

Study of the release of Ca, Mg, Fe, and Mn from a biologically active soil due to microbial eradication and a chelating agent

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May 23, 2007

Submitted to the Geology Department of Carleton College in partial completion of a Bachelor of Arts degree

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ABSTRACT

Because of the health threats posed by trace metals in both groundwater and edible flora, a great deal of study has been done on the remediation of soils with high metal content by both bacterial input and chemical washing. This study focuses on the potential for the extant microbial community in a biologically active soil to trap Ca, Mg, Fe, and Mn cations. Of four homogenous soil samples, two were exposed to temperatures of one hundred degrees Celcius for twenty four hours to eradicate the microbial community. H₂O was run through one biologically active and one biologically inactive sample, and EDTA, a common chelating agent, was run through the two other samples. Results show an increase in leached cation content in all three altered samples. Microbial eradication alone had a minor increase in leached cation mass, and both EDTA washing and EDTA washing after microbial eradication had a large increase in leached cation mass. Analysis of the data shows a significant facilitation of cation release early in the EDTA wash after a microbial eradication, suggesting the possibility of a release of held cations in soils after eradication events.

Keywords: soil, chelant, EDTA, cations, trace metals, leaching, bacteria, remediation.

Introduction

Small amounts of trace metals in soil can pose serious health threats to humans and other animals alike. Toxic chemicals can leech into groundwater and be ingested or be taken up by edible plants and eaten. EPA standards for drinking water are 0 mg/L for both Pb and As, and allow only 0.002 mg/L Hg, 0.004 mg/L Be, and 0.005 mg/L Cd (EPA, 2006). In addition, chemicals like fluoride and copper have higher limits, but are equally poisonous.

Because such small concentrations of dangerous trace metals can be damaging to an organism's health, there has been a great deal of research on the remediation of soils that contain these metals, and several techniques using chemical chelants have been developed for that purpose (Davis, A. P. and Singh, I., 1995, Wasay et al., 1998, and Finžgar, N. and Leštan, D., 2007). Additionally, methods of remediation by bacterial adsorption of trace metals have been found effective in dealing with many trace metals present in soil (Huang, Q. et al., 2005). In these cases, the trace metals are bound by bacterial composites, and the bacteria and their composites are removed from the soil.

Research has shown that soils containing a metal for a long period of time can develop microbial communities that absorb those metals into composites (Chander, K. et al., 2001, Kao, P., Huang, C., and Hseu, Z., 2006). These communities will then help trap trace metals in a soil, preventing them from being leached down into the groundwater or up into the root of a plant. Thus far, little work has been done to examine the ability of a soil and its associated microbial communities to trap ions. Likewise, there has been little study of the potential for the release of the ions trapped by microbial communities in soils. This study is an attempt to develop a method of testing the

potential release of ions from soils after a microbial eradication and/or the addition of a chelating agent to the soil that would break ions out of the bacterial composites and leach them from the soil.

In the experiment, we would expect to see an increase in the amount of ions leached in both cases – the eradication of the microbial community in the soil and the addition of a chelating agent to the soil. Relatively, due to the effectiveness of the chelating agent in soil remediation relative to biological methods, the chelant should leach a much greater concentration of ions than water, whether the microbial community is active or deceased. The study supports these hypotheses, though additional factors are shown to be affecting the leaching of cations from the soil in most cases.

Use of EDTA in Soil Science

In several recent studies, ethylenediamine-tetraacetic acid (EDTA) has been used as a chelating agent to assist in remediation and phytoremediation of soils with significant trace metal content (Tejowulan and Hendershot, 1998 and Vangronsveld, J., 2001). Experiments run using EDTA have found that, in some cases, it can increase the mobility of ions by 2500% when compared to pure bacterial remediation (Papassiopi, N. et al., 2003). Among chemical chelants, EDTA, citric acid, and nitrilotriacetic acid (NTA) are most commonly recommended for remediation use in soils, though techniques using alternative methods of remediation and analysis (Elliott et al., 1989, Elliott and Brown, 1989, Hendershot et al., 1993, Maynard, D.G. and Kalra, Y.P., 1993, Davis and Singh, 1995, Mulligan, C. N. et al., 2001). Among these, EDTA is most effective in the remediation of a wide range of trace metals (Zeng et al., 2005). EDTA has also been

used in several studies along with bacterial remediation to study the potential benefits of combining remediation treatments (Chen, H. and Cutright, T., 2002, Vaxevanidou, K., Papassiopi, N., Paspaliaris, I., 2002, and Papassiopi, N. et al., 2003). EDTA has been shown to bind strongly with alkaline-earth and transition metal cations including Ca, Fe, Mg, and Mn, elements common to many soils and easily measurable in solution by atomic absorption spectrometry (Zeng et al., 2005). Because of its widespread recent use in research, its ease of use, and the range of metals with which it will bind, EDTA was chosen to be the chelating agent in this study.

Area of Study

The soil for this study was taken from the Carleton College Arboretum in Northfield, southeastern Minnesota (Fig. 1). Samples are of the A1 and A2 horizons of the Ankeny Series with possible input from the Rushriver Series (Rice County Atlas, 2000). The Ankeny Series is classified as a coarse-loamy, mixed, mesic Cumulic Hapludolls. The A horizon of the Ankeny Series is well drained with moderately rapid permeability. Situated on a flood plain, soil input is limited to drainage and flood deposits from the nearby Cannon River.

