Investigating the Effects of Rare Earth Elements on the Soil Microbial Activity
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Dear Dr. Manderville,

I am a third year biomedical toxicology co-op student and I have just completed my fourth and final co-op term working at Environment and Climate Change Canada as a laboratory student in their Soil Microbial Assessment and Genomics Laboratory. While working there I was under the supervision of Jane Smith.

My job was to assist Jane Smith with her Chemical Management Plan - Rare Earth Element project which looked into the effects that rare earth elements (REE) (Lanthanides) have on the soil microbial activity in boreal Canadian soil. This project is to help fill in the data gaps about the subject since not much research or data exists for this topic. In recent years REE is becoming more prevalent in many industries and therefore is becoming more present in the environment, although naturally occurring in the soil, it appears that there are increases in concentrations. These increases are suspected to be due to the increase in use in different industries (medical, technologies, and agriculture), increased mining and leeching from landfills.

This project involved running tests that gathered information about the microbial activity in soil contaminated with REEs. This included nitrification activity testing, organic matter, pH, and moisture contents.

My work term report will be about the section of the project I assisted with which, is the effect of Yttrium and Neodymium on soil microbial activity in soil.

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Abstract

Rare Earth Elements (REEs) are naturally occurring elements found commonly in the environment. They are used in many different industries including agricultural, biomedical technologies and green technologies. Their prevalence in the environment is increasing as their uses and applications in society have increased and therefore so has the mining of REE and the leaching of REE from landfills (Gonzalez, V. *et al*. 2014). There is not much research into the ecotoxicological effects of REE. Furthermore, of the research that has been conducted there have been conflicting results and several gaps in the data. Due to the lack of knowledge on the effects of the REE on the environment and biological systems, there are not many regulations set for the uses of REE in industry (Gonzalez, V. *et al*. 2014). Increasing levels of REE have shown to affect the microbes in the soil, however the details of the correlation are not very clear (Chao, Y. *et al*. 2016). With the increased presence of REEs in the environment and the limited knowledge and consistency of data on their toxicological effects, research into REE toxicities is essential (Gonzalez, V. *et al*. 2014). Environment and Climate Change Canada have been conducting research to help fill in the knowledge gaps regarding the effects of REE on the environment, more specifically, the effects on soil microbial health. They are accomplishing this using four REEs, Pr, Sm, Y, and Nd, and a variety of tests that assess and quantify their effects on soil. The experiments conducted examining Y and Nd contaminated soil typically found that the chemicals negatively affected the soil microbial health. However, under certain circumstances and conditions the chemicals can be beneficial to the soil.

1.0 Introduction

Rare Earth Elements (REE) are comprised of a metal group, the Lanthanides (La (z=57)-Lu(z=71)), yttrium (Y) and, scandium (Sc) which are naturally occurring in the environment (Gonzalez, V. *et al*. 2014). Although the name suggests they are sparse they are actually common in the environment; the name refers to the quantity in which they are found to occur in (Allison, J.E. *et al.* 2015). Lanthanides have been observed to follow two phenomena. The first being the Oddo Harkins rule, where even atomic numbered elements are more abundant than their neighboring odd atomic numbered elements (Gonzalez, V. *et al*. 2014). The second being lanthanide contraction which is the relationship where ionic radius is indirectly proportional to the atomic number. This is caused by the attraction between 4d electrons and the increasingly stronger pull of electrons to the nucleus as the atomic number increases (Gonzalez, V. *et al*. 2014).

REE are used in several different industries including medical technologies, agricultural purposes, green technologies, and industrial manufacturing (Gonzalez, V. *et al*. 2014). As a result, they are prevalent in the environment from the increased mining of REE, leaching into the soil from landfills, and from applications in agriculture where they are used in fertilizers. Limited research has been conducted to determine the toxicological effects of REE and so far, the results observed have been inconsistent and contradicting. This has resulted in knowledge gaps and caused problems with providing the proper regulations and guidelines for REE in industry. It is hazardous to continue to use them and increase their prevalence in society and the environment without knowing the risks associated with their effects on human and environmental health (Gonzalez, V. *et al*. 2014). Increasing levels of REE have shown to affect the microbes in the soil, however the details of the correlation are not very clear (Chao, Y. *et al*. 2016).

With their increased presence in the environment and the limited knowledge and consistency of data on their toxicological effects, research into REE toxicities and chemical

properties is essential (Gonzalez, V. *et al*. 2014). Environment and Climate Change Canada have been conducting research to help determine the missing information regarding the effects of REE on the environment, more specifically, the effects on soil microbial health. They are accomplishing this using four REEs, Pr, Sm, Y, and Nd, and a variety of tests that assess and quantify their effects on soil. This paper focuses on the experiments conducted using Y and Nd, completed during the four month period of September 2018 to December 2018. These experiments include soil characterization tests, organic matter decomposition, soil nitrification activity testing, and enzyme assays.

2.0 Materials and Methods

2.1 The Soil and Its Preparation

The soil used in the study was from the Jack Pine Forest in the Saskatchewan region. The sampling site of the soil is found in the Boreal Plain Ecozone. The soil was sandy in texture. Once the soil arrived it was sieved through a 2 mm sieve, homogenized, and split into different pails. Subsamples from each pail were taken for the soil pre-emptive tests.

