# The Effect of Salinity on Soil Fungi

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# I. Background

Soil is an extremely important factor in the health of an ecosystem. It consists of many different microbes, including two types of fungi: mycorrhizal and saprotrophic. These fungi are involved in many processes that affect the health of the ecosystems in which they reside, including cycling nutrients through decomposition, playing various roles in the carbon cycle, and even providing medical drugs to humans (Speer et al, 1994).

The first of these types of fungi, mycorrhizal, has a symbiotic relationship with plants that benefits both organisms (Davies, n.d.). The fungi attach to the different kinds of plants by bonding to the cells of their roots. Once they are in this relationship, the fungi colonize the root cells and exchange different nutrients with them. Fungi have the ability to easily absorb nutrients from the soil, such as nitrogen and phosphorus compounds, that many plants have a difficult time absorbing on their own, and the fungi provide these nutrients to the plant through their attachment to the roots. In return, the plants give the fungi the carbohydrates from photosynthesis that allows the fungi to have the energy to reproduce and grow.

Together, the fungi and plants bring the nitrogen and phosphorus into the food chain that all living things need to build their cells. Nitrogen is absorbed through the roots of the plant, and is a major component in photosynthesis, and therefore plant growth (NEALS, 2014). Phosphorus, on the other hand, is a critical part of the nucleic acid in a plant's cell structure, which controls protein synthesis (University of Nebraska Cooperative Extension, 1999). Together, then, the nitrogen and phosphorus that a plant absorbs create the proteins that control the chemical reactions in a cell, which make and break chemical bonds using energy to perform the four tasks that a cell does. Hence, without nitrogen and phosphorus, plant cells could not reproduce, engage in homeostasis and photosynthesis, or create proteins. In other words, the plant could not survive, and therefore the

biological molecules of the plant would not be available for the primary consumers who eat the plants (and so on up the trophic levels). Since mycorrhizal fungi has a relationship with approximately 90% of vascular land plants, harming the mycorrhizal fungi could damage the entire rest of the food chain (Pace, 2003).

Another kind of fungi, called saprotrophic fungi, do not share the same symbiotic relationship with plants, but are also vital to the health of the soil. Saprotrophic fungi play an important role in the carbon cycle where they decompose dead organic matter, which acts as an energy source for the fungi, and release the element carbon back into the atmosphere in the form of carbon dioxide (AFTOL, 2005). This process in the carbon cycle is important because it releases the carbon in a form that plants can use to photosynthesize. This allows the plants to make energy, and after they die, the carbon goes back into the soil through decomposition where the process is repeated (The National Center for Atmospheric Research, 2014). This recycling of dead organisms allows the carbon to keep moving through the food chain as plants photosynthesize the carbon dioxide into the rest of the biological molecules. Hence because all organisms depend on plants to live, and the plants depend on saprotrophic fungi for their carbon, these fungi also help prevent the collapse of the rest of the ecosystem.

However, many things can damage the different soil fungi and the jobs they perform in the environment, posing a threat to ecosystems, and one action that humans do to contribute to the possible damaging of the microbes is applying salt to roads in the winter. During snow and ice storms, people commonly place a form of sodium chloride on the ground to melt the ice faster than what it normally would, (Senese, 2010), and after large snow and ice storms, clearing the roads is necessary for people to drive safely. So large amounts of salt are needed to melt all of the ice that people wish to dispense with, and in the year 2014 alone, with a total of 19 storms, Baltimore County by itself used 185,503 tons of salt to clear ice from roadways, costing over 17 million dollars (Department of Public Works, 2014).

However, while melting the ice is a necessary process for the safety and convenience of people, it creates runoff with a high degree of salinity from the sodium chloride. The amount of runoff is so large that, in 2013, it had an estimated effect on the drinking water of 90% of Baltimore County (Advisory Commission on Environmental Quality, 2013). This can have damaging effects on a number of different organisms and processes, and it is important to consider and investigate long-term impacts that this behavior could have on the health of the soil and plants that receive the runoff. (Advisory Commission on Environmental Quality, 2013).