Method

Four cylinders of two-inch PVC pipe, each five hundred centimeters in length, were cut and placed on raised stands. The bottom of each column was covered with four layers of cheesecloth so samples would filter down out of the columns. Soil from the Carleton Arboretum was collected and manually homogenized. Four one-kilogram samples were weighed out and placed in separate containers. Two of these containers

were placed in an oven and left at one hundred degrees Celsius for twenty four hours to eliminate the bacterial community present in the soil, then each of the four soil samples was placed in one of the four prepared columns (Fig. 2). The soil in each column was saturated with H₂O (Type I DI water). Approximately every twelve hours, 150 mL of H₂O was added to one of the cooked columns and one of the uncooked columns (Fig. 40). To the remaining two columns, 150 mL of 0.05 M EDTA solution was added. The drainage from each column was collected. Each sample had its volume recorded and was run through the Atomic Absorption Spectrometer to measure the concentrations of Ca, Fe, Mg, and Mn. Six samples were taken from each column for a total of twenty four solutions.

Results

Ca and Mg

Ca and Mg, both alkali-earth metals, best exhibited the expected trends of decreasing concentration over time. In columns one, two, and four (Fig. 2), the measured values trended downward over time, though cation concentration occasionally rose from one sample to the next (Fig. 3, 4, 6-8, 10). Column three, however, increased in concentration over time (Fig. 5, 9). Total leached cation mass, measured by multiplying the cation concentration of each sample by the volume of that sample, showed less well-defined trends (Fig. 19-26). For both Ca and Mg, the lowest observed concentration was observed in column one, and the highest was observed in columns three and four.

Fe and Mn

Fe and Mn, transition metals, did not exhibit expected trends of either cation concentration change or total leached cation mass. Columns one and two showed trends of little change in concentration over time, but spikes of increased concentration at irregular intervals in the experiment (Fig. 11, 12, 15, 16). Columns three and four showed trends of increasing concentration over time (Fig. 13, 14, 17, 18). Similarly, total leached cation mass was irregular in columns one and two, and had an upward trend in columns three and four (Fig. 27-34). As with Ca and Mg, concentration values were exponentially higher in columns three and four than their values in columns one and two.

Discussion

This experiment tested two different effects on a soil, both of which should produce an increase in the amount cations leached from that soil. As predicted, both the extermination and the addition of EDTA as a chemical chelating agent increased the cation concentrations in the samples taken. It was also confirmed that the EDTA had a much greater remedial effect on the soil than the presence of the natural microbial community in the soil. This supports the results found in previous comparative studies of soil remediation (Chen, H. and Cutright, T., 2002, Vaxevanidou, K., Papassiopi, N., Paspaliaris, I., 2002, and Papassiopi, N. et al., 2003). However, in the case of both the extermination and the added chelating agent, the samples exhibited trends of concentration change that suggest more complex factors involved in soil leaching.

Microbial Eradication

The eradication of bacteria in the soil sample caused an increase in the observed cation concentration in both Ca and Mg for all four columns. Graphs of the total leached mass of Ca show a general trend downward, though peaks in the graph of column one are difficult to interpret. The downward trend suggests that the water running through the system is likely picking up Ca ions that are either very weakly bonded to soil minerals or not bonded at all, and the supply of those available Ca ions decreases without ever being replenished. The difference in values between column one and column two indicates that, due to the decomposition of the molecules associated with the dead microbial community in column two, more Ca atoms are freed in column two to be washed away by the flow of water. Graphs of total leached Mg mass are nearly identical to the graphs of Ca. Ca and Mg are both alkali-earth metals, so it should be expected that they would bond similarly within a soil.

The Fe concentration values both peak in the first sample, then drop and maintain a fairly steady concentration. If the initial peak is meaningful, it may represent a very limited amount of unbound Fe that is quickly leached out of the soil. Once that Fe is gone, small amounts of remaining, weakly-bound Fe could be providing the observed concentration in the remainder of the samples. It is difficult to tell whether or not this is the case, because a peak in the graphs of Mn concentration occurs in sample five of column one, and it is unclear what the sudden increase in leached Mn might be attributable to. It is possible that the baking stage may have dehydrated Fe- and Mn-based molecules, freeing them from the soil. In most cases, the graphs of the total leached cation mass will resemble the graphs of cation concentration. For this reason, the

apparent peak in the third sample of column two is actually a peak in the second sample, and that could be attributable to the same cause I have proposed for the early Fe peaks.

Chemical Chelating Agent

For each tested cation, the use of EDTA washing in the soil completely changed the observed trends of that cation. In each case, EDTA washing produced an increase in leached cation mass over time. This trend implies that the addition of a chelating agent not only increases the amount of cation leached from the soil, but it makes it easier for repeat washings of that chelant to remove cations from the soil. It may be that, as the chelant reacts with cations bonded to the soil, the chemical structure of the soil is weakened, and it becomes easier for new molecules of the chelant to pull cations out of the soil. The graphs of Ca and Mn show the trend beginning to level off in samples five and six, so it is possible that either the EDTA reached the limit of its ability to free these cations or they began to become scarce in the soil sample. For all cations except Mn, column three had the single highest observed cation concentration value among all four columns.

