DATA *Nugget*

Salty sediments? What bacteria have to say about chloride pollution
Featured scientist: Lexi Passante from the University of Wisconsin-Milwaukee

Research Background:

In snowy climates, salt is applied to roads to help keep them safe during the winter. Over time, salt - in the form of **chloride** - accumulates in snowbanks. Once temperatures begin to warm in the spring, the snow melts and carries chloride to freshwater lakes, streams, and rivers. This runoff can quickly increase the salt concentration in a body of water.

Lexi taking water quality measurements at Cedar Creek in Cedarburg, WI.

In large amounts, salt in the water is harmful to aquatic organisms like fish, frogs, and invertebrates. However, there are some species that thrive with lots of salt. Salt-loving bacteria, also known as **halophiles,** grow in extreme salty environments, like the ocean. Unlike other bacteria and organisms that cannot tolerate high salinity, halophiles use the salt in the environment for their day-to-day cellular activities.

Lexi is a freshwater scientist who is interested in learning more about how ecosystems respond to this seasonal surge of chloride in road salts. She thought that there may be enough chloride from the road salt after snowmelt to change the bacteria community living in the sediment. More salt would support halophiles and likely harm the species that cannot tolerate a lot of salt.

By taking a water sample and measuring the chloride concentration, we can see a snapshot in time of how toxic the levels are to organisms. However, the types of bacteria in sediments take a while to change. Halophiles may be able to tell us a long-term story of how aquatic organisms respond to chloride pollution. Lexi's main goal is to use the presence of halophiles as a measure of how much chloride has impacted the health and water quality of river or stream ecosystems. This biological tool could also help cities identify areas that may be getting salted beyond what is necessary to keep roads safe.

Lexi expected that there would be few, or maybe no, halophiles in rural areas where there are not many roads. She also thought halophiles would be widespread in urban

environments where there are many roads. Because salt impacts the streams year after year, she expected that halophiles would become permanent members of the microbial community and increase in winter and spring. Therefore, she also wanted to track whether halophiles remain in the sediment throughout the year, increasing in numbers when salt levels become high.


A sediment sample taken at Honey Creek, in Milwaukee WI.

She began to sample sediments from two different rivers in Southeastern Wisconsin. The urban Kinnickinnic River site is in Milwaukee, WI, and the Menomonee River site is in a rural environment outside of the city. She selected these sites because they offer a good comparison. Because there are more roads, and thus saltier snowmelt, the Kinnickinnic site in the city should have higher chloride concentrations than the Menomonee site.