The potential impact of different levels of salinity on soil fungi has been explored already, but the findings have not been conclusive. For example, in an experiment by Piloto (1997), fungi were grown in two different environments, one with sodium chloride and one without. The one with sodium chloride had more branching hyphae, many cells, and a differently structured cell wall, while the one deprived of it grew thin, without bulbous cells. A similar experiment by Singh et al (2013), though, had different results. In their experiment, as the fungi begin to receive excess sodium chloride, they began to have less enzyme activity. Since enzymes are responsible for starting and stopping chemical reactions, which ultimately control the four tasks that a cell performs in its life they are crucial to the health of an organism. Therefore, while Piloto (1997) would seem to show sodium chloride is good for fungi, Sing et al (2013) seems to show that sodium chloride threatens the fungi's survival (Malloch, 2013).

Because of the extreme impact that soil fungi have on plants, we decided to do an experiment that will allow ability to determine the actual effects that salinity has on fungi. If the hypothesis of this experiment is correct, many of the mycorrhizal fungi and the plants it has relationships with will die, and the saprotrophic fungi will be suddenly presented with an excess food source from all of the dead matter (AFTOL, 2005). We predict that this would cause a rise in the density of the saprotrophic fungi microbes and a decrease in the density of the mycorrhizal fungi microbes. The results will be able to determine whether this hypothesis is incorrect or correct, giving us options of

how to further act on this potentially threatening issue; it will allow for future modification possibilities to melting ice and will give people options of reasonable actions to participate in to lessen dangerous impact on the earth's cycles, processes, and general ecosystem.

# II. Experiment

**Problem:** Does the addition of road salt to soil alter the density of Mycorrhizal and Saprotrophic fungi in the soil?

**Hypothesis**: Increasing the salinity in the soil will decrease the density of Mycorrhizal fungi and increase the density of saprotrophic fungi.

**Independent Variable:** Pouring sodium chloride salt on the soil plots

**Dependent Variable:** Amount of mycorrhizal and saprotrophic fungi in 1 cc of soil and the chloride levels in the soil

**Negative Control:** No salt on the soil plots

**Positive control:** The density of fungi microbes in 1 cc of soil before adding the independent variable

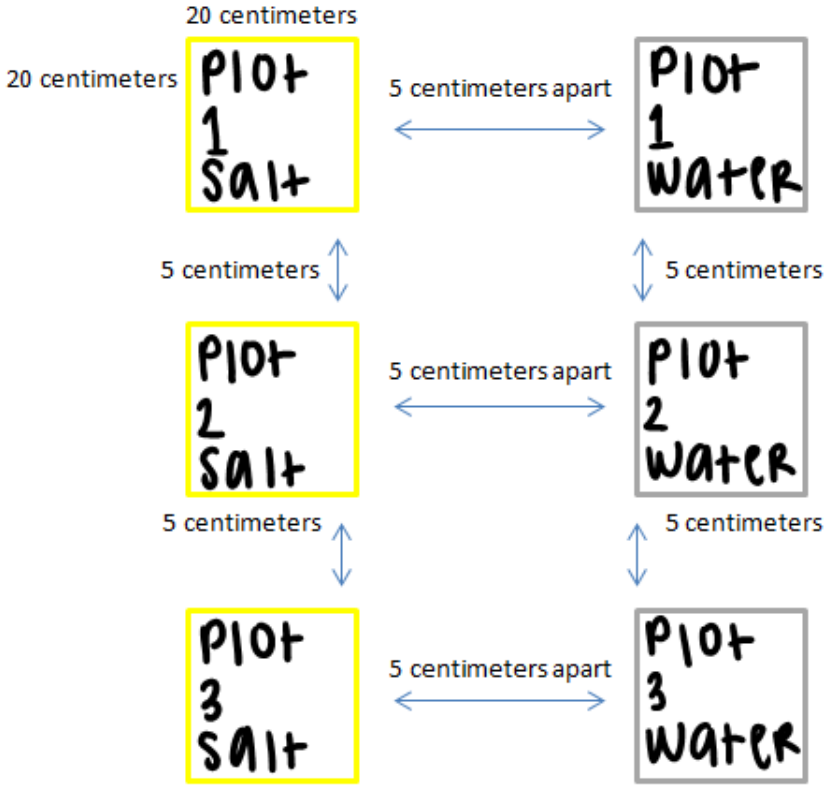
**Controlled Variables:**

Location of soil, size of plot, distance of plots from each other, how plot is marked, materials used, how to record location, method of testing chloride, number of grams of salt put on the independent variable plots, method of mimicking road salt, amount of time left in environment, time allotted for fungus to grow, how much the soil is diluted, amount of samples taken for fungus dilution, equation used, amount of water, amount the soil is mixed, type of extracting d, amount put on nutrient agar plates, type of nutrient agar plates, amount of nutrient which dilutions plated

**Step by Step:**

**Getting Soil:**

1. At N 39.35706 degrees and W 76.6305 degrees, locate six 20 centimeters by 20 centimeters squares of grass 5 centimeters apart. Label one “Plot 1 salt”, one “Plot 1 water”, one “Plot 2 salt”, one “Plot 2 water”, one “Plot 3 salt”, and one “Plot 3 water.” Use yellow flags to mark the salt plots and white flags to mark the water plots. (See diagram)
2. All samples of soil need to be taken at the same time on the same day from all 6 plots. Use a soil core extractor to take three separate 14.5 cm deep and 2.5 cm width samples from each square plot of grass. Each sample should be taken 2 centimeters apart. Place each sample in a separate plastic bag. Make sure bag is labeled with the corresponding plot number (1,2, or 3), plot type (salt or water), sample number (1,2, or 3), and “before the addition of the solution/water.”




3. Serial dilution and chloride tests have to be done on the same day at the same time for all samples. (for example, all #1's from each plot need to be done)

4. Do serial dilution for all plots following serial dilution instructions below. Sample 1 from each plot needs to be done on the same day at the same time. Sample 2 from each plot needs to be done on the same day at the same time. Sample 3 from each plot needs to be done on the same day at the same time.

5. Do chloride test for all plots using the Model STH-14 Outfit (Code 50150). Modification: Use 10 mL of demineralized water instead of 5 mL. Multiply results by 2 because of the doubled amount of the demineralized water. Sample 1 from each plot needs to be done on the same day at the same time. Sample 2 from each plot needs to be done on the same day at the same time. Sample 3 from each plot needs to be done on the same day at the same time.

6. Record amount of each type of fungi in data table.

7. Record results in data table.

8. Pour 70 grams of salt on each of the “salt” plots.

9. Allow plots to be rained on (each plot will receive the same amount of water)

10. 96 hours later, use a soil core extractor to take three separate 14.5 cm deep and 2.5 cm width sample from each plot on the same day at the same time. Each sample should be taken 2 centimeters apart. Place each sample in a separate plastic bag. Make sure bag is labeled with the corresponding plot number (1,2, or 3), plot type (salt or water), sample number (1,2, or 3), and “after the addition of the solution/water.” All samples need to be extracted on the same day at the same time.

11. Repeat step 3-7 for each sample collected from each plot.

**Serial Dilution for Fungus:**

1. Take a clean pipette and add 10 mL of sterile water to a 15 mL culture tube. Label the culture tube “$10^{0}$” and the corresponding plot number (for example, plot 1 salt #1).

2. Use the pipette (same one from the first step) to add 9 mL of sterile water to another 15 mL culture tube. Label this culture tube “$10^{-1}$” and the corresponding plot number (for example, plot 1 salt #1).

3. Use the same pipette to add 9 mL to another 15 mL culture tube labeling the new culture tube “$10^{-2}$” and the corresponding plot number (for example, plot 1 salt #1).

4. Put 1 cc of the corresponding soil sample into the culture tube labeled “$10^{0}$” (for example, plot 1 salt #1).

5. Cap the culture tube and shake forcefully.

6. Using a clean and different pipette, take out 1 mL of the soil/water mixture from the "$10^{0}$" culture tube and put it into the culture tube labeled "$10^{-1}$."