Microbial Eradication + Chemical Chelating Agent

Because the EDTA had such a greater effect on the amount of cations leached out of the soil than the presence of the microbial community, it is very interesting to observe the differences between the data for column three and column four. Both columns have values in similar ranges, but while the values in column three show an increasing trend in cation concentration with time, and peak in either sample five or sample six, the peaks in

the Ca and Mg graphs of column four occur in the first two samples. The data clearly shows that the eradication and decomposition of the microbial community in the soil significantly increased the initial effectiveness of the EDTA in washing Ca and Mg out of the soil. The difference in concentration between column three and column four was much greater than the difference in concentration between column one and column two, even though the variable changed was the same between both sets of columns. Because of the ineffectiveness of the water in leaching cations bound firmly in molecules in the soil, many of the decomposed bacterial composites may not have affected the leached cation content of the second column. However, this data shows that, while the metals may still be too well-bound for water to leach, cations held in decomposed bacterial composites are significantly easier to weather out of a soil. In some cases, including cases involving trace metals, the difference may be enough for water to show a significant increase in cation leaching after some sort of a microbial eradication.

Conclusion

This study suggests that both the extinction of the microbial community in a soil and the input of a chelating agent can increase the leaching of metal cations from that soil. While in the natural world, the danger of a chelant being introduced to a soil system might be very low, there is a potential for damage to the microbial community in a soil from many sources. Changes in O₂ content of the soil, forest fires, or the introduction of a competing strain of bacteria could all harm the extant microbial community, so the potential for the release of metals is not discountable.

Understanding the mechanisms for the capture of trace metals by a microbial community in soil, and the potential dangers of instant release of those metals, may be

extremely useful for the monitoring of contaminants in groundwater, especially in cases where a groundwater supply may exhibit low, but acceptable levels of contaminants like fluoride or copper. In those cases, a harmful concentration of those contaminants may be trapped in the soil, and remediation may be required in order to prevent the sudden release of a harmful amount of the contaminant in question.

To gain understanding of the mechanisms of trace metal capture, more study needs to be done in this area. Studies on the potential held cation concentration of common soil bacteria, the potential for the eradication of those bacteria, and the possible reactions that could free the large amount of cations that were measured in this experiment given reasonable environmental input in the study area would all contribute to a more well-defined concept of this phenomenon.

Acknowledgements

There are a few people to whom this study and I owe a debt of gratitude. I would like to thank my comps advisor, Bereket Haileab, for being scary. I would also like to thank Clint Cowan, whose support and willingness to brainstorm after all seemed lost helped create this study and convinced me there was still hope. I also owe a great deal to Mary Savina, the professor who showed me how to turn that idea into a meaningful study, and who came through in the clutch with a pretty-looking soils map of Northfield.

Thanks to Tim Vick for being able to hunt down EDTA, unclog the AA, and leap tall buildings with a single bound. A big thanks to everyone in the department, including my fellow majors, for putting up with me for four years. Thanks to Cliff for the formatting help, thanks to Dixon 200 for moral support, thanks to Rold Gold for making such tasty pretzels, and thanks to Spider-Man for always watching my back. Go Brewers!