When visiting her sites throughout the year, Lexi collected multiple water and sediment samples. Every time she visited, she also recorded important water quality characteristics such as pH, conductivity, and temperature of the water. She then brought the samples to the laboratory and analyzed each for its chloride concentration. To measure the quantity of halophiles in the sediment, Lexi used a process where the sediment is shaken in water to separate the bacteria from the sediment and suspend them in the water. Samples from the water were then plated on a growth medium that contained a very high salt concentration. Because the growth medium was so salty, Lexi knew that if bacteria colonies grew on the plate, they would most likely be halophiles because most bacteria do not thrive in salty environments. Lexi counted the number of bacteria colonies that grew on the plates for each sample she had collected.
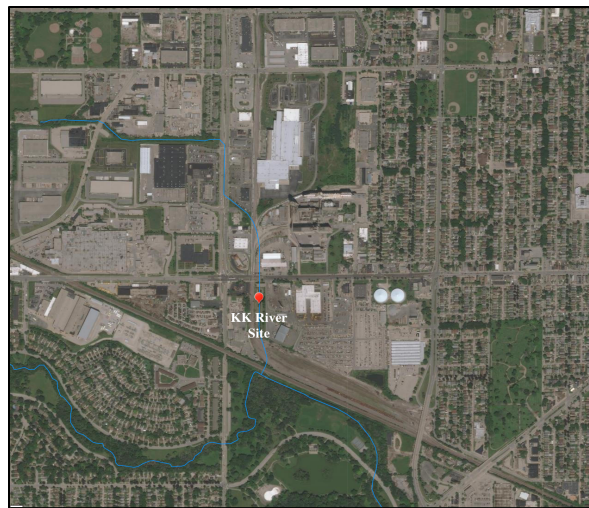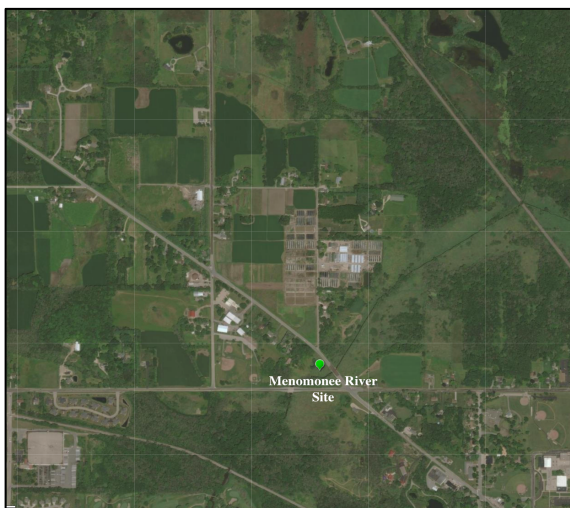
Satellite images of Lexi's field sites: rural Menomonee River Site (left) and urban Kinnickinnic River Site in Milwaukee, (right). Lexi is interested in the halophile communities in these two different environments.
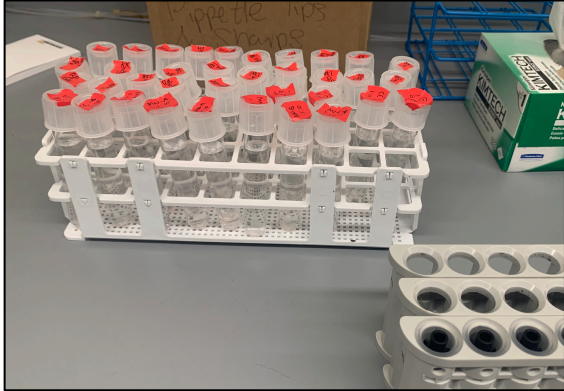
Filtered sediment and water samples ready to be measured for chloride concentration.
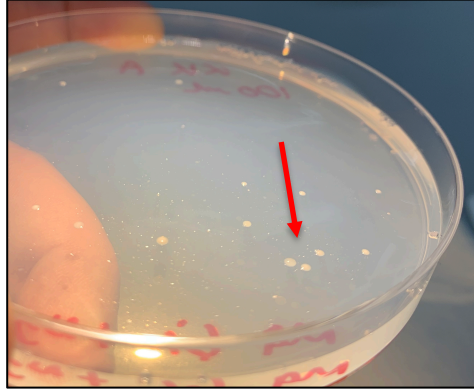
For each sample, Lexi counts the number of bacteria colonies found on salty growth media.

Scientific Question: Are halophiles useful as biological indicators of chloride pollution based on their quantity in urban and rural environments?

Scientific Data:

Use the data below to answer the scientific question:

| Sampling site	Site description	Date sampled	Month	Average chloride concentration (mg/L)	Number of bacteria colonies per gram of sediment			Average Number of bacteria colonies per gram of sediment
					Sample A	Sample B	Sample C	
Kinnickinnic River	urban	5/12/21	Early May	781.9	2210	1720	1690	
Kinnickinnic River	urban	5/26/21	Late May	911.3	1580	1120	1530	
Kinnickinnic River	urban	6/23/21	Late June	595.4	190	160	240	
Kinnickinnic River	urban	7/26/21	Late July	453.2	280	475	270	
Kinnickinnic River	urban	8/31/21	Late August	452.5	65	45	55	
Menomonee River	rural	5/14/21	Early May	150.8	0	3	0	
Menomonee River	rural	5/25/21	Late May	145.4	7	7	10	
Menomonee River	rural	6/22/21	Late June	147.4	73	167	77	
Menomonee River	rural	7/27/21	Late July	125.4	17	13	7	
Menomonee River	rural	9/1/21	Late August	125.0	97	73	63	

What data will you graph to answer the question?