7. Cap the culture tube and shake forcefully.

8. Using the same pipette from step 6, take out 1 mL of the soil/water mixture from the "$10^{-1}$" culture tube and put it into the culture tube labeled "$10^{-2}$."

9. Cap the culture tube and shake forcefully.

10. Plate 100 µl samples from each of the culture tubes onto its own separate pieces of 3M Petrifilm™ Yeast and Mold Count Plate nutrient agar papers labeled with the corresponding plot number, sample number, salt or water plot, and before or after (for example, plot 1 salt #1).

11. Let fungi grow for 72 hours on nutrient agar papers.

12. Examine each of the pieces of nutrient agar papers for separate saprotrophic (yeast) and mycorrhizal (mold) fungi colonies and choose the most diluted plate which has the type of fungi present (if one type of fungi is not present on the plate that the other is on, that is okay, just move to the next plate and find that type of fungi you are looking for) to estimate the amount of fungi in 1 cc soil sample. Use this equation:

# microbes in 1 cc of soil = # colonies on sheet x $10^{2}$ x $10^{|\text{dilution \# at which these colonies were found}|}$

**Data Table:**

The Impact of Road Salt on Soil Fungi Density

| **Plot and Condition of Soil** | **Number of Saprotrophic Fungi Microbes in 1 cc of soil** | **Number of Mycorrhizal Fungi Microbes in 1 cc of soil** |
|---|---|---|
| Salt plot 1 before | 30,000 | 20,000 |
| Salt plot 2 before | 40,000 | 30,000 |
| Salt plot 3 before | 30,000 | 50,000 |
| Water plot 1 before | 20,000 | 3,000 |
| Water plot 2 before | 20,000 | 4,000 |
| Water plot 3 before | 2,000 | 3,000 |
| Salt plot 1 after | 10,000 | 20,000 |
| Salt plot 2 after | 6,000 | 10,000 |
| Salt plot 3 after | 6,000 | 7,000 |
| Water plot 1 after | 10,000 | 20,000 |
| Water plot 2 after | 20,000 | 20,000 |
| Water plot 3 after | 20,000 | 40,000 |

Amount of Chloride in Plots with and without the Impact of Road Salt

| **Plot and Condition of Soil** | **Chloride Levels in Parts per Million (ppm)** |
|---|---|
| Salt plot 1 before | 100 |
| Salt plot 2 before | 200 |
| Salt plot 3 before | 200 |
| Water plot 1 before | 50 |
| Water plot 2 before | 100 |
| Water plot 3 before | 100 |
| Salt plot 1 after | 400 |
| Salt plot 2 after | 1000 |
| Salt plot 3 after | 400 |
| Water plot 1 after | 200 |
| Water plot 2 after | 100 |
| Water plot 3 after | 100 |

Chloride Average Table:

Averages of Amount of Chloride in Salt and Water Plots

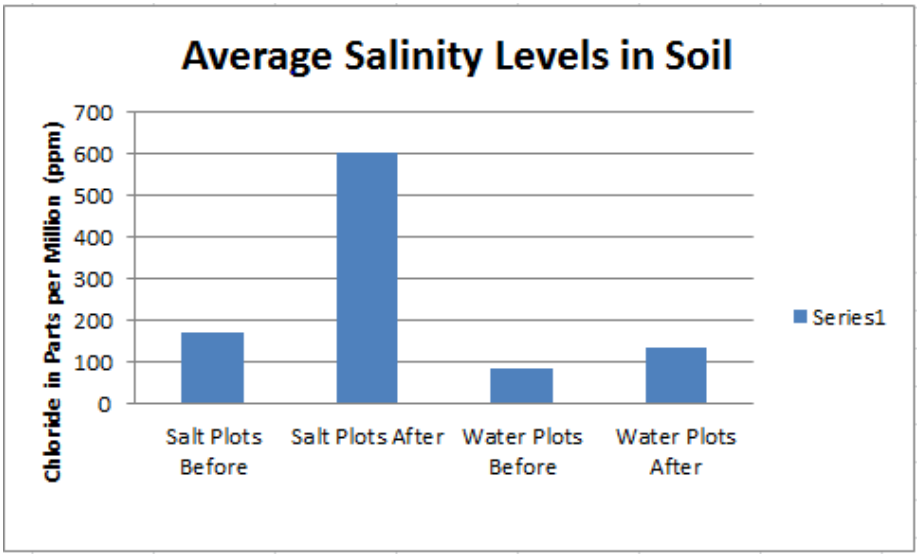
| **Condition of Soil** | **Soil Chloride Levels in Parts per Million (ppm)** |
|---|---|
| Salt plots before | 166.667 |
| Salt plots after | 600 |
| Water plots before | 83.333 |
| Water plots after | 133.333 |

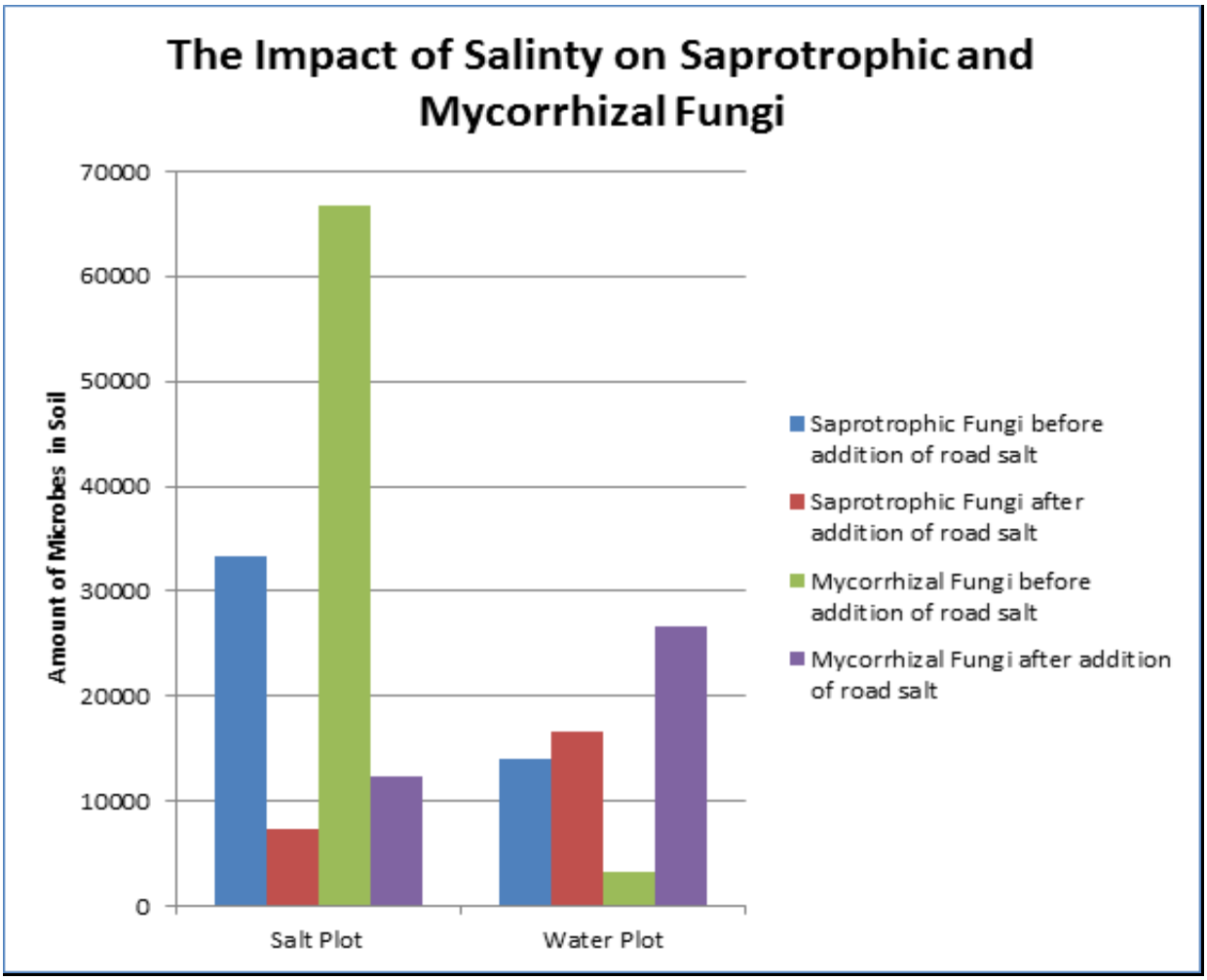
Average Soil Fungi Density Table:

Averages of Soil Fungi Density

| **Plot and Condition of Soil** | **Number of Saprotrophic Fungi Microbes in 1 cc of soil** | **Number of Mycorrhizal Fungi Microbes in 1 cc of soil** |
|---|---|---|
| Salt plot before | 33,333.333 | 66,666.666 |
| Salt plot after | 7,333.333 | 12,333.333 |
| Water plot before | 14,000 | 3,333.333 |
| Water plot after | 16,666.666 | 26,666.666 |

Graph:






# III. Conclusion

Our hypothesis was disproved. The density of saprotrophic and mycorrhizal fungi decreased after the addition of road salt. Salt impacted both saprotrophic and mycorrhizal fungi but was more harmful to the mycorrhizal fungi. As seen in the graph, the decrease from the mycorrhizal fungi before the addition of salt to after the addition of salt was more dramatic of a decrease than the one for saprotrophic. For saprotrophic fungi, before the addition of road salt the number of these microbes in 1 cc of soil was 33,333.33 and it decreased to 7,333.333 of these microbes. For mycorrhizal fungi, before the addition of road salt, the number of these microbes

in 1 cc of soil was 66,666.666 and it decreased to 12,333.333 of these microbes. We can draw these conclusions because we took a chloride test which demonstrated that we actually increased the salinity of the soil. If the salinity did not increase then our conclusions would not be accurate.

Our data has shown us that the addition of road salt negatively impacts saprotrophic and mycorrhizal fungi, causing both types of fungi to decrease in density. This means that when humans use sodium chloride to melt ice and snow, it damages the fungi microbes in the soil. Each type of fungi contributes to plant life and when there is a decrease in their density the plants struggle to live. The role that saprotrophic fungi play in the environment is the decomposition of dead organic matter. This decomposition releases carbon from the soil and into the atmosphere in the form of carbon dioxide, which plants use in photosynthesis (AFTOL, 2005). If plants cannot complete photosynthesis they cannot get the energy that they need to live. This causes plants to die, and since plants are producers it could cause the whole ecosystem to collapse. As well as saprotrophic fungi, mycorrhizal fungi also have an important role that is crucial to the health of an ecosystem. These soil microbes have a relationship with 90% of vascular land plants and provide them with nutrients, so if the density of mycorrhizal fungi decreases dramatically, the plants would stop receiving the nutrients that allow them to live and could die (Pace, 2003). Since plants are the support for the food chain, killing them could cause a collapse in an ecosystem because all living organisms depend on plants to live. This means that saprotrophic and mycorrhizal fungi have an indirect yet significant impact on the health of an ecosystem and the survival of all organisms.

For future research, we will want to find the amount of sodium chloride road salt that is safe for soil fungi microbes. We would use similar plots but put different amounts of salt onto each plot and then collect the density of both types of fungi to determine which amount is the

safest for soil fungi microbes. If we were to figure out the amount of road salt that is safe for fungi microbes, we can develop a limit of road salt that can be put down on the road. More future research could include testing the sodium chloride road salt on different soil microbes. When testing on different microbes, we would find if other microbes are harmed by road salt and how much road salt impacts each microbe's density. If we were to figure out that other soil microbes are harmed by road salt, this problem will become more dangerous and the amount of road salt used during snow and ice storms will have to be drastically reduced. Future research will allow us to decrease the harm that road salt causes soil fungi microbes and an entire ecosystem.

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