

The Effect of Moisture on Bacteria Populations and Manganese Oxide in the Soil



By: Alexandra Cirelli, Alexa Wolfe, Brittany
Jones, & Jordan Tapp

Abstract

Manganese is a nutrient that is both essential and potentially hazardous to plant life. Plants use manganese for their metabolisms and photosynthetic processes, but high amounts of manganese can cause unusually short roots and chlorosis. Soil bacteria also employ manganese and tend to oxidize it under wet soil conditions to use for their own metabolic functions. In the 2013 E.S.S.R.E. biota survey, anomalously low levels of manganese were observed in the highly acidic soils in Sites 1 and 2 while the normal correlation between manganese and acidity was observed in E.S.S.R.E Site 4. We identified that the key environmental difference between the three sites is the presence of large amounts of additional water in Site 4. We hypothesized that this additional water was causing increased levels of bacteria in the soils there and that, consequently, the extra bacteria were oxidizing the manganese present there more frequently. 10 soil core samples that were 15 centimeters deep and 2 centimeters wide were collected from Site 4 each day over the course of 4 days. The samples were then tested to find the percentage of moisture, the density of bacteria (#/cc), and the level of manganese (ppm). The results showed that bacteria were causing manganese levels to decrease as expected but that the more moist soils contained higher than expected levels of manganese. In the future, we would test the soil in Site 4 for acidity as well as moisture, bacteria, and manganese levels.

Introduction

Manganese is an element that makes up nearly 0.14% of the earth's crust, most of it in the form of manganese dioxide (Incitec Pivot, 2003). This inorganic form of manganese is one of the nine essential nutrients plants use for their metabolic needs, and it plays a role in everything from the absorption of carbon dioxide to the synthesis of chlorophyll to electron transport in photosynthesis. (Spectrum Analytic Inc, 2013). However, while manganese is essential to a plant's homeostasis, high amounts of the micronutrient can be lethal (Hong, Ketterings, McBride, 2010), and the consequent toxicity can cause necrotic lesions, chlorosis, stunted roots, and the collection of lethal levels of manganese dioxide in plant cells (Spectrum Analytic Inc, 2013).

Access to this critical nutrient depends on key ecological processes in the soil. For example, when large populations of bacteria are present under moist conditions, almost all free manganese available in the soil is reduced to manganese oxide; whereas in drier soils, the manganese remains in forms more accessible to the organisms living there (LaMotte, 1994 Incitec Pivot, 2003). However, the solubility of all forms of manganese depends heavily on the prevailing pH in the soil. As acidity increases, the lower pH more easily allows the ionic bond in MnO to dissociate, releasing Mn^{+2} into the soil, making the manganese more available to plants and the many soil microbes. In fact, the availability of manganese increases 100-fold for every unit increase of acidity (e.g. from 5pH to 4pH) which is one of the reasons why high acidity can cause manganese poisoning in plants. In soils with neutral acidities, on the other hand, there is less dissociation of the MnO, causing the amount of Mn^{+2} in the soil to be less, which is why manganese deficiency is most commonly found in soils with a pH of 6.5 or more (Schulte and Kelling, 1999). But in either case, any excess Mn^{+2} that is available is used by plants and many types of bacteria for their respective metabolic purposes. (e.g. Mn^{2+} can be used by bacteria to reproduce and form endospore, the exterior of their cell structures) (He, Zhang, Jin, Zhu, Liu, 2008).

During the E.S.S.R.E. 2013 Biota Survey (E.S.S.R.E., 2013), anomalous levels of available manganese were found in E.S.S.R.E Sites 1 (N 39.35794; W 076.63977) and 2 (N 39.35740; W 076.63893). The average soil pH in each of these was extremely acidic (5.4 in Site 1; 5.1 in Site 2). Yet the average level of available manganese in these sites was quite low (6.2 ppm in Site 1, and 5 ppm in Site 2). Given that the normal relationship between soil pH and manganese levels is an inverse one, not a direct one, we wondered what could be the source of this anomaly.

One possible explanation we observed was the unusually dry soil conditions in these sites. In another one of the E.S.S.R.E. research sites, which is extremely close in proximity (Site 4: N 39.35733; W 076.63840), a normal relation between soil pH (average 6.7pH) and available manganese (average 12.6ppm) was observed. Since the only dominant feature distinguishing the soil between the sites is a small stream that runs through Site 4 that makes the soil much more moist than the steeper and dryer areas in Sites 1 and 2, we decided to investigate whether it was the moisture difference that might account for the anomaly.

Methods

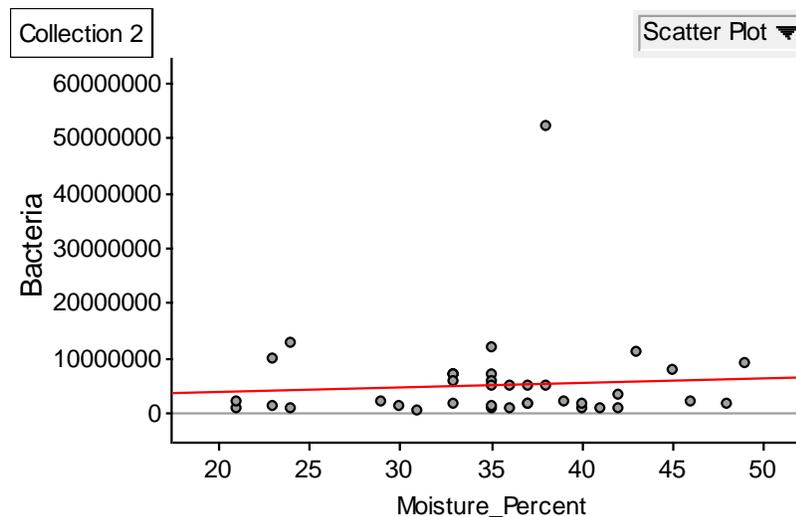
5 locations were marked in ESSRE Site 4 (N 39.35733 W 076.63840) based on observable differences in moisture at each site. The first of these locations contained dense mud with no plant life and was located 1.7 meters north of the northwest corner of Site 4. This location was used as the negative control. The second location was 5 meters southeast of the northwest corner of Site 4, and the remaining 3 locations were established in a straight line 4 meters apart from each other due southeast of the second location.

5 additional locations were marked based on their estimated bacterial density according to the 2013 soil biota survey (E.S.S.R.E., 2013). The first of these locations contained the highest population of bacteria and was located in Quadrant 4 of Site 4, 1.5 meters northeast of the southwest corner. (E.S.S.R.E., 2013). This location was used as the negative control. A second test site was established in Quadrant 4, 2 meters northeast of the negative control site. There was one testing location established in the center of each of the remaining three quadrants.

A soil sample that was 15cm deep and 2cm wide was taken from each of the 10 marked locations each morning over the course of 4 days. All 30 samples were serially diluted to the 10^{-4} dilution, and 100 μ L of the 10^{-2} , 10^{-3} , and 10^{-4} dilutions of each sample were plated on separate 3M Petrifilm™ Aerobic Count Plates. After 48 hours, the population density of bacteria was calculated. Simultaneous with the serial dilutions, all samples were tested for manganese (ppm) using the LaMotte Combination Soil test kit Model STH-14, and each sample was tested for its moisture content by air drying for 24 hours and then baking at 110°C for 2.5 hours.

Results

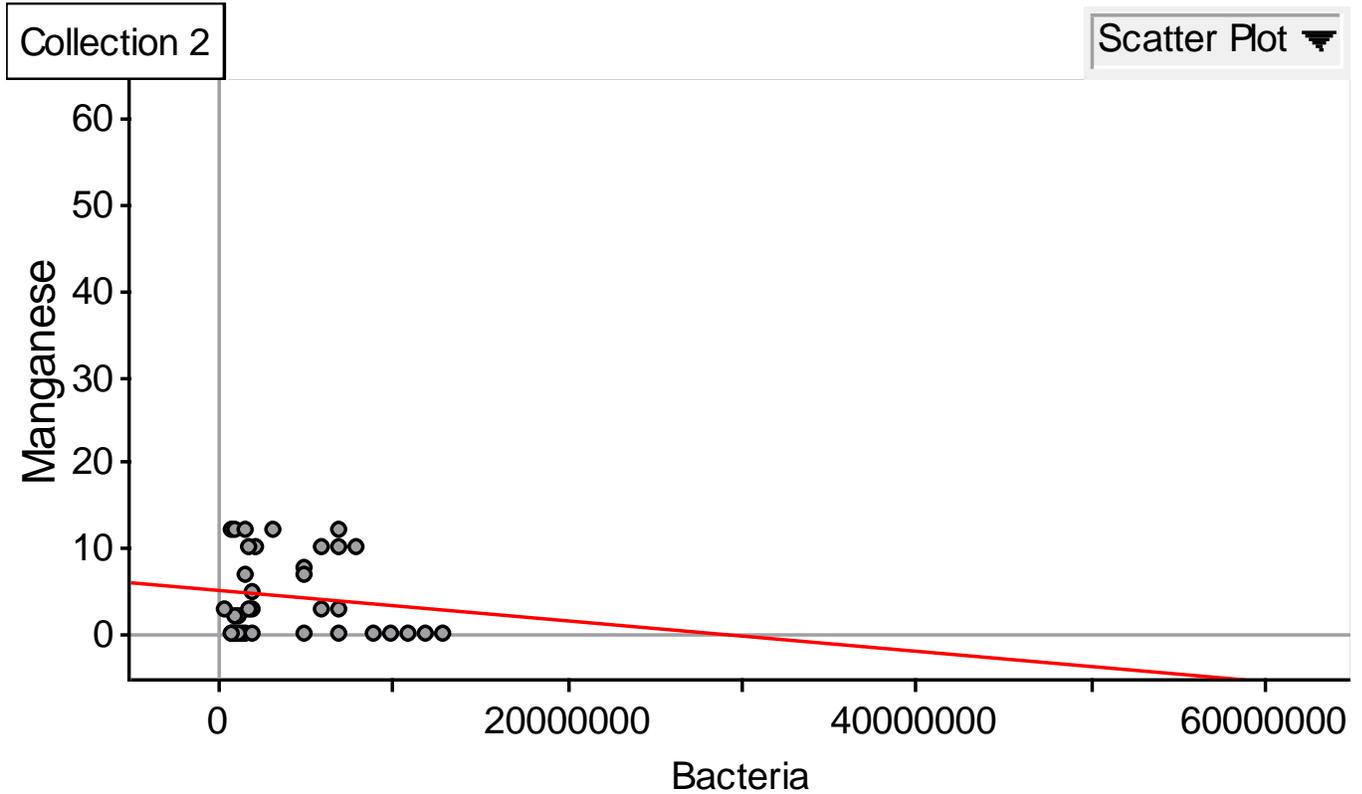
Figure 1 (Below): Percentage of Moisture vs. Bacteria Population



$$\text{Bacteria} = 81400\text{Moisture_Percent} + 2600000; r^2 = 0.0047$$

The graph in figure 1 shows the relationship between the percentage of moisture and the population of bacteria in the soil. The graph shows that there is a slight positive correlation between the percentage of moisture in the soil and the bacteria populations in the soil.