Independent variable(s): _____

_____

Dependent variable(s): _____

_____

Draw your graph(s) below: Identify any changes, trends, or differences you see in your graph(s). Draw arrows pointing out what you see and write one sentence describing what you see next to each arrow.
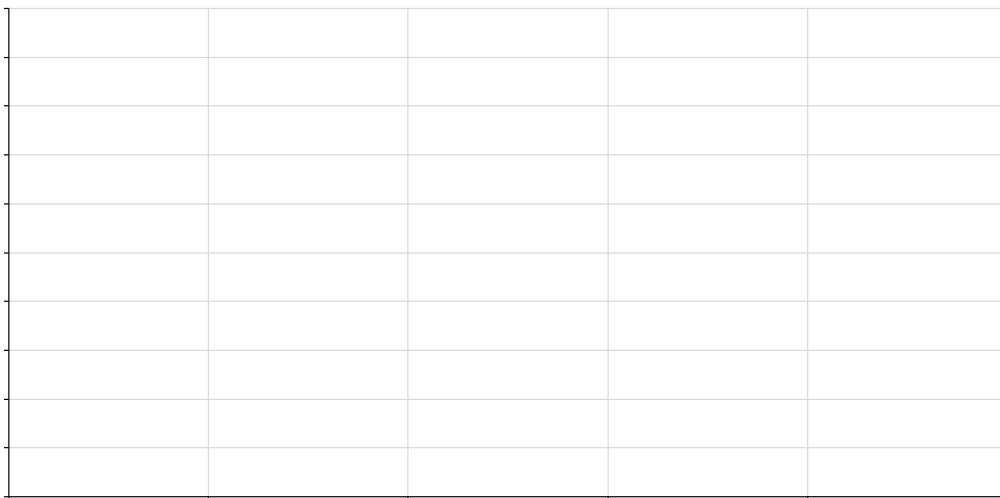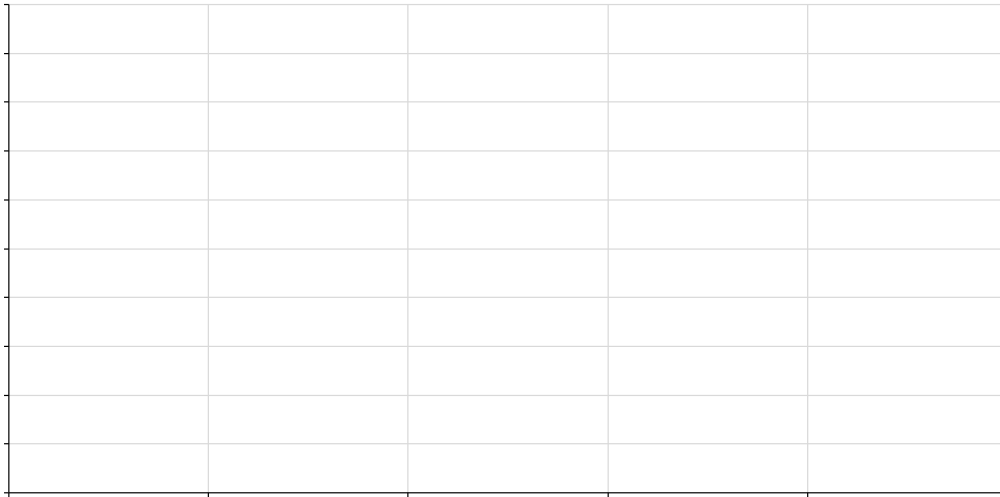
Interpret the data:

Make a claim that answers the scientific question.

What evidence was used to write your claim? Reference specific parts of the table, graph(s), or maps.

Explain your reasoning and why the evidence supports your claim. Connect the data back to what you learned about halophiles and road salt pollution.

Your next steps as a scientist: Science is an ongoing process. What new question(s) should be investigated to build on Lexi's research?

What future data should be collected to answer your question?

        Independent variable(s): _____

        _____

        Dependent variable(s): _____

        _____

For each variable, explain why you included it and how it could be measured.

What hypothesis are you testing in your experiment? A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies.