

Abstract)

Papers in the past have investigated the effects of Lithium on bacterial respiration. However few to none have studied the impacts on microbial colonies as a whole, and fewer still have examined these effects in the frame of Lithium EV batteries. Here we report that the introduction of Cobalt, Nickel, Manganese and Lithium ions into microbial systems had adverse impacts on bacterial growth with Cobalt having the highest effect on bacterial growth. In this experiment, solutions of agar and metal ions (selected from a previous study on battery leaching) at varying concentrations were plated. Microbiome samples taken from Carnegie Lake were incubated in these agar plates for a day. The bacteria was scraped from the plates and their DNA was extracted using a Qiagen DNA Extraction kit. Subsequently, the DNA was replicated through a polymerase chain reaction (PCR). This DNA was then analyzed through the use of an Oxford Nanopore Flongle. Due to the high sensitivity of the Nanopore device, the number of colonies were counted. Knowing the impacts of metals commonly found in lithium batteries can help future assessments on the environmental impacts of technologies such as EV Cars, and storage of power created from renewable sources.

Introduction:

Electric Vehicles, also known as EV's, have been growing in popularity at an exponential rate. [1] Their popularity stems from their touted environmental benefits, attributed to their lack of combustible engines – which eliminates carbon emissions – and use of rechargeable Lithium Ion batteries. Leading brands such as Tesla, BMW, Ford, and more have pivoted towards producing EVs in response to this growing demand. [2] Despite their eco-friendly reputation, their impact on the environment is still substantial. Methods to minimize the negative impact EV cars have need to be further investigated to optimize their usage. After EV Cars have been used, only around 5% of the batteries within them are recycled [3]. If disposed of improperly, metals such as Lithium, Nickel, Manganese, Cobalt, Zinc, and more can leach out of them and make their way into local ecosystems [4]. Limited research has explored the effect of metals from batteries in microbial systems. However, Porter and Bernot have found that increased lithium levels will lower the rate of respiration in microbial systems [5]. In light of these considerations, this study aims to address the gap in literature by investigating the impact of metals from EV Batteries on microbial systems found in river ecosystems. By illuminating the potential ecological consequences of metal leaching from EV batteries, this research seeks to contribute valuable insights towards mitigating the environmental impact of EV technology and influence potential regulation regarding recycling batteries.

Methods:

Water Collection:

A water sample was collected from Carnegie Lake near Princeton, NJ, approximately 20 feet from the shore using a collection bottle. The sample was taken from a pier extending into the lake and immediately capped to prevent contamination. The sample was then taken to the lab and stored in a cold room that maintained a temperature of -4°C until further processing. In order to mimic the conditions of the river microbial system, the water was not treated in any way. Prior to use of the water, the sample bottle was gently shaken in order to ensure the bacteria was uniformly distributed throughout the water.

Plates:

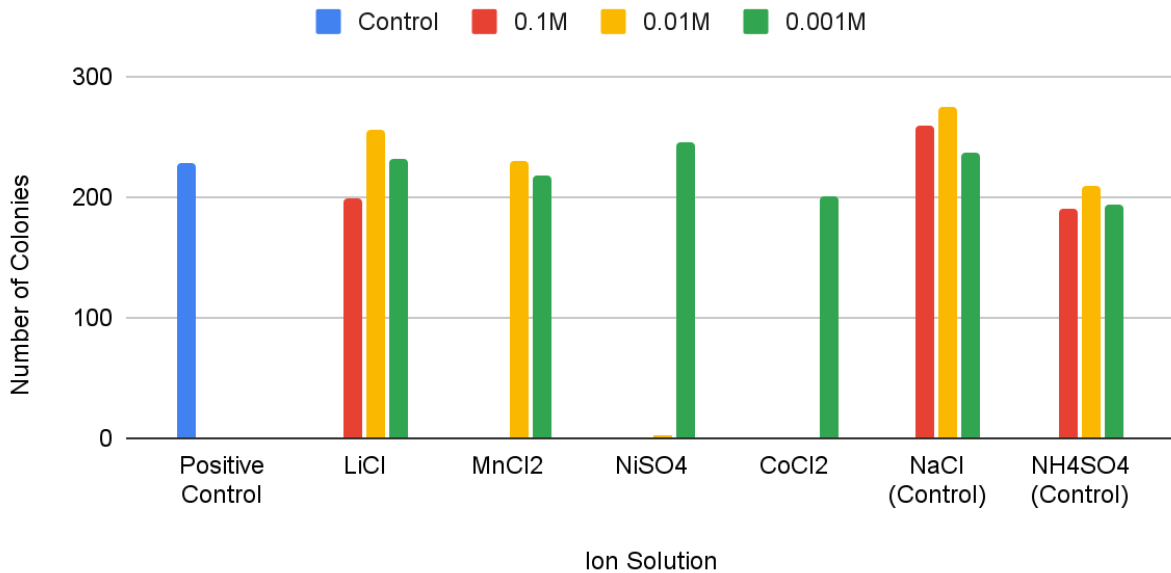
The four metals used in the study were Lithium, Nickel, Cobalt, and Manganese in the forms of LiCl , NiSO_4 , CoCl_2 and MnCl_2 respectively. Controls of NaCl and NH_4SO_4 were used to ensure that the secondary ion in the compound was not responsible for any effects used. Metal ions and agar were mixed at varying levels of concentrations of 0.1M, 0.01M, and 0.001M. The following day, the water samples were plated onto agar plates containing the respective metal concentrations. Following this step, the plated samples were stored in an incubation chamber of approximately 30°C , for three days to allow for bacterial growth.

Bacteria Extraction and Analysis

Samples of the plates were taken and spun down using a QiaGen DNA Extraction Kit. These samples were then analyzed through an Oxford Nanopore. Due to the high sensitivity of the Nanopore device, there were a few inaccuracies with the results (Ex: Nanopore suggested similar levels of microbial diversity for 0.1M Ni and Positive Control, even though 0.1M Ni had 0 colonies present on the plate while the Positive Control had 200+), on the suggestion of the supervising professor, the number of colonies was counted instead.

Data:

Number of Colonies vs Concentration of Ion Solutions for EV Battery Ions



Average Number of Colonies vs Concentration of Ion Solutions for EV Battery Ions

	Control	0.1M	0.01M	0.001M
Positive Control	228	–	–	–
LiCl	–	198	255	232
MnCl ₂	–	0	230	217
NiSO ₄	–	0	2	245
CoCl ₂	–	0	0	200
NaCl (Control)	–	260	275	237
NH ₄ SO ₄ (Control)	–	190	209	193

Discussion:

The results of our study reveal significant differences in the toxic effects of various metals on bacterial growth. The data suggested that Cobalt had the most toxic effect on the bacteria plated, due to a lack of bacteria on both the 0.1M plates and the 0.01M plates. Nickel followed closely, as it had no bacteria on the 0.1M plates, however contained extremely trace amounts of bacteria on the 0.01M plates. Manganese also displayed inhibitory effects, as it had plates that

contained no bacteria at a concentration of 0.1M. Lastly, Lithium had no significant impact on the growth of bacteria.

Interestingly, our analysis suggests neither the SO₄ ions or Cl ions were the cause of this effect, as the control plates containing those ions had no significant difference in the number of colonies present, especially when compared to Nickel, Cobalt, or Manganese. This indicates that the observed toxicity is likely attributed to the metals themselves rather than their associated ions.

In summary, our findings highlight the differential toxicity of metals commonly found in Lithium batteries on microbial systems. Cobalt emerged as the most toxic, followed by Nickel, Manganese, and Lithium. Understanding the relative toxicity of these metals is essential for assessing their environmental impact and implementing appropriate mitigation measures.

Significance:

Given the rise of EV cars, as well as reports of increased levels of Lithium and other metals believed to be from batteries entering river systems, it is important to understand the effects of these changes in order to accurately build proper evaluations of pollution. Micro Bacteria acts as a basal food source for many organisms in river ecosystems, additionally they recycle nutrients and participate in various nature cycles. Metals that harm these bacteria would cause harm to the ecosystem as a whole, preventing the degradation of pollution and killing many organisms that depend on bacteria as a food source. On the other hand, oversaturating an ecosystem with bacteria could cause harm to the river in terms of Eutrophication and algal bloom from the bacteria's ability to breakdown nutrients. Exploration into the long-term ecological consequences of metal contamination in the environment is critical given the rise in EV sales. Future steps in research could involve testing different metals from batteries, taking direct samples from EV manufacturing sites, testing a battery directly in order to observe any potential interactions between the ions, as well as doing further trials to verify the results provided.

Sources:

1. IEA, Global sales and sales market share of electric cars, 2010-2021, IEA, Paris
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4. Mrozik, W., Rajaeifar, M. A., Heidrich, O., & Christensen, P. (2021). Environmental impacts, pollution sources and pathways of spent lithium-ion batteries. *Energy & Environmental Science*, (12). <https://doi.org/10.1039/D1EE00691F>
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