toxicity_m/Tolerance.htm.

Methods and Materials

Description of Sites

A stream ran through all three sites from which the samples were collected. Two of the sites were also classifiable as forest, and the third was a defined wetland or marsh. E.S.S.R.E. site 3 had a monoculture of rhododendron on the steeply sloping east side of the stream. The flatter west side was covered predominantly with Kenilworth ivy and erect knotweed, with interspersed trees. The stream was lined with very slippery rocks on the east side. The stream flowed from the north-northeast to the south-southwest corners of site 3. E.S.S.R.E. site 2, similarly, had a great deal of rocks surrounding a central stream, sourced at an artificial waterfall. The banks on either side of the stream sloped very steeply in site 2, but quickly gave way to flat forest. E.S.S.R.E. site 4 had a monoculture of jewelweed covering most of its area. A stream flowed through it diagonally from the northeast so southwest corners, spreading out to saturate much of the ground, making site 4 a wetland. The coordinates of these sites were: 39.35845N, 76.638475W (site 3); 39.35837N, 76.63892 (site 4); and 39.35772N, 76.6387225W (site 2).

Procedures

Based on the previously conducted biota survey (E.S.S.R.E. 2005), we decided which quadrats in which sites to test for Al, Fe, and mold. Because we wanted to observe mold levels over a range of 5 different Al and Fe levels from low to high (relative to the levels in our three sites), we chose 5 areas each for Al and Fe that represented such a range. We chose site 2, quadrat 1 because, according to the biota survey, it had the highest Al average out of all the quadrats in our three sites (125 ppm). Similarly, we chose site 2, quadrat 4 as our medium-high area (110 ppm); site 2, quadrat 3 as our medium area (70 ppm); site 2, quadrat

3 as our medium-low area (43.3 ppm) and site 4, quadrat 2 as a low area (8.33 ppm). For Fe, we chose site 3, quadrat 2 as a high-iron area (28.75 ppm); site 3, quadrat 3 as our medium-high area (22.92 ppm); site 3, quadrat 1 as our medium area (5 ppm); site 2, quadrat 2 as our medium-low area (2.5 ppm); and site 4, quadrat 2 as our lowest area (0 ppm).

Using soil core samplers, we extracted cylindrical soil samples from the ground in our chosen quadrats. We pulled these samples from the ground vertically. Each was 15 cm long and 2 cm in diameter. We took three samples at random from each of the nine chosen quadrats. One by one, we tested each soil sample for ferric iron and aluminum and performed serial dilutions. We made sure to perform all three of these processes simultaneously for each sample. For the chemical tests, we used a LaMotte Combination Soil test kit (model STH-14). For the serial dilutions, our dilution levels were 10^0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. We placed 100µl of each of the diluted solutions on a 3M Petrifilm™ Yeast and Mold Count Plate. We left the plates undisturbed at room temperature out of direct sunlight, and examined them for molds 3-4 days later. After calculating the number of mold per cubic centimeter of soil for each of the samples, we looked at the relationship between the mold density and the Al and Fe levels for every sample.

Results

In order to see if there was a possible linear relationship between the aluminum levels and the mold density, we made a scatter plot (Figure 1.2). Then we performed a linear regression analysis (Figure 1.1) to see if the variation in aluminum was accounted for by the mold density in the soil. As the r-squared value indicates mold does not have a linear relationship with aluminum.

Figure 1.1

Estimate of Collection 1 — Linear Regression

Independent attribute (continuous): mold
Dependent attribute (continuous): aluminum

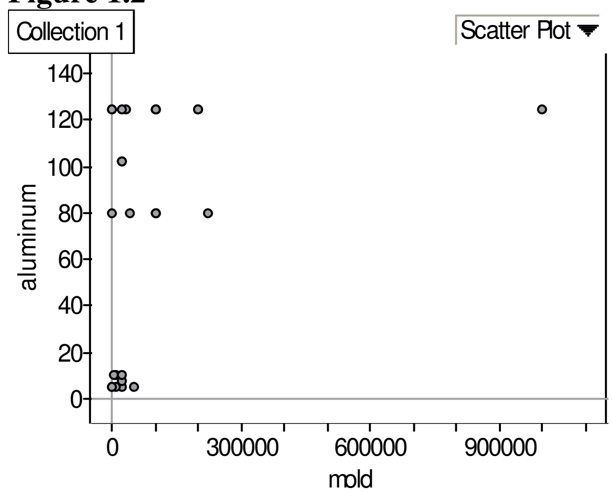
Independent attribute: **mold**
Dependent attribute: **aluminum**
Sample count: **27**

Equation of least-squares regression line:
aluminum = **8.77014e-05 mold + 65.906**
Correlation coefficient, r = **0.329857**
r-squared = **0.10881**, indicating that **10.881**% of the variation in **aluminum** is accounted for by **mold**.

The best estimate for the slope is **8.77014e-05** +/- **0.000103387** at a **95** % confidence level. (The standard error of the slope is **5.01993e-05**.)

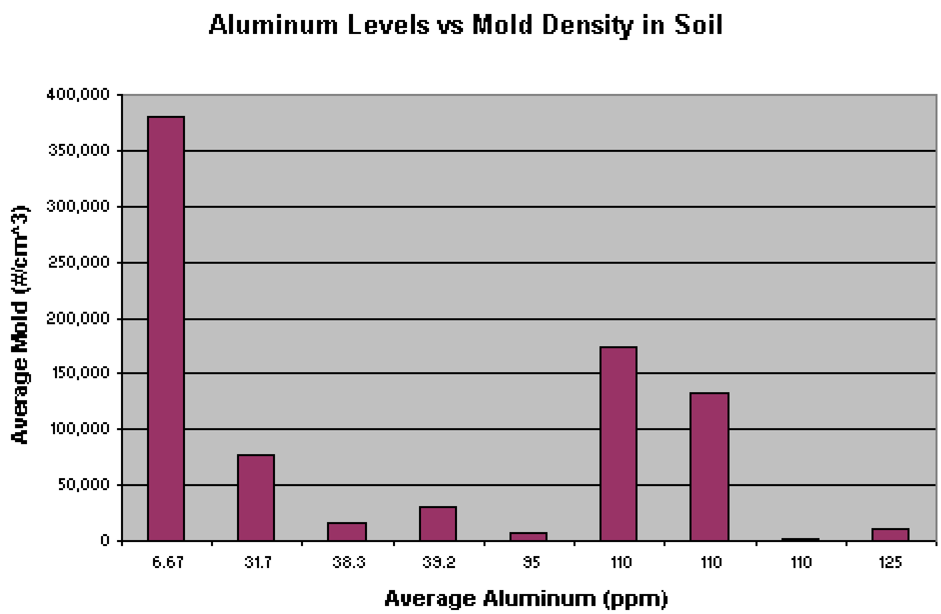
When **mold** = **0** , the predicted value for a future observation of **aluminum** is **65.9065** +/- **103.975**.

Figure 1.2



Then we used a bar graph (Figure 1.3) to see if another type of relationship existed between the aluminum levels and mold density. As can be seen in figure 1.3, there does appear to be an inverse exponential relationship between mold density and aluminum levels.

Figure 1.3



In order to see a possible linear relationship between the iron levels and the mold density, we made a scatter plot (Figure 1.5). Then we performed a linear regression analysis (Figure 1.4) to see if mold density accounted for the variation in iron. As the r-squared value indicates, mold does not have a linear relationship with iron.

Figure 1.4

Estimate of Collection 2 — Linear Regression

Independent attribute (continuous): mold
Dependent attribute (continuous): iron

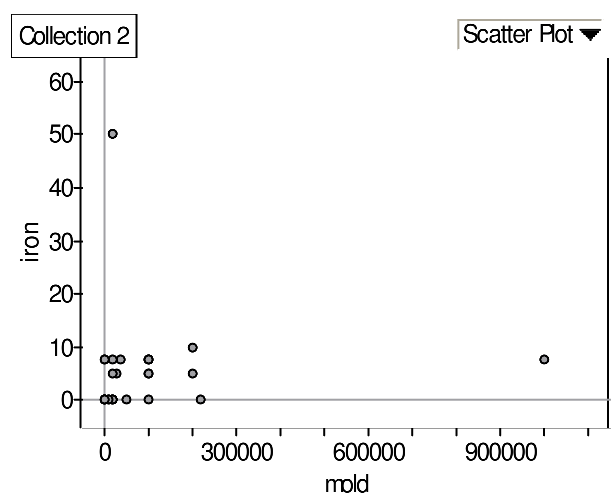
Independent attribute: **mold**
Dependent attribute: **iron**
Sample count: **27**

Equation of least-squares regression line:
iron = **2.1784e-06 mold + 5.1698**
Correlation coefficient, r = **0.0437629**
r-squared = **0.0019152**, indicating that **0.19152**% of the variation in **iron** is accounted for by **mold**.

The best estimate for the slope is **2.1784e-06** +/- **2.0484e-05** at a **95** % confidence level. (The standard error of the slope is **9.94594e-06**.)

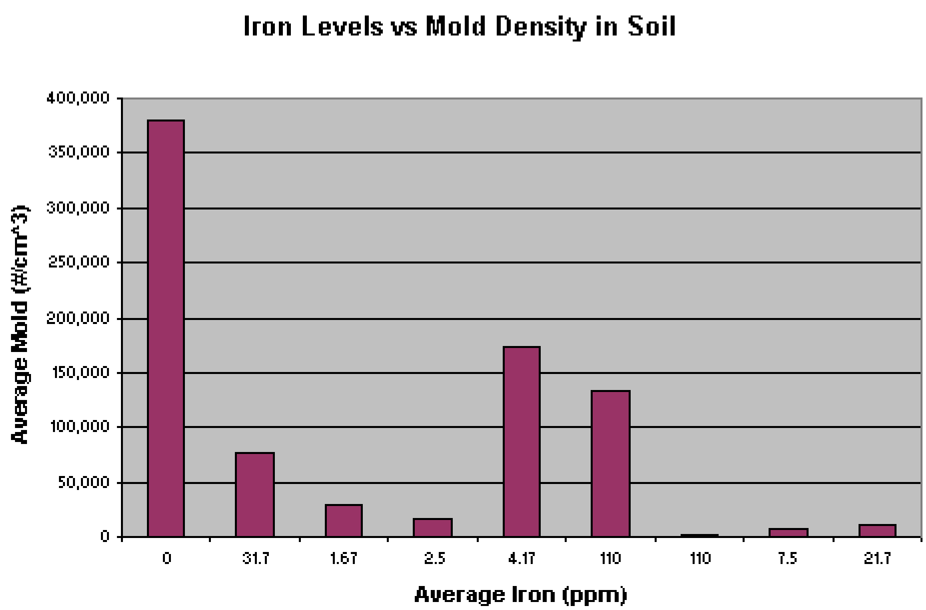
When **mold** = **0** , the predicted value for a future observation of **iron** is **5.1698** +/- **20.6006**.

Figure 1.5



Then we used a bar graph (Figure 1.6) to see if any other type of relationship existed between the iron levels and mold density. As can be seen in figure 1.6, there does appear to be an inverse exponential relationship between mold density and iron levels.

Figure 1.6



After we found the mold density and the aluminum and iron levels, we also t-tested the mold densities between the various plots we studied to see if any of them were significantly different. The samples that tested to be significant (p value of .1 or lower) are highlighted in Figure 1.6.

Figure 1.6

Tests	P Value	Tests	P Value	Tests	P Value	Tests	P Value	Tests	P Value
2.4-2.3	0.12	2.3-3.4	0.31	3.4-4.2	0.14	4.2-3.2	0.04	3.2-3.3	0.47
2.4-3.4	0.17	2.3-4.2	0.28	3.4-3.2	0.05	4.2-3.3	0.06	3.2-3.1	0.04
2.4-4.2	0.09	2.3-3.2	0.05	3.4-3.3	0.08	4.2-3.1	0.48	3.2-2.2	0.05
2.4-3.2	0.1	2.3-3.3	0.07	3.4-3.1	0.1	4.2-2.2	0.72	3.3-3.1	0.05
2.4-3.3	0.24	2.3-3.1	0.04	3.4-2.2	0.18			3.3-2.2	0.06
2.4-3.1	0.08	2.3-2.2	0.39					3.1-2.2	0.24
2.4-2.2	0.1								

We wanted to see if these significantly different plots displayed the expected relationships between mold density, iron levels, and aluminum levels. To do so we used bar graphs to compare the mold density, iron levels, and aluminum levels of the samples between these significant plots. The graphs in the left column represent the normal expected relationship. The graphs in the right column do not show the expected relationship.

Figure 2.1

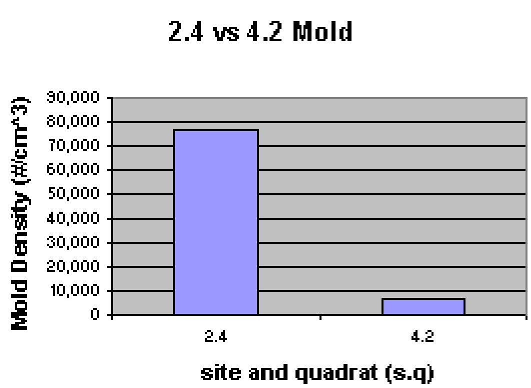


Figure 2.2

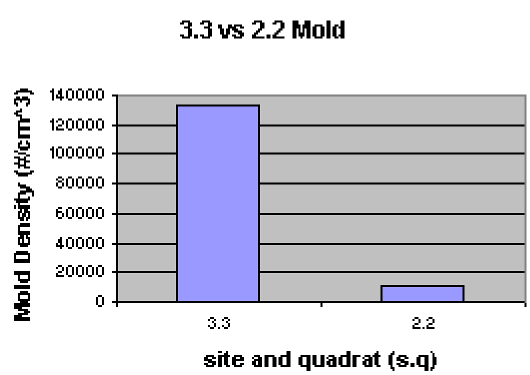


Figure 2.3

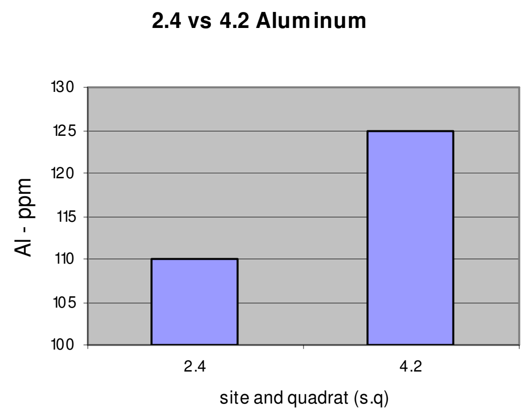


Figure 2.4

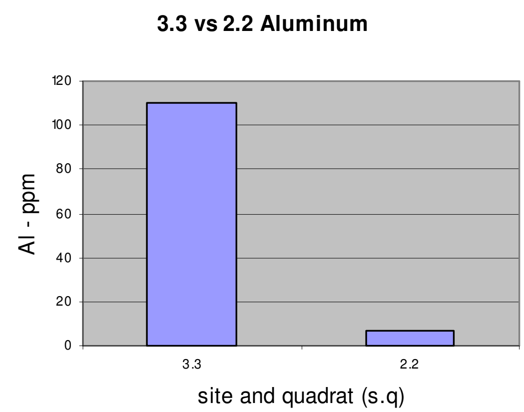


Figure 2.5

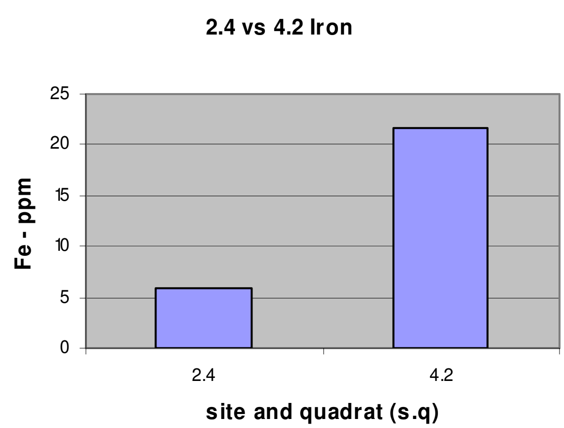
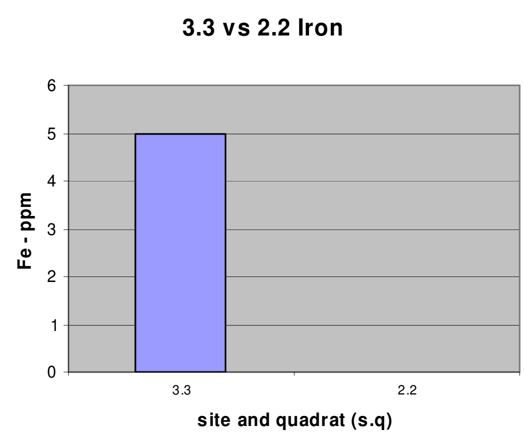


Figure 2.6



The only samples that showed the expected relationship were 4 samples from plot 4.2. The other 16 samples did not show the expected relationship.

Discussion

As shown in figures 1.1 and 1.2, mold and aluminum did not have a linear relationship. However, further data analysis showed that aluminum and mold appeared to have an inverse exponential relationship (figure 1.3). This further analysis supports our hypothesis, since decreasing mold densities coincide with increasing aluminum levels. However, two quadrats from our collected data did not fit this general curve. These two outliers were site 3, quadrat 2 and site 3, quadrat 3. Our data comparing mold densities to ferric iron levels was similar: figures 1.4 and 1.5 show that iron and mold do not have a linear relationship. However, like aluminum, iron and mold do appear to have