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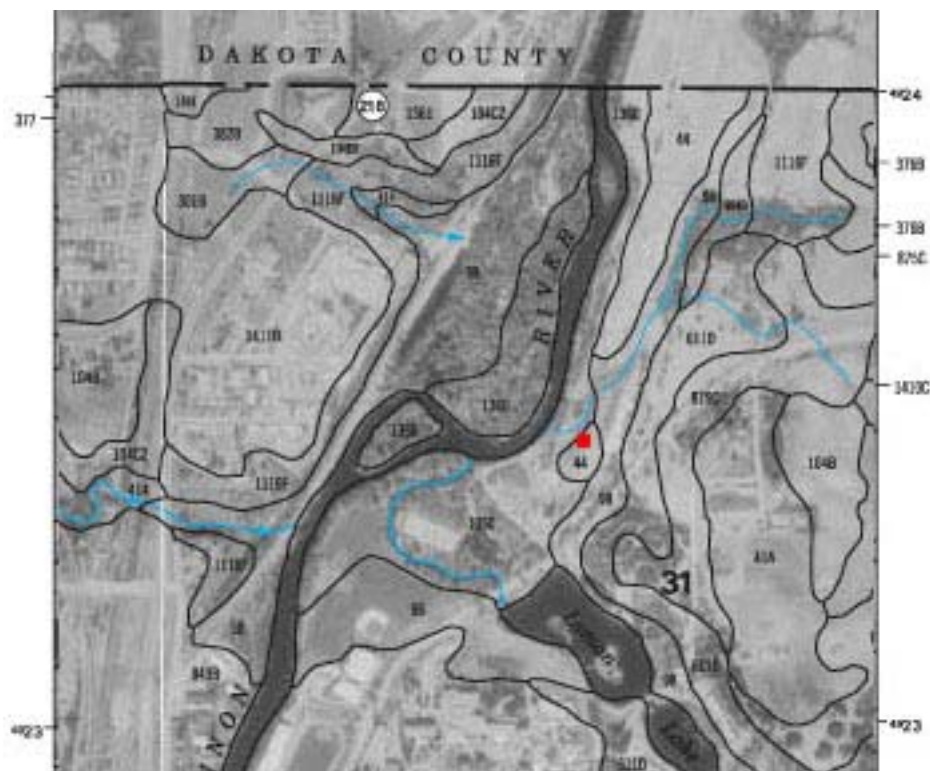
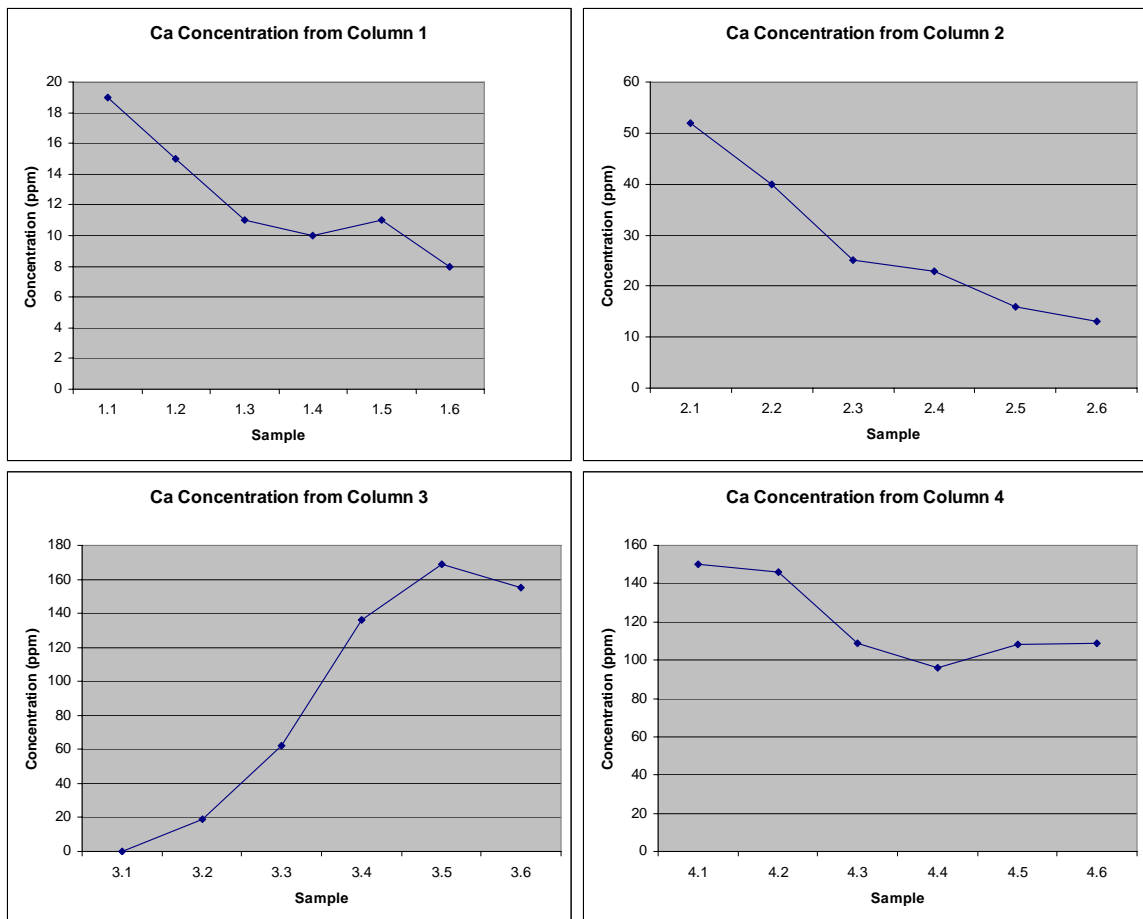