2.2 Soil Preemptive tests

2.2.1 Moisture Content

To determine moisture content, samples of soil were taken and wet weights were recorded before putting them in the oven to dry overnight at 105 ˚C. The next day the dry weight was measured, and the moisture content was calculated.

$$\%\, moisture = \left(\frac{(Wet\ weight - Dry\ Weight)}{Dry\ weight}\right)(100\%)$$

2.2.2 Water Holding Capacity (WHC)

To determine water holding capacity 25 grams of oven dried soil (at 105˚C) was mixed with 25 mL of deionized water and then run through glass funnels with filter papers that had been pre-weighed together. The funnels were then covered with aluminum foil and left to drain for 3 hours. Then, the funnel, soil, and filter paper were weighed together, and the weight was recorded.

$$Water\ Holding\ Capacity = (\frac{Final\ Weight - Initial\ Weight}{Dry\ Weight\ of\ soil})(100\%)$$

2.2.3 pH

For each sample, 8.00 g ± 0.01 g of soil was weighed out into 50mL beakers. Forty milliliters of a 0.01 M calcium chloride buffer was added to each sample and then the mixtures were stirred intermittently for 30 minutes then left to sit undisturbed for 1 hour before the pH was measured using Fisher Scientific AR20 pH meter. Each treatment had three replicates.

2.2.4 Conductivity

For each sample, 8.00 g ± 0.01 g of soil was mixed with 40 mL of ultrapure water in a 50 mL beaker. The samples were intermittently stirred for 30 minutes using a glass rod, then left undisturbed for 1 hour before having their conductivities measured and using Fisher Scientific AR20 conductivity meter. Each treatment had three replicates.

2.2.5 Preemptive mixing

Several different percentages of water holding capacities (WHC) were investigated to determine the conditions the soil would be most optimal to work with during the experiments. The amount of water that needed to be added to 200 grams of the soil to attain different desired percentages of water holding capacity was calculated using the predetermined water holding capacity and moisture content. The % of WHCs that were tested were 30, 35, 40, 45, 50, 55, 60. The calculated water to add to attain the % of WHC was added to 200 g of soil in a large steel mixing bowl and mixed until homogenized. The soil was observed to determine the optimal WHC.

2.3 Spiking

The chemicals Neodymium (III) Chloride Hexahydrate (Nd) (200 mg/mL) and Yttrium (III) chloride hexahydrate (Y) (200 mg/mL) were added to the soil to give the 8 experiment treatments, leached Reference (0 ppm), unleached reference (0 ppm), Nd 1493 (1493 ppm), Nd 4571 (4571 ppm), Nd 8000 (8000 ppm), Y 1493 (1493 ppm), Y 4571 (4571 ppm), and Y 8000 (8000 ppm). Each treatment was mixed for 5 minutes. After mixing was complete, subsamples

were set aside for further analysis by Maxxam Analytics (Ottawa, ON) and pH, conductivity, and moisture content experiments. All tests were performed as described above. Conductivity and pH were measured before and after leaching to determine if and how the leaching process affected either one. Once the soil was chemically spiked, the bowls were weighed, covered, and put into the incubator to sit for a week. During the incubation week, moisture contents of the bowls were determined, and the pH and conductivity were tested using the sub samples that were set aside.

2.4 Leaching

After one week in the incubator the bowls were removed and rehydrated to their original weights. Then, the soil treatments were placed into large Nalgene bottles lined with wire mesh and holes in the bottom of the containers. Tubes were hooked up into a peristaltic pump that was set up to have a water flow of 6mL per minute. Figure 1 shows the leaching set-up. The soil was leached 8 hours per Kg of soil and afterwards the soil was left to drain for 30 minutes before putting the soil out to dry. The leachate was collected, volumes were recorded, and observations were noted. Once the soil was dry enough to work with, the moisture contents were determined, and they were rehydrated to experimental conditions. After the soil had been hydrated, the treatments were aliquoted for organic matter decomposition testing, pH, conductivity, soil nitrification activity testing, Maxxam Analytics (Ottawa, ON), and enzyme assay experiments.

Figure 1: Leaching Set Up

2.5 Soil Nitrification Activity Testing

2.5.1 Nitrification Set up

The soil treatments were manually mixed with alfalfa meal at a rate of five mg of alfalfa meal for one gram of soil with a spoon for five minutes. The soil in each beaker was then separated into three replicates in Nalgene bottles and put into the incubator.

2.5.2 Nitrification Collections

For all of the collections, the Nalgene bottles were rehydrated and then 7.90 grams from each bottle was weighed into a 50mL falcon tube and then 35 mL of 0.1 M potassium chloride (KCl) buffer solution was added to each. Then the falcon tubes were put into the shaking incubator for one hour at 20 ˚C and 150 RPM. After shaking, the samples were filtered through a Whatman 41 filter paper using a vacuum pump and 50 mL tube top filters. The filtrate was put aside in the freezer (-20 ˚C) to be sent for analysis at Caducean Environmental Labs (Ottawa, ON) for levels of nitrite, nitrate, and nitrate and nitrite. Nitrification collections were performed every two weeks for a total of four collections on day 0, day 14, day 28, and day 42.

2.6 Enzyme Assays leucine aminopeptidase and β-1,4-glucosidase

For each sample 1.13 grams of soil was weighed into Nalgene bottles that contained 15-20 glass beads (for homogenization). Each treatment had 3 replicates. Then, 125 ml of a 10 µM sodium phosphate buffer solution was added to each Nalgene bottle. The bottles were then put into the shaker for 24 minutes at 20 ˚C at 150 RPM. After the soil samples were finished being homogenized, they were poured into 1 L plastic beakers and put on a stir plate. The soil samples were then plated into black 96 well plates in their appropriate wells, pipetting from the plastic beaker. The phosphate buffer was also pipetted into the plates. After, the standard, either 4-methylumbelliferone (MUB) for β-1,4-glucosidase (BG) or 7-amino-4-methylcoumarin (MC) for leucine aminopeptidase (LAP), was added to the appropriate plates and wells. And finally, the substrate, LAP or BG, was added to the appropriate plates. The time the substrate was added to each plate was recorded and after the addition, the plates were covered and put in the incubator

to sit. The incubation time for the BG plates was 2 hours and for the LAP plates was 20 hours. After two hours, the BG plates were taken out of the incubator and 10 µM of NaOH was added to each well to stop the reaction. The optical density (OD) was measured using a fluorometer at fluorescence intensities of 300 over 40 and 400 over 40. The enzyme assays were completed every other week for eight weeks.

The assay fluorescence (F_A) was the OD from the soil and substrate (columns 5,6,8,9,11,12). Negative Control (NC) was the mean OD of the buffer and substrate (column 3). The sample blank (SB) was the OD obtained from the buffer and soil plate. The quench (Q) is the mean fluorescence of the soil and standards (column 4,7,10). The reference standard (R_S) is the mean of the buffer and standard (column 2). The soil weight (W_S) is the ratio of dry soil weight per wet soil weight. These were used to determine the enzyme activity measured in fluorescence emission.

$$Quench\ Coefficient\ (Qc) = \frac{Q}{R_S}$$

$$Emmision\ Coefficient\ (Ec) = \frac{R_S}{0.5\ nmol\ per\ well}$$

$$Net\ Fluorescence(N_{Fluoro}) = \frac{F_A - SB}{Qc - NC}$$

$$Enzyme\ Activity\ _{Fluoro} = \frac{N_{Fluoro} * Volume\ of\ sample}{Ec * Volume\ of\ well * Incubation\ Time * W_S}$$

96 Well Plate Overview

	1	2	3	4	5	6	7	8	9	10	11	12
A	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
B	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
C	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
D	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
E	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
F	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
G	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP
H	250µL buffer	200µL buffer + 50µL MUB/MC	200µL buffer + 50µL BG/LAP	200µL Soil R1 + 50µL MUB/MC	200µL Soil R1+ 50µL BG/LAP	200µL Soil R1 + 50µL BG/LAP	200µL Soil R2 + 50µL MUB/MC	200µL Soil R2+ 50µL BG/LAP	200µL Soil R2 + 50µL BG/LAP	200µL Soil R3 + 50µL MUB/MC	200µL Soil R3 + 50µL BG/LAP	200µL Soil R3 + 50µL BG/LAP

Figure 2: Enzyme Assay Plate Overview

2.7 Soil Organic Matter Testing:

2.7.1 Organic Matter Decomposition Set Up

Soil from each treatment was separated into 25 falcon tubes (5 replicates and 5 take downs). For each sample, 11.3 g ± 0.02 g of soil was weighed into each of the falcon tubes and then flattened on the top, then a pre weighed filter paper was placed on top of the flattened soil and then another 11.3 ± 0.02 g of soil was weighed on top of the filter paper. A water blank was set up for each takedown as well. The final weights of the falcon tubes were recorded for rehydration purposes. The falcon tubes were then placed in the incubator and soil was rehydrated weekly.

2.7.2 Organic Matter Takedowns

For each takedown, five replicates of each treatment were taken out of the incubator as well as the water blank for the specific time point. For each tube, the contents were emptied into a

dish and the filter paper was removed from the soil pile and all of the soil particles were removed using brushes and purified water. Once the filter paper was clean it was placed in a pre-weighed aluminum weigh boat. The boats were placed in the oven at 105˚C to dry overnight. Soil from the first 3 replicates of each treatment was sampled for moisture content. These samples were also placed in pre-weighed aluminum boats and then put in the oven to dry overnight. The following day the samples of filter paper and soil for moisture content were removed from the oven and placed in the desiccator for 20 minutes before having their final weights recorded. Takedowns occurred every 4 weeks. The calculation as follows:

$$\%\ Mass\ Loss = \left(\frac{Initial\ Filter\ Paper\ Weight - Final\ Filter\ Paper\ Weight}{Inital\ Filter\ Paper\ Weight}\right)(100\%)$$

3.0 Results and Discussion

3.1 Soil condition

It was determined through incremental addition of water to soil that the optimal % WHC for the soil to work with during the experiments was 45%. The soil resembled the desired ground beef texture at this WHC. The average moisture content that all of the treatments were maintained at throughout the experiments was 12.9% which corresponded to the optimal WHC. It was important to keep the moisture content consistent throughout the experiments so any change in microbial activity would not be due to moisture loss.

Table 1: Average pH measurements and conductivities of the treatments at different time points throughout the experiments

Treatment	Average pH			Average Conductivity (units)		
	Before leaching	After Leaching	Week 8	Before leaching	After Leaching	Week 8
Reference	6.56	6.13	6.40	11	8.36	17.4
Nd 1493	6.28	6.22	6.20	261	20.4	16.9
Nd 4571	5.58	5.70	5.62	787	41.4	39.3
Nd 8000	5.38	5.60	5.50	1313	93.9	90.5
Y 1493	6.07	6.18	6.18	319	23.2	21.9
Y 4571	5.49	5.62	5.54	927	41.0	39.2
Y 8000	5.35	5.58	5.47	1550	79.7	76.2
Reference Unleached	6.68	6.52	6.42	10	11.3	22.4

3.2 pH

The pH was tested because the pH affects the soil microbial activity. The pH remained relatively constant before and after leaching and throughout the experimental period of 8 weeks for each treatment (Table 1). When comparing the pHs within a treatment at the different time points, the pHs all were within 1.5 of each other. The reference treatments consistently had pHs above 6. The contaminated soils with the lowest concentrations (Nd 1493 and Y 1493) had the highest pHs. It was observed that the higher the concentration of chemical (Nd or Y) the lower the pH. Nd treatments had a slightly higher pH than the Y treatments of the same concentration at all time points. A more acidic environment is less favorable for microbial communities; therefore, it is possible that differences in microbial activity could be attributed to differences in pH.

3.3 Conductivity

The conductivities of all of the treatments were measured before and after leaching to determine how the leaching process affected the ion content. The conductivity measurements were used as an indicator for the amount of ions lost from leaching the soil, specifically the chloride ions, following the assumption that the change in conductivity is due to losses of chloride ions from leaching. The purpose of the leaching process was to purge the soil of the chloride ions that were bound to the chemicals, Y or Nd, when they were added to the soil. It had been suggested that the chloride ion could have been interfering with and disrupting the validity of the results in previous un-leached tests. The reference soils had much lower conductivities than the treated soils before leaching (Table 1). After the leaching process the conductivities of all treated soils decreased by over 90%. The soil conductivities measured after the soil was leached and after 8 weeks were very similar to each other, suggesting the conductivities remained constant throughout the experimental period. For both chemicals, as the concentration increased the conductivities did as well. In general, the Y treatments had slightly higher conductivities than the Nd treatments of the same concentration.

3.4 Leaching

The clarity of the leachate is affected by the soil aggregate stabilities. The weaker the aggregates are the less they stick together and the cloudier the leachate is due to some organic matter being lost as the soil is leached. The stronger the soil particles bind together means that less organic matter is being lost as the soil is leached, and the clearer the leachate is. The soil biological activity, nutrient cycling, and organic matter composition are all influenced by the stability of the soil aggregates (USDA Natural Resources Conservation Service, 2008). The leachate collected showed as the concentration of both Nd and Y increased, the clearer the leachate was. The reference soil leachate was the cloudiest and the only opaque one. Rare earth elements can form strong complexes with organic compounds (Davranche, M. *et al*. 2015). This

could explain why with increasing concentrations of REE, the soil aggregates may have been more stable and therefore less organic matter leached out.

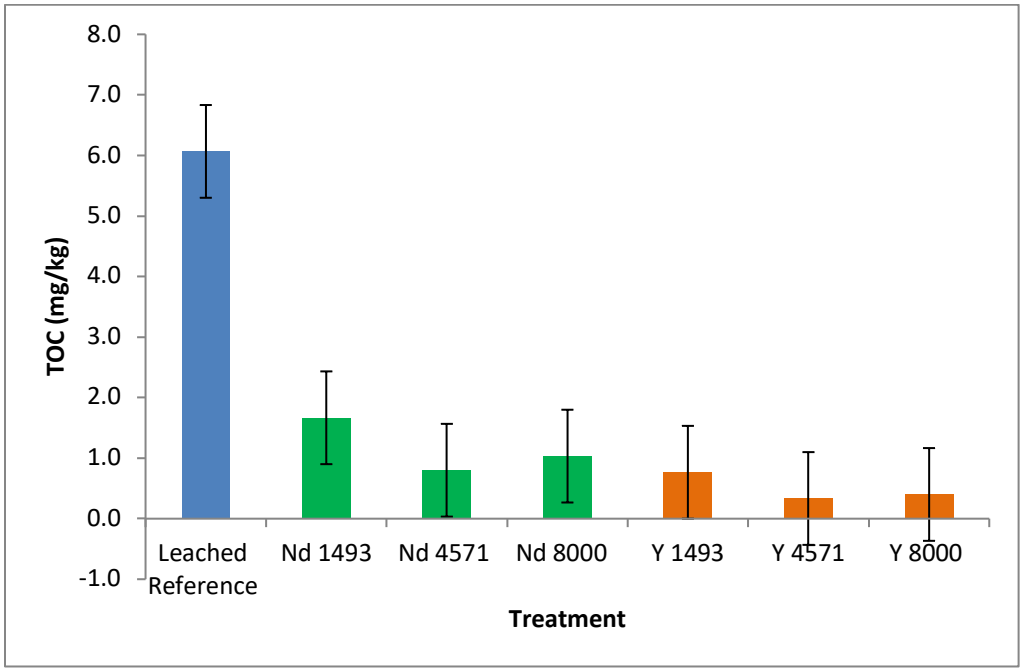
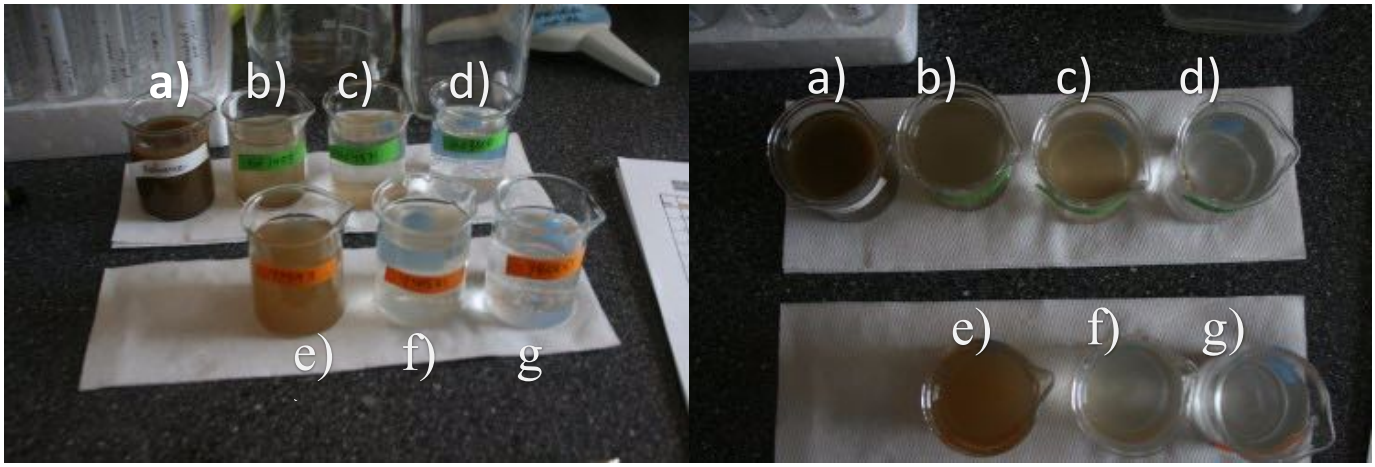


Figure 3: Bar graph displaying total organic carbon (TOC) of the leachate for all of the treatments. SE is shown (n=3).



Figures 4.a) & 4.b): Photos of leachate samples from each treatment. a) Reference, b) Nd 1493, c) Nd 4571, d) Nd 8000, e) Y 1493, f) Y 4571, g) Y 8000

3.5 Organic Matter Decomposition

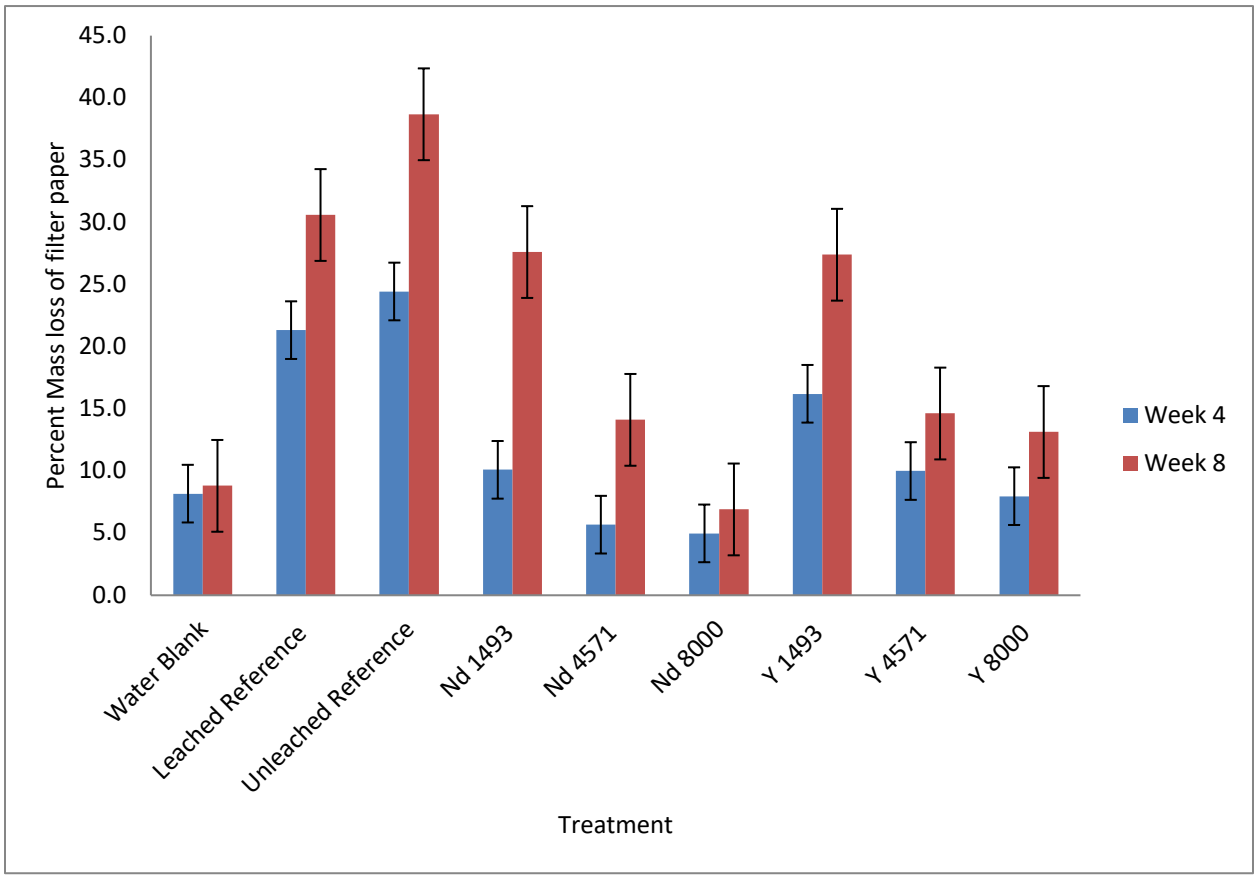


Figure 5: Bar graph of the organic matter decomposition testing results SE is shown (n=5).

The organic matter (OM) decomposition was tested to observe if the chemicals affected microbial activities in the soil, specifically ones that contribute to carbon cycling. OM decomposition was measured by calculating how much mass loss occurred in the filter paper discs over the test period. Organic matter decomposition is used as an indicator to determine soil microbial health and functionality. The reference treatments had higher rates of organic matter decomposition than the Nd and Y treatments, suggesting that they had more microbial activity. It was observed that the higher the concentration of chemical in the treatment, the lower the rate of decomposition of organic matter. This indicates that the chemicals interfere with the microbes involved with carbon cycling in the soil. As well, Y treated soil consistently had higher mass losses than the Nd treated soil of equal concentration. This demonstrates that Nd has a more inhibiting effect on the organic matter decomposing microbes.

3.6 Nitrification

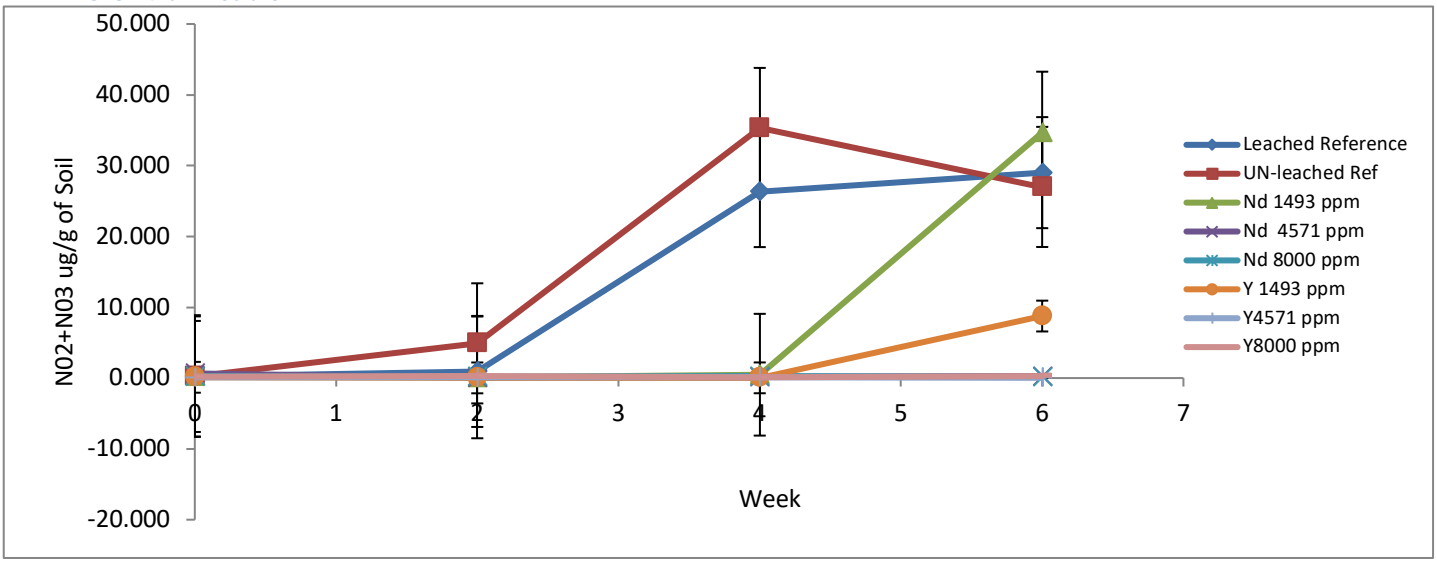


Figure 6: Scatter plot of NO2+NO3 (ug/g of soil) measured in the soil at 2 week intervals. SE is shown (n=3).

Soil nitrification activity was tested every two weeks for 6 weeks to observe and monitor the effects of the chemicals on the soil microbial health, specifically the nitrogen fixing bacteria, which are important contributors to the nitrogen cycle. Nitrification is an essential function of soil microbes for environmental health and maintenance. Therefore, it is important to determine if and how the chemicals affect the soil's nitrifying abilities. It was observed that for the first two weeks the nitrifying bacteria activities in the soil were low in all of the treatments. After, in the fourth week there were large spikes in the nitrate and nitrite levels in the reference soils. These levels remained high at week six as well. The contaminated soils had very little nitrifying activity for the first 4 weeks. The higher concentrated soils (4571 ppm and 8000 ppm) for both chemicals had low nitrate and nitrite levels throughout the whole trial period. However, for the lowest concentration of chemicals, Nd 1493 ppm and Y 1493 ppm, there was a spike in nitrogen levels at the fourth takedown during week 6. This could indicate the presence of resistance microbes. The Nd 1493 ppm treatment had a much larger increase in nitrites and nitrates than the Y 1493 ppm treatment, suggesting that the effect is greater in Nd contaminated soil than Y contaminated soil. This indicates that at high levels the chemicals interfere with nitrifying activity and can have

a negative effect on the soil microbial health. However, at low concentrations the chemicals may cause a positive effect on the nitrifying bacteria in the soil after a period of time.

3.7 Enzyme Assays

BG and LAP enzyme activities are used as an indicator of soil microbial health. They are both enzymes involved in cell degradation, cell turnover and, cellular maintenance. They are important for carbon cycling in soil and are used as indicators for soil microbial health. The enzyme assays were conducted every two weeks investigating the activity of BG and LAP in the soil.

3.7.1 BG

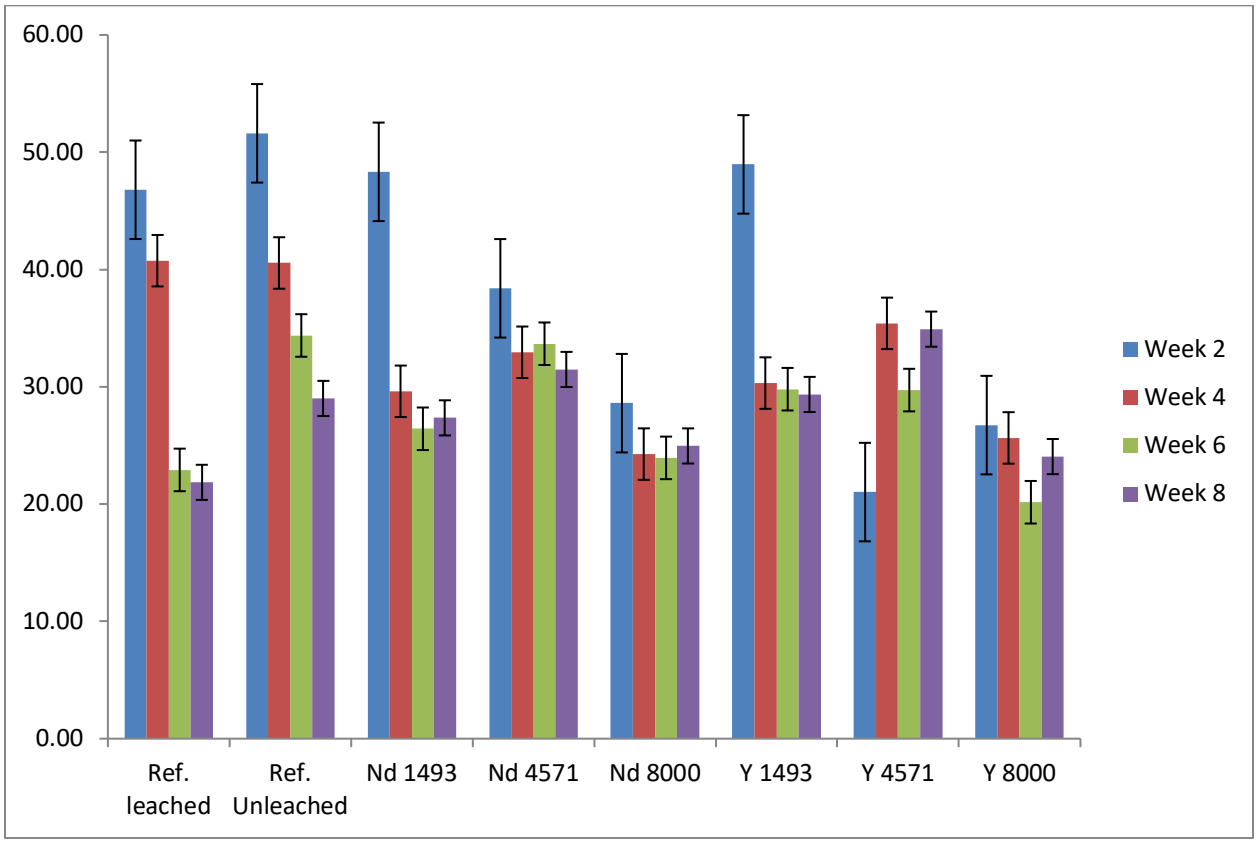


Figure 7: Bar graph of BG activity measured in fluorescence every two weeks, SE is shown (n=3).

For the Nd contaminated soil, the first week followed the trend that the higher the concentration, the lower the activity. For the rest of the weeks the highest concentration had the lowest activity and the middle concentration had the highest activity, suggesting there was a resistance effect with the Nd treated soil. The references typically had higher activity than the

contaminated soil. For the first week of Y treatments the lowest concentration had the highest activity and the middle concentration had the lowest activity. The rest of the weeks had similar results to Nd, suggesting Y also has a resistant effect on the soil microbial activity.

3.7.2 LAP

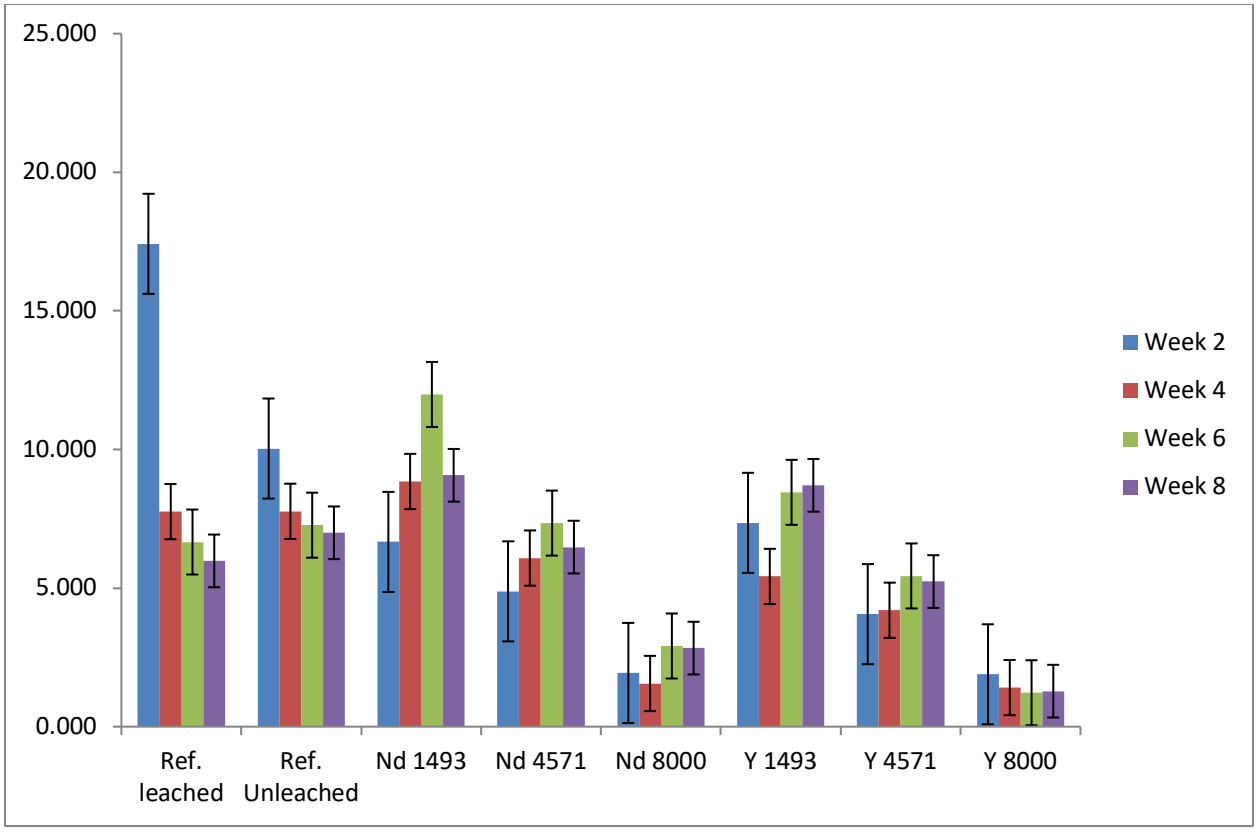


Figure 8: Bar graph of LAP activity measured in fluorescence every two weeks

The activity in the reference soils decreased as time passed. For both Nd and Y contaminated soils, as the concentration of chemical increased the activity of LAP decreased. This indicates that the chemicals negatively affected the soil microbial activity of LAP.

4.0 Conclusion

For both organic matter decomposition and LAP enzyme activity, as the concentration of Nd and Y increased, the activity of the enzyme and decomposing bacteria decreased. This relationship suggests that the chemicals have a negative effect on the soil microbial health, however, other trends were observed that suggest a resistance occurs in the soil and at certain concentrations the chemicals appear to have a beneficial effect on soil health. At higher

concentrations of REE, soil nitrifying bacteria activity were very low, however at low levels of REE the nitrifying bacteria activity was high. BG enzyme activity was highest in the treatments with the middle concentrations of Nd and Y. Therefore, although the chemicals appear to have negative effects on the soil microbial health, there is a possibility that under the right conditions they can have beneficial influences on soil.

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