# The Potential Effect of Ferric Iron and Bacteria Levels on Mold Density in Soil

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## Abstract

Fungi are essential organisms in soil because they decompose dead matter and replenish the soil with nutrients. Mold, a form of fungi, utilizes ferric iron ($Fe^{+3}$) in a process that creates energy which allows it to grow and reproduce Therefore, when ferric iron levels drop in the soil mold levels also decrease. One thing that can cause this to happen is when bacteria convert ferric iron ($Fe^{+3}$) to ferrous iron ($Fe^{+2}$), a form of iron mold cannot use. When the 2014 E.S.S.R.E. Biota Survey revealed varied and unexpected mold densities across the microclimate research sites, we hypothesized it was due to high levels of bacteria in the soil. Soil core samples were extracted from quadrants 2 and 4 of Site 4 and tested for ferric iron levels while serial dilutions were also performed to determine the densities of mold and bacteria in those soil samples. Although some of the results showed the expected correlations between the amount of bacteria, mold, and iron in the soil, these correlations had low statistical significance. To extend this study, we would explore potential correlations between light, water, bacteria densities, and mold densities in Site 4 due to the fact that light and water also vary in this location significantly and are critical environmental factors that influence the viability of these two critical soil microbes.

## Introduction

Molds play an important role in soil health by decomposing and preventing the buildup of organic matter. They thrive in moist, warm soil (North Carolina Department of Health and Human Services, 2014), and when there is a lower density of mold in the soil, matter is not decomposed efficiently, causing plants dependent on these released nutrients to suffer. One factor that influences the health of soil molds is the amount of $Fe^{+3}$ present. Mold utilizes ferric iron ($Fe^{+3}$) in its mitochondria to aid in growth and reproduction (Isaac, 1997); therefore, when there is a lack of ferric iron in the soil, mold cannot grow and by extension reproduce, depleting the levels of mold in that particular site.

One of the environmental factors that can influence $Fe^{+3}$ levels in the soil is the amount of bacteria living there, specifically members of the genera *Pseudomonas*, *Bacillus*, *Bacteroides*, and *Desulfovibrio*. Species of these bacteria groups can reduce $Fe^{+3}$ to $Fe^{+2}$, thereby making the $Fe^{+3}$ which molds need less available. Ferric iron reduction is most commonly seen in the neutrophilic, heterotrophic bacteria of these groups (Johnson and McGinness, 1991), and while the causes of ferric iron reduction are not fully known, there is evidence that bacteria may induce ferric iron reduction by using ferric iron as a hydrogen sink to produce energy (Johnson and McGinness, 1991). Regardless, where there are high levels of these bacteria in the soil, there can be low levels of ferric iron and, therefore, less mold present.

Based on the findings of the E.S.S.R.E 2014 Biota survey (E.S.S.R.E, 2014), we observed that mold density in the soil of microclimate Site 4 (4,808.33 /cc) was significantly lower than those in the other three microclimate sites. But given that Site 4 is a partial wetlands, this finding is highly unexpected. Upon examining the rest of the survey data, though, we noticed that the $Fe^{+3}$ levels in Site 4 were also significantly lower (11.04 ppm) while the bacteria density was unusually high (12,174,166.67 /cc). Therefore, in this experiment we investigated the correlations between the quantities of bacteria, mold, and $Fe^{+3}$ present in different parts of Site 4. We hypothesized that the higher amounts of bacteria in Site 4 are inversely affecting the amount of ferric iron and mold in the soil because of bacteria's ability to convert ferric iron into ferrous iron.

## Methods

For 3 consecutive days 5 separate soil samples each 15 cm deep with a diameter of 2 cm were randomly extracted from both quadrant 2 and quadrant 4 In E.S.S.R.E. Site 4 (E.S.S.R.E., 2001) for a total of 10 samples per day. All soil samples were tested for Fe+3 (ppm) using The LaMotte Model STH-14 Series Test Kit while simultaneously each sample was serially diluted to 10-4 and 100 µl aliquots of the 100, 10-1 and 10-2 dilutions were plated on separate 3M Petrifilm™ Yeast and Mold Count agar sheets and 100 µl aliquots of the 10-2, 10-3 and 10-4 dilutions were plated on separate 3M Petrifilm™ Aerobic Count Plates agar sheets. All plates

were incubated at room temperature for 72 hours, and then the density of mold (#/$cm^3$) and bacteria (#/$cm^3$) in each soil sample was calculated.

**Results**

**Table #1**

Data Averages

| | Quadrant 2 | | | Quadrant 4 | | |
|---|---|---|---|---|---|---|
| | Iron (ppm) | Mold (#/$cm^3$) | Bacteria (#/$cm^3$) | Iron (ppm) | Mold (#/$cm^3$) | Bacteria (#/$cm^3$) |
| Day 1 | 8.25 | 2960 | 17262000 | 11 | 18400 | 26300000 |
| Day 2 | 15.5 | 2100 | 568000 | 7.25 | 4220 | 1754000 |
| Day 3 | 6.25 | 2875 | 2746000 | 13.5 | 5400 | 1550000 |

Table 1 indicates the correlations observed between iron levels and mold and bacteria densities in the soils sampled over the course of the 3 days of the experiment.

**Table #2**

p-values

| | Quad 2 | | Quad 4 | |
|---|---|---|---|---|
| | Day 1-Day 2 | Day 2-Day 3 | Day 1-Day 2 | Day 2-Day 3 |
| Iron | 0.576 | 0.576 | 0.2898 | 0.565 |
| Mold | 0.698 | 0.9237 | 0.203 | 0.995 |
| Bacteria | 0.3346 | 0.4373 | 0.0296 | 0.821 |

Table 2 values determined by t-testing

**Figure #1**

The effect of the amount of Iron on the amount of Bacteria.

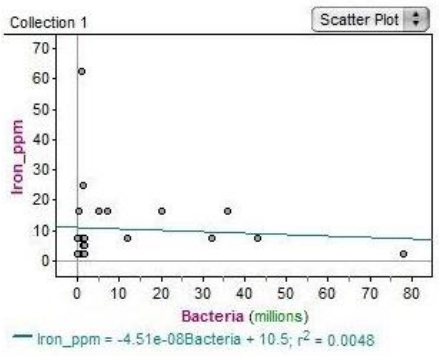


Figure 2 shows that the relationship between the amount of bacteria and amount of iron as a negative correlation, meaning that as the bacteria levels go up the Ferric iron levels go down. The $r^2$ value of .0048 shows a very weak negative correlation between the levels of bacteria and iron.

**Figure #2**

The effect of the amount of Iron on the amount of Mold.

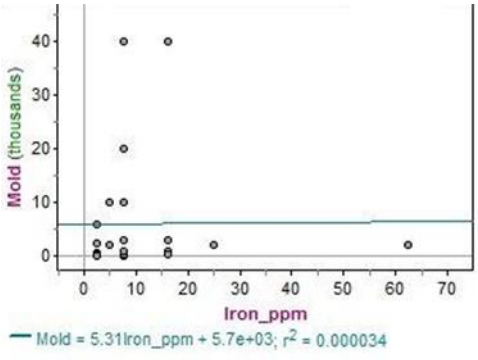


Figure 3 shows a positive correlation between the amount of mold and iron, meaning that as the iron levels go up the mold levels also go up. The $r^2$ value of .000034 shows a positive weak relationship between the levels of mold and iron.

**Discussion**

As Table 1 shows, the changes we observed in mold and bacteria densities and iron levels in the tested soil samples produced only partial support for our original hypothesis. While the changes in the bacteria densities show the expected inverse relationship with $Fe^{+3}$ levels from one day to the next in quadrant 2, there is no statistical significance to this trend ($p = 0.576$ for iron and $p=0.3346$ for bacteria). Furthermore, while the mold densities and Fe+3 levels in quadrant 4 do show the hypothesized direct correlation, they do so significantly only between Day 1 and Day 2 of the experiment ($p = 0.2898$ for iron and $p = 0.203$ for mold). Finally, as Figures 1 and 2 indicate, the trends we predicted were observed during our research, but the anticipated correlations are very weak at best ($r^2 = 0.0048$ for the relationship between bacteria and iron and $r^2 = 0.000034$ for the relationship between iron and mold). Hence, while there is data to support our original hypothesis, it is minimal at best.

However, the data in Table 1 do show two interesting trends. The first trend observed was that in quadrant 2, the bacteria densities fluctuated unsteadily while the mold densities remained at a steady level of about 2,500 per cm3over the course of three days. Conversely, in quadrant 4, it was the bacteria densities that stabilized at a steady level of 1.8 million per $cm^3$ while the mold densities fluctuated unsteadily. These two trends suggest that there is an alternative factor other than $Fe^{+3}$ levels that may be at work in these two different quadrants.

One possibility is the physical environmental differences between quadrant 2 and 4 in Site 4. For example quadrant 4 lacks trees and is therefore exposed to more sunlight than in quadrant 2. In addition, quadrant 2 has a stream going through it, whereas quadrant 4 does not have any water flow in it. Given that both of these factors can play a role in the health of both bacteria (E.S.S.R.E., 2005) and molds (Nyberg, 1987), it would be interesting to explore the potential correlations between light, water, bacteria densities, and mold densities in Site 4. In the future, we would include gathering data on the levels of sunlight and moisture in both quadrants as well as measuring changes in the growth rates of mold and bacteria in these locations.

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**Acknowledgments**

We would like to extend our thanks to Dr. Heine, Larry Jennings, and Human Capital Development, Inc. for giving us the opportunity and ability to participate in the E.S.S.R.E program and helping become successful young women in the sciences. We would also like to thank all of our teaching assistants for answering our questions. Finally, we would also like to thank Mr. David Brock for all of his amazing hard work and unfaltering dedication in running E.S.S.R.E.