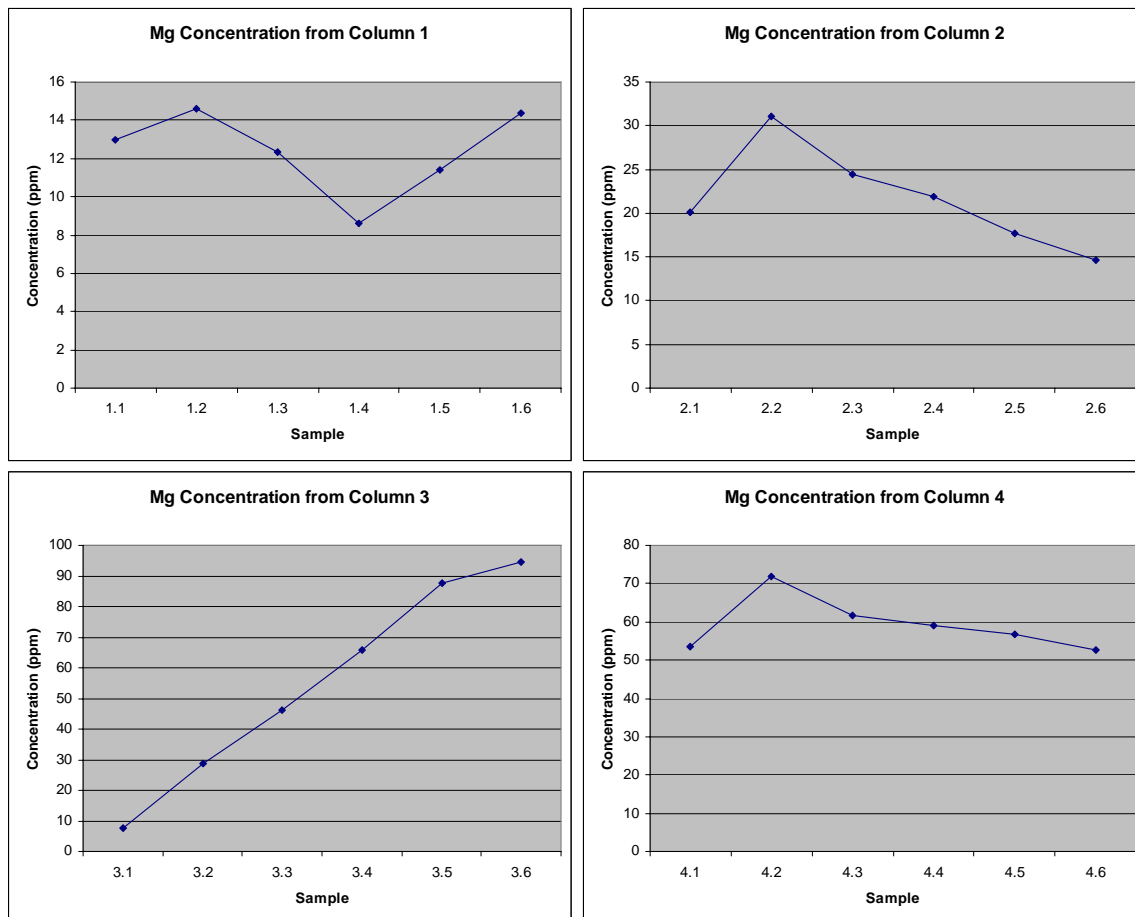
Fig. 1 – Soils map of the Carleton Arboretum. The sample location is marked in red. The number 44 corresponds to the Ankeny Series; adjacent to it is 1360, the Rushriver Series.

X	Uncooked Soil	Cooked Soil
H2O Added	Column 1	Column 2
EDTA Added	Column 3	Column 4

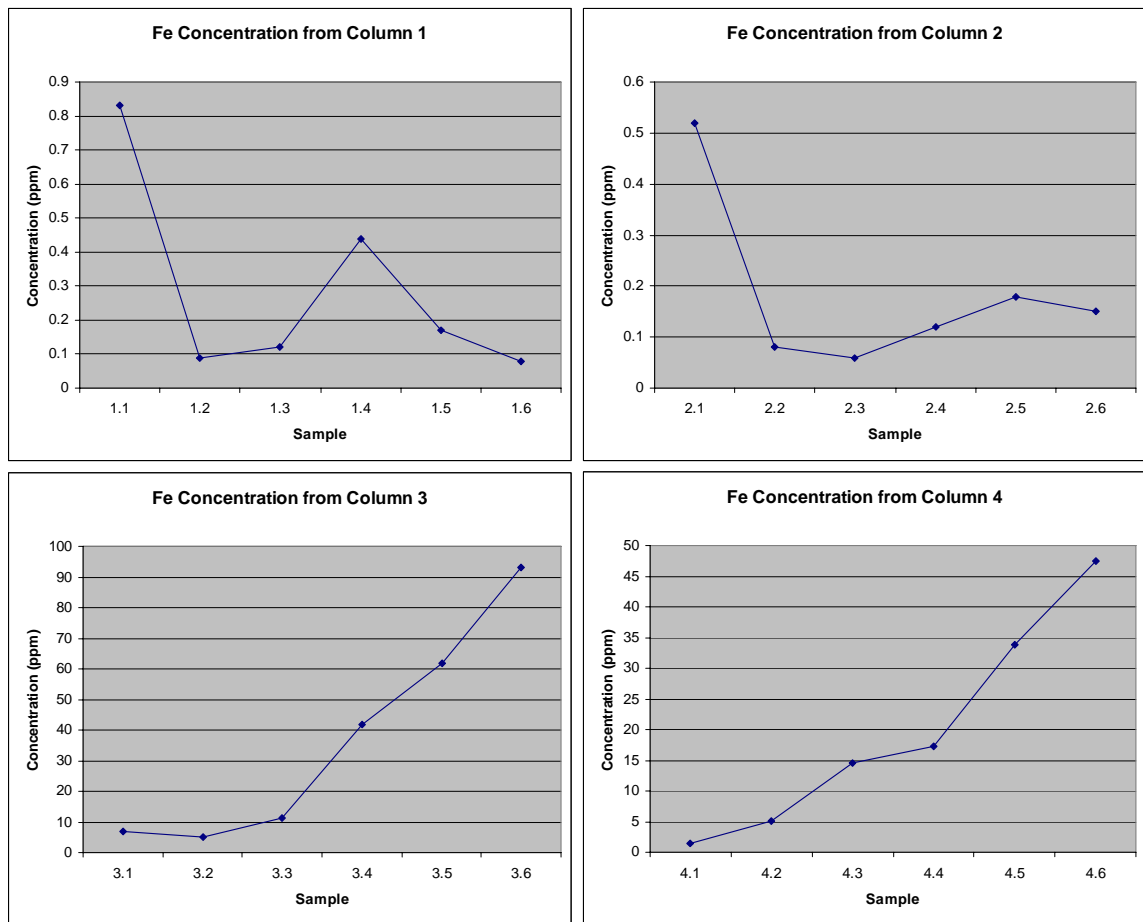
Figure 2 – Table of column treatments.



