

Effect of Different Concentrations of Road Salt on Earthworm Burrowing

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Abstract:

The purpose for this experiment was to see how environmental factors such as road salt can affect our environment and organisms living in it. To see how road salt affects organisms, we tested how fast worms burrow based on the difference of salinity they are exposed to and for how long they are exposed to the salt water solutions. The setup of the lab consisted of 4 plastic cup burrowing chambers filled half full with potting soil. Placing 2 red earthworms into cups with 0%, 10%, 20%, and 29% salt water concentrations, we timed the worms for 2.5, 5, 10, and 20 minutes. Once the worms finished soaking in the salt water solutions, they were placed into their respective burrowing chambers and timed for the time that each worm was completely burrowed, only cut off after a time of over 20 minutes. The discovery was that the higher the concentration of salt water, the longer it took the 2 worms to burrow compared to their respective control group. However, some worms burrowed faster than their partner worm or tried to climb out of the cup instead of burrowing immediately, causing the results to vary between the predictions. The significance of this experiment shows the devastating effect of road salt on the nervous system of the worms.

Introduction:

The purpose of this experiment is to test the effects of road salt concentrations on earthworm burrowing since road salt is used heavily on Wisconsin's roads. Road salt is spread on roads to melt ice and provide friction for car tires. Runoff from the melted ice that contains road salt increases pollution in drinking water and soil. This is especially a problem in Ohio; the water in a town called Gamden became so salty that it was forced to abandon its wells (Road Salt Pollutes Ohio Drinking Water, 2011). The damage road salt does is largely caused by the

chemicals that give it its ice-melting capabilities. Chloride, one chemical used in road salt, is toxic to aquatic life at levels above 230 mg/ml (“Road Salt and Water Quality” 2016). In addition, a five-year study was conducted and the research group found that road salts are toxic to plants, wildlife, lakes, streams and groundwater at levels higher than 800 ppm. This led the Canadian Environmental Protection Act (CEPA) to add road salt to the list of toxic substances (“Road Salt Blues” 2001). Another invertebrate that has been studied for effects of road salt is the monarch caterpillar. Monarch caterpillars (*Danaus plexippus*) raised on the sodium-boosted plants grew into males with extra thoracic muscle and females with bigger eyes (probably a sign of bigger brains) than butterflies that feed on plants without a sodium-boost. Stronger thoracic muscles could mean males can outfly the competition. A butterfly's brain is about 75 percent devoted to vision, so bigger brains may improve butterfly botanizing (“Road salt reshapes butterfly form” 2014). Overexposure to road salt can have detrimental effects on both human health and the health of earthworms. Earthworms have very moist skin and need to keep it that way to be able to breathe. They will die if their skin dries out because of the hypertonic solution forcing the water out of the worms which they need to keep their skin moist so they can breathe. Earthworms have such a high sensitivity to salt the overexposure can result in reduced growth and their sensitive skin being destroyed. All this is because the worms do not have control over their osmotic regulation. Like earthworms, overexposure to road salt also negatively affects human health. Overexposure raises the amount of sodium in the blood which alters the fragile balance of sodium and potassium affecting the ability of the kidney to remove water. (Jenner, Katharine. “Salt and the kidneys.” *Consensus Action on Salt And Health*) Because less water has been removed it results in higher blood pressure which causes strain on delicate blood vessels resulting in possible heart attacks, strokes, and kidney disease (“Why salt is bad” 2008).

Earthworms are important since their burrows create pores through which oxygen and water can enter and carbon dioxide can leave the soil ("Earthworm functions" 2017). Also, earthworms are responsible for mixing soil layers and incorporating organic matter into the soil. This mixing improves the fertility of the soil by allowing the organic matter to be dispersed and the nutrients held in it to become available to bacteria, fungi and plants to use. We are testing to discover the different effects of road salt concentrations on earthworm burrowing. The salt on the worms should be causing a physical change in their locomotion, so we can infer that road salt has a harmful effect on the environment. If a worm is exposed to the highest concentration of a road salt solution, then the worm will take the longest amount of time to burrow because the road salt will alter the ganglia of the worm, leaving it with delayed reactions and paralysis.

Methods:

We ground road salt into powder with a mortar and pestle and mixed it with water to create a saltwater solution. This water will be added to a plastic cup that contains two earthworms to test the effect of road salt on earthworm burrowing. The salinity will steadily increase, with the second round of cups containing the least and fourth round of cups containing the most.