an inverse exponential relationship despite two outlier quadrats: site 3, quadrat 2 and site 3, quadrat 3 (figure 1.6). The fact that these outlier quadrats are the same for both Al and Fe caused us to suspect that an environmental factor other than mold, iron, or aluminum is either causing mold densities to be unexpectedly high, or aluminum and iron levels to be unexpectedly low in these quadrats (in fact, iron levels in the two outlier quadrats did reverse over the course of the experiment). Although we did have two outlier quadrats, the data from site 4, quadrat 2 supported our hypothesis completely. As stated in *Results*, the only four samples out of twenty that showed the expected relationship were from site 4, quadrat 2. However, the graphs in figures 1.3 and 1.6, which take all the quadrats into account, indicate that higher aluminum and iron levels are generally accompanied by low mold densities. Therefore, we consider our hypothesis supported by our findings: low mold densities do seem to be linked to high active aluminum and ferric iron levels in the soil, with the exception of the two outlier quadrats.

Further research on this topic might explore the outlier quadrats. A research team might look for environmental factors other than mold that might be causing the Al and Fe levels in site 3, quadrat 2 and site 3, quadrat 3 to be unusually high. An example of such a factor could be decomposers other than mold. Resource shortages limited our research to mold, but truer results might be obtained by measuring bacteria densities as well as mold densities, since bacteria, as decomposers, also produce the organic acids that prevent Al and Fe from being toxic to plants. Further research might also investigate the reason for the area's plants' apparent good health given such high Al and Fe levels. Researchers might also want to study the reason that the data from site 4, quadrat 2 adheres so closely to the pattern projected by our hypothesis, while other sites do not.

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