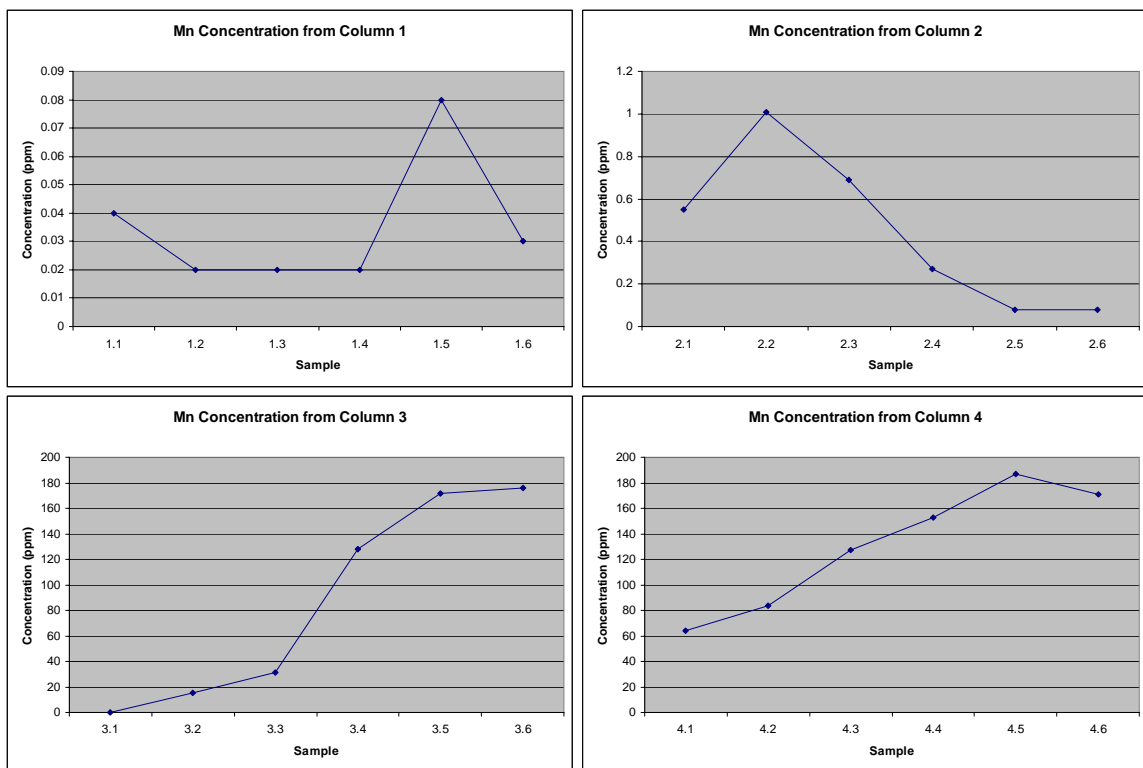
Figures 3-6 – Ca cation concentrations measured by atomic absorption spectrometry in each column.



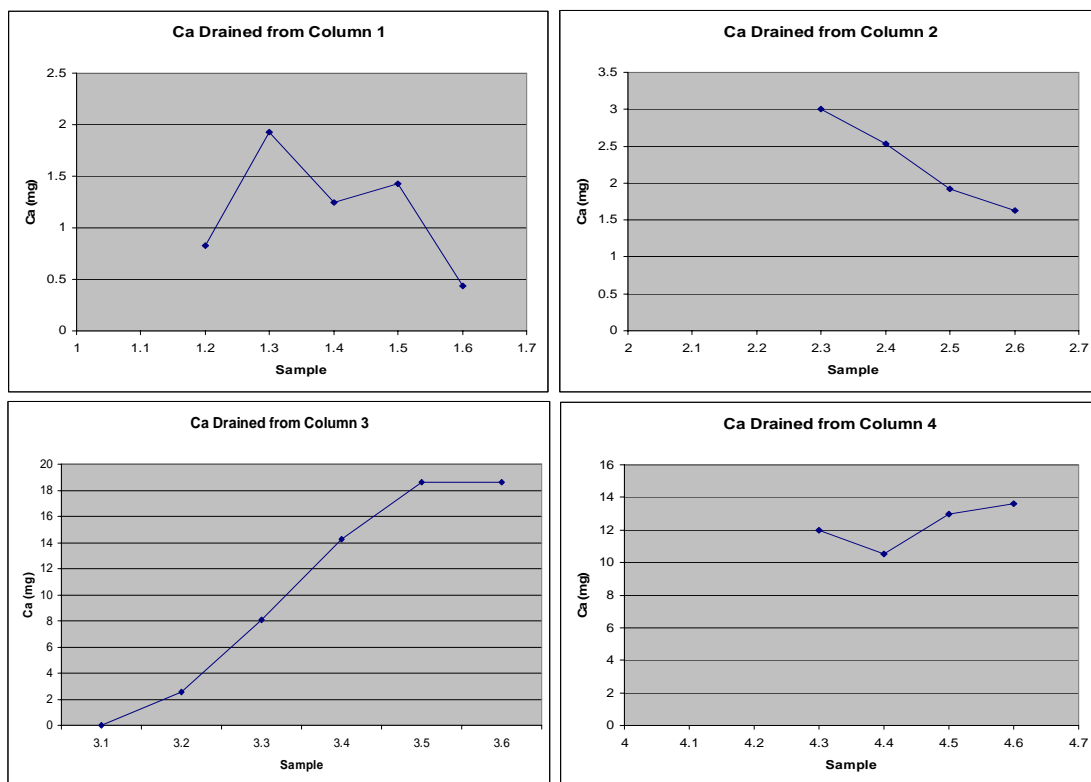
Figures 7-10 – Mg cation concentrations measured by atomic absorption spectrometry in each column.



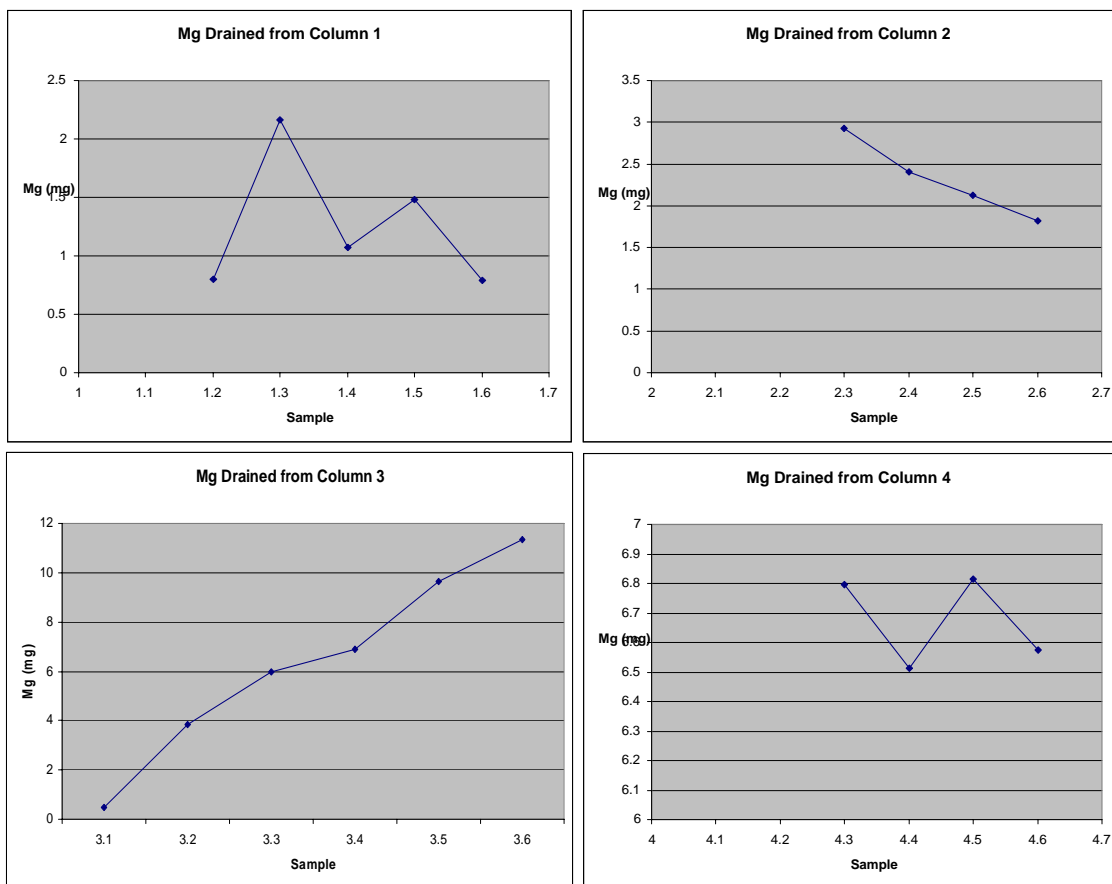
Figures 11-14 – Fe cation concentrations measured by atomic absorption spectrometry in each column.