Materials:

- Road salt
- Three 250 mL beakers
- Distilled water
- Mortar and Pestle
- 12 Plastic cups
- Filter paper
- Timer
- Potting soil
- Trout worms

- Tweezers
- Scale
- Stirring rod
- Parafilm

Procedure for Creating Salt Water Solutions:

1. To create salt water solution, fill a beaker with 250 mL of distilled water.
2. Use a mortar and pestle to break up rock salt into a fine powder dust.
3. Start by adding 2.5 grams of the salt powder to the beaker full of water and stir til dissolved.
4. Cover beaker with parafilm and set aside
5. To calculate salinity: $\text{salt mass}/(\text{salt mass} + \text{water mass}) * 1000 = \text{salinity}$
6. Repeat steps 1-5 with 5 grams and 7.5 grams of rock salt powder.

Procedure for Testing Earthworms:

1. To prepare the exposure chambers, take one plastic cup for each chamber's exposure time and desired solution and place a piece of filter paper within it
2. Fill each exposure chamber with about 1 cm of the solution that is being tested
3. For Control Group, add two worms to the separate exposure chambers containing just water and time it for 2.5, 5, 10, and 20 minutes
4. Add another piece of filter paper on top of the worm
5. Place another cup over the worm filled cup to keep worm covered
6. Repeat steps 1-4 with the 2.5 gram, 5 gram and 7.5 gram concentrations.
7. To create the burrowing chamber, place enough potting soil to halfway fill plastic cup.
8. Once the worms have been exposed to their respected solutions, place the worms into the burrowing chamber
9. Record the amount of time it takes each of the worms to become completely buried (20 minutes max burrowing time allowed for worms)
10. Repeat steps 7-9 for the other experimental worms.

Salinity of Cups:

Cups In Group 1 (Control): distilled water (0% salinity)

Cups In Group 2 (Experimental): 10% salinity

Cups In Group 3 (Experimental): 20% salinity

Cups In Group 4 (Experimental): 29% salinity

Data Tables and Graphs:

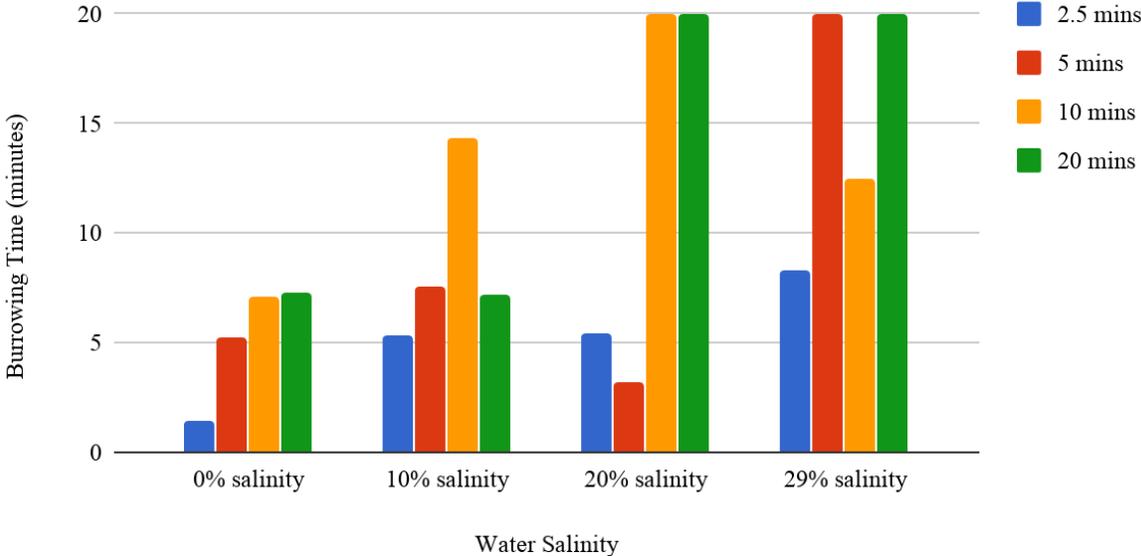
Time It Takes Earthworms To Burrow In Soil After Exposure to Road Salt Concentrations

Water Quality	Exposure Time	Exposure Time	Exposure Time	Exposure Time
	2.5 minutes	5 minutes	10 minutes	20 minutes
Distilled Water	Worm 1: 1 minute 41 seconds Worm 2: 1 minute 41 seconds Average: 1 minute 41 seconds	Worm 1: 5 minutes 25 seconds Worm 2: 5 minutes 25 seconds Average: 5 minutes 25 seconds	Worm 1: 7 minutes 13 seconds Worm 2: 7 minutes 13 seconds Average: 7 minutes 13 seconds	Worm 1: 7 minutes 4 seconds Worm 2: 7 minutes 50 seconds Average: 7 minutes 27 seconds
10% Salinity	Worm 1: 4 minutes 3 seconds Worm 2: 6 minutes 55 seconds Average: 5 minutes 29 seconds	Worm 1: 5 minutes 5 seconds Worm 2: 10 minutes 1 second Average: 7 minutes 53 seconds	Worm 1: 8 minutes 54 seconds Worm 2: 19 minutes 36 seconds Average: 14 minutes 35 seconds	Worm 1: 7 minutes 0 seconds Worm 2: 7 minutes 40 seconds Average: 7 minutes 20 seconds
20% Salinity	Worm 1: 3 minutes 52 seconds Worm 2: 7 minutes 38 seconds Average: 5 minutes 45 seconds	Worm 1: 2 minutes 0 seconds Worm 2: 3 minutes 54 seconds Average: 3 minutes 17 seconds	Worm 1: 20+ minutes Worm 2: 20+ minutes Average: 20+ minutes	Worm 1: 20+ minutes Worm 2: 20+ minutes Average: 20+ minutes
29% Salinity	Worm 1: 7 minutes 30 seconds	Worm 1: 20+ minutes	Worm 1: 4 minutes 10 seconds	Worm 1: 20+ minutes

	Worm 2: 8 minutes 46 seconds Average: 8 minutes 28 seconds	Worm 2: 20+ minutes Average: 20+ minutes	Worm 2: 20+ minutes Average: 12 minutes 5 seconds	Worm 2: 20+ minutes Average: 20+ minutes
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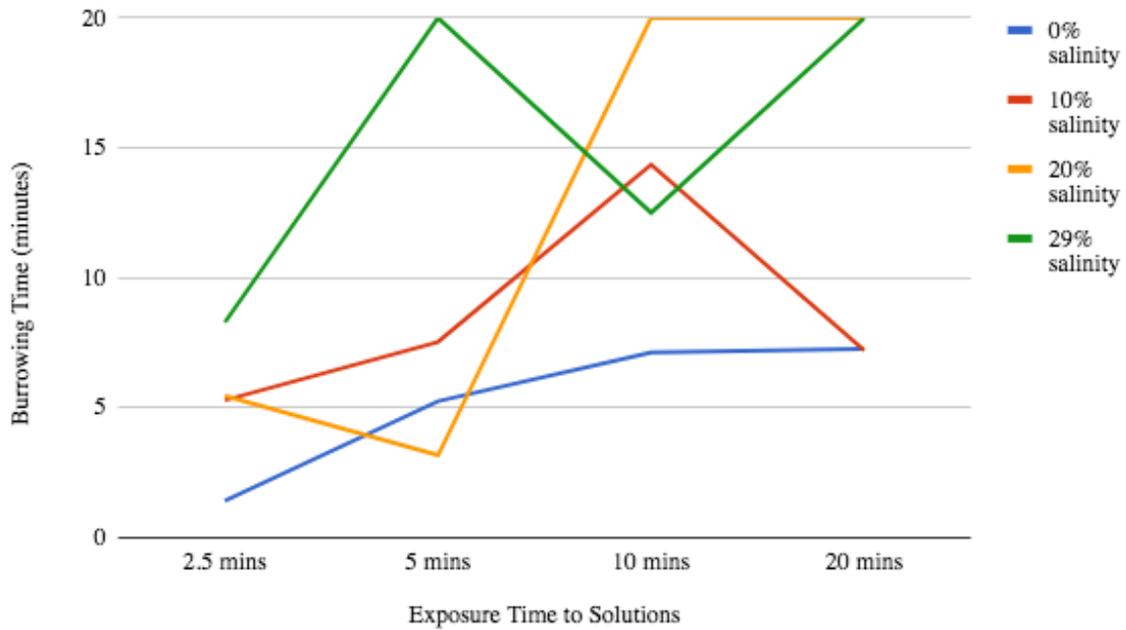
Graph 1

Average Time Taken to Burrow by Earthworms Exposed to Different Concentrations of Salt Water



Graph 2

Averages of Earthworm Burrowing Time Compared to Salinity



Observations:

	2.5 minutes	5 minutes	10 minutes	20 minutes
Control Group: 0% salinity	The worms were alert and burrowed quickly.	The worms were alert and burrowed quickly.	The worms were alert and burrowed quickly.	The worms were alert and burrowed quickly.
Group 2: 10% salinity	The worms were alert, (slightly frantic) and started burrowing after crawling a bit.	The worms started crawling around the cup more before they started burrowing and speed was showing signs of slowing.	The second worm tried to climb out of the cup multiple times.	Worms were very slow and seemed to be stunned and slightly unresponsive.
Group 3: 20% salinity	Both worms were quite responsive but only worm was	Both worms were very active and started to burrow after they	When the worms would start burrowing they would head to	Worms were unresponsive to burrowing. Toward end of

	more active toward burrowing while the other tired to climb up the side of the cup.	had crawled to the side of the cup.	the edge of the cup instead of starting in the middle.	time, worms began to slowly move.
Group 4: 29% salinity	Worms moved fairly well but their speed was shown to be affected and motion was slowed.	Both worms took 20+ minutes to burrow and were extremely unresponsive.	Both worms were resistant to move at first but after a couple minutes one began to burrow quickly and the other took 20+ minutes.	Worms were possibly dead, and there was no movement after placed in cup.

Results:

From this experiment we can see how salt affects earthworm burrowing. The independent variable of this experiment was the varied amounts of salinity in the water each worm was placed in and how long we kept each worm in such salinity. The dependent variable was the earthworms burrowing speed, while the controlled variable was the amount of earthworms. In graph 1, the salinities were the same in each group, but the exposure times changed. At 0%, the data shows that the water had little effect on the burrowing times since the exposure time and burrowing times were similar. At the 10% salinity, the solutions also had a very minimal effect on the earthworm's burrowing, besides the 10 minute outlier. However, at 20% the burrowing time was greatly influenced. At 10 minutes in the exposure chamber in the 20% salinity solution, the burrow time increased to over 20 minutes. Also, the 29% salinity solution yielded a dramatic increase in burrowing time compared to exposure time. The worms only lied on the dirt, without burrowing for over 20 minutes. Graph 2 compared the overall salinities to each other. 0% salinity had the lowest overall burrowing time, while 29% salinity had the overall highest burrowing

times. The last worms tested in the 20% salinity and 29% salinity took over 20 minutes to burrow.

Figure 1

0% salinity for 2.5 minutes



Fastest worms to burrow compared to all other data points.

Figure 2

29% salinity for 10 minute exposure time



Took over less time than both 5 minutes and 20 minutes, it is an outlier.

Figure 3

20% salinity for 20 minutes exposure time



Took over 20 minutes for worms to burrow.

Figure 4

10% salinity for 15 minutes exposure time



Delayed burrowing time compared to other tested worms and outlier of the data.

Discussion:

Our data confirms the hypothesis; high concentrations of road salt have more effect on a worm's nervous system compared to the lower concentrations of salt. For example when worms were exposed to 20% salinity solutions for 10 and 20 minutes and 29% solutions for 5 and 20 minutes, the worms took over 20 minutes to burrow in each case (Graph 1). The main outlying points are the 10% salinity for 10 minutes and 29% salinity for 10 minutes (Graph 1). The reason

for the abnormally long time it took the worms to burrow in 10% salinity with a 10 minute exposure time was the second worm in the cup refused to burrow and tried to climb out. Next time this experiment is done, a clear plastic cup should be placed over burrowing worms to prevent escaping earthworms. For 29% salinity for 10 minutes, one worm burrowed quickly while the other layed on the surface of the dirt (Figure 2). One reason this could have been was that one of the worms was exposed to more of the solution compared to the other. Some other limitations to the data were the amount of worms per experiment, amount of time available, and the quality of the supplies used. Suggestions to solve the limitations above include, using more than 2 worms per salinity, only using days with extended class periods to record data, and using clear plastic burrowing cups to see the progress of worms in exposure chambers and burrowing chambers. One main reason for this experiment being conducted was to highlight the negative effects road salt causes the environment. For instance, if road salt runoff were to infect a farm field near a road, worms could be killed causing the soil quality to decrease. A soil quality decrease can risk a farmers income, increase local food prices because of lack of fertile farm fields, or just kill off future vegetation that could grow in that area. Furthermore, some governments are taking action against abuse of road salt. Canada has placed road salt as a toxic substance and its usage will be heavily monitored in future winters.

Further question:

What specific earthworm species has the highest tolerance to salt water? The future lab would use the same testing system testing a variety of different species of earthworms and comparing the other worms' results to the red earthworms' results.

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