Figure 2 (Below): Bacteria Populations vs. Amount of Manganese



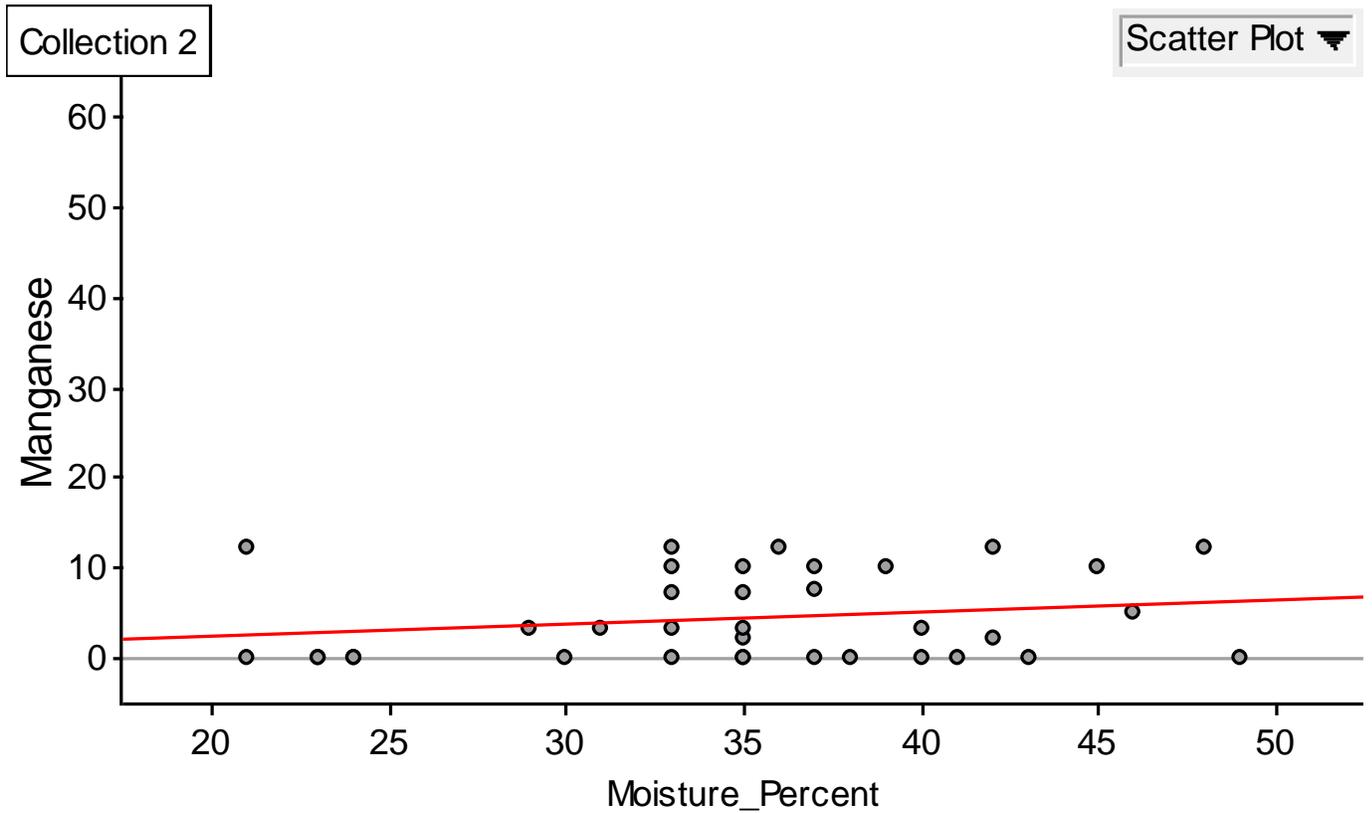
$Manganese = -0.000000170Bacteria + 5.0; r^2 = 0.017$

The graph in figure 2 shows the relationship between the population of bacteria and the amount of manganese (ppm) in the soil. There is a clear negative correlation between the amount of bacteria and the amount of manganese.

The following outliers were removed from the original data set:

	Moisture	Number of Bacteria	Manganese (ppm)
Moisture Sample D taken on 7/22/13	32%	7,000,000	50
Bacteria Sample 4 taken on 7/18/13	38%	52,000,000	0
Moisture Sample C taken on 7/22/13	35%	1,400,000	30

Figure 3 (Below): Percentage of Moisture vs. Amount of Manganese



$Manganese = 0.129Moisture_Percent - 0.21; r^2 = 0.041$

The graph in figure 3 shows the relationship between the amount of moisture and the amount of manganese (ppm) in the soil. The linear regression line for the graph clearly shows that there is a positive correlation between the percentage of moisture and the amount of manganese in the soil.

The following outliers were removed from the original data set:

	Moisture	Number of Bacteria	Manganese (ppm)
Moisture Sample D taken on 7/22/13	32%	7,000,000	50
Moisture Sample C taken on 7/22/13	35%	1,400,000	30

Discussion

Our original hypothesis predicting that soil moisture levels have an impact on the amount of manganese in the soil was supported though not in the way we expected. Figure 1 shows that the expected direct relationship between the amount of moisture in the soil and the density of the bacteria living there was observed; while figure 2 also indicates that the predicted inverse correlation between bacteria and manganese was observed. Therefore, the expected correlations seen in figures 1 and 2 support our prediction that the bacteria in Site 4 contribute to the observed manganese levels in the original biota survey (E.S.S.R.E, 2013).

However, figure 3 shows a highly unexpected relationship between the amount of moisture and manganese levels found in the soil in Site 4 given the expected correlations documented in figures 1 and 2. We would expect that in the more aqueous soil samples that the pH levels would increase, the acidity of soil would become more neutral, and the amount of manganese would consequently decrease. In fact, this was why we hypothesized that higher moisture levels in Site 4 were the cause of the amounts of manganese that were found there. Figure 3, though, clearly indicates that as the moisture levels in the soil in Site 4 go up, the amount of manganese in the soil is actually increasing as well. This failure to support the supposition behind our original hypothesis leads us to believe that we need to research the acidity in Site 4 as well as the amount of moisture. It is possible that the stream in Site 4 could be bringing acidic runoff from further up the hillside in Site 3, augmenting the manganese content. In the future, we would test the soil in Site 4 for acidity as well as moisture bacteria and manganese levels.

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