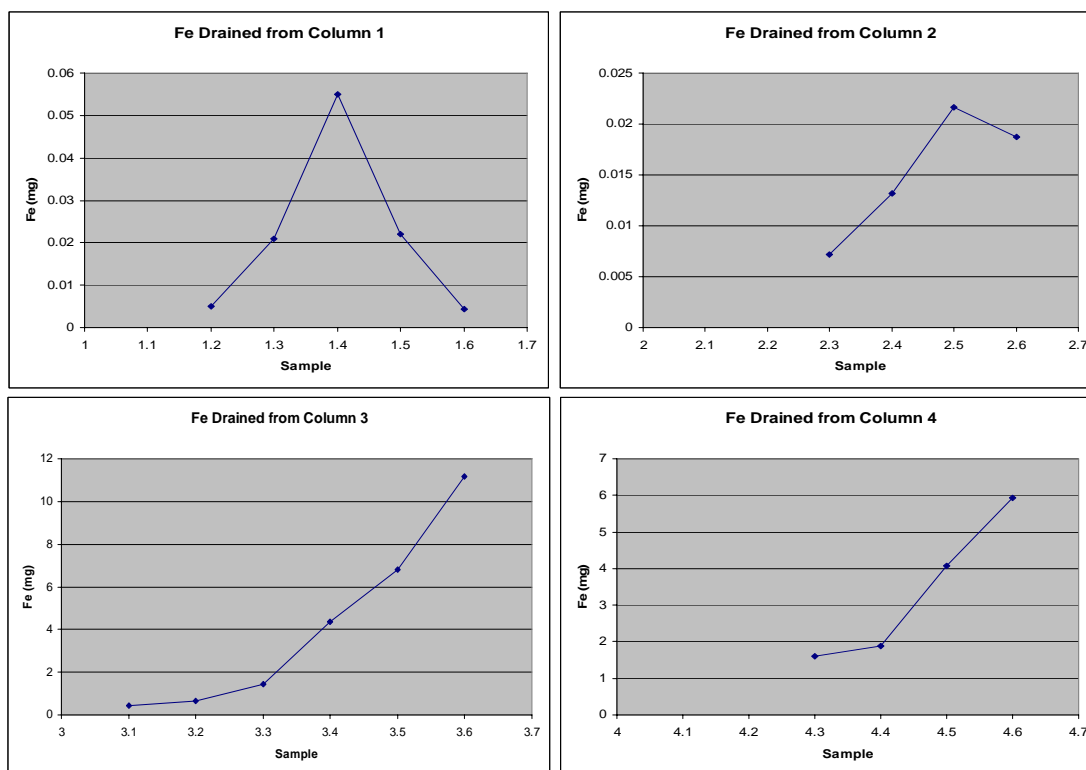
Figures 15-18 – Fe cation concentrations measured by atomic absorption spectrometry in each column.



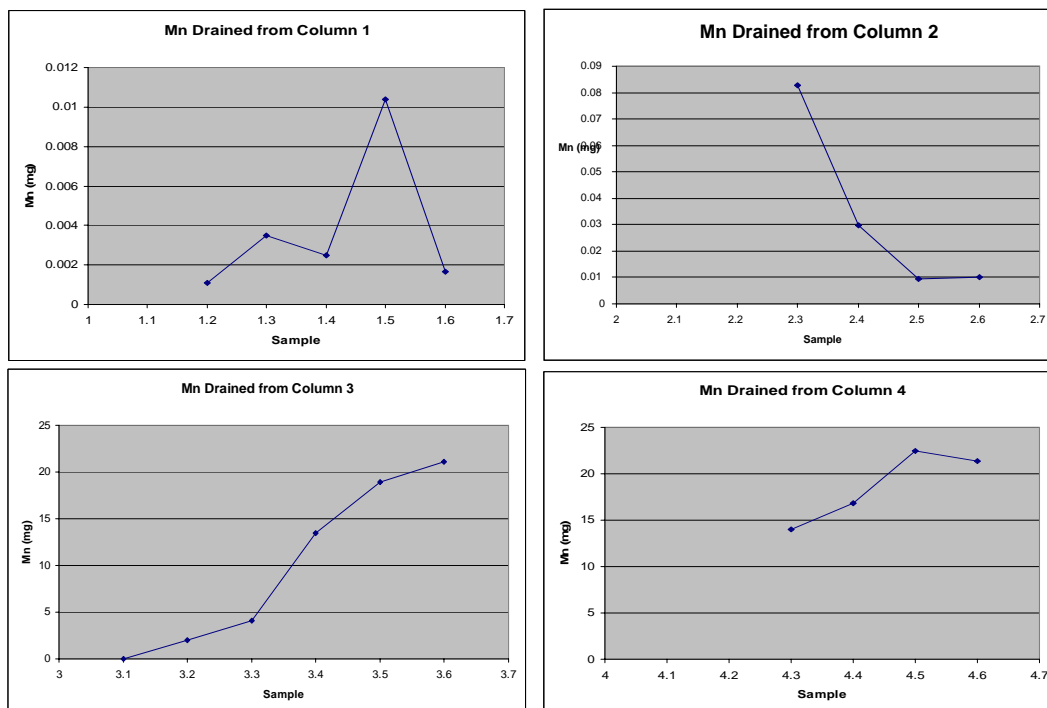
Figures 19-22 – Total Ca cation mass leached from the columns. Note that samples 1.1, 2.1, 2.2, 4.1, and 4.2 have zero values because they did not have their volumes measured, so no calculations could be done.



Figures 23-26 – Total Mg cation mass leached from the columns. Note that samples 1.1, 2.1, 2.2, 4.1, and 4.2 have zero values because they did not have their volumes measured, so no calculations could be done.



Figures 27-30 – Total Fe cation mass leached from the columns. Note that samples 1.1, 2.1, 2.2, 4.1, and 4.2 have zero values because they did not have their volumes measured, so no calculations could be done.



Figures 31-34 – Total Mn cation mass leached from the columns. Note that samples 1.1, 2.1, 2.2, 4.1, and 4.2 have zero values because they did not have their volumes measured, so no calculations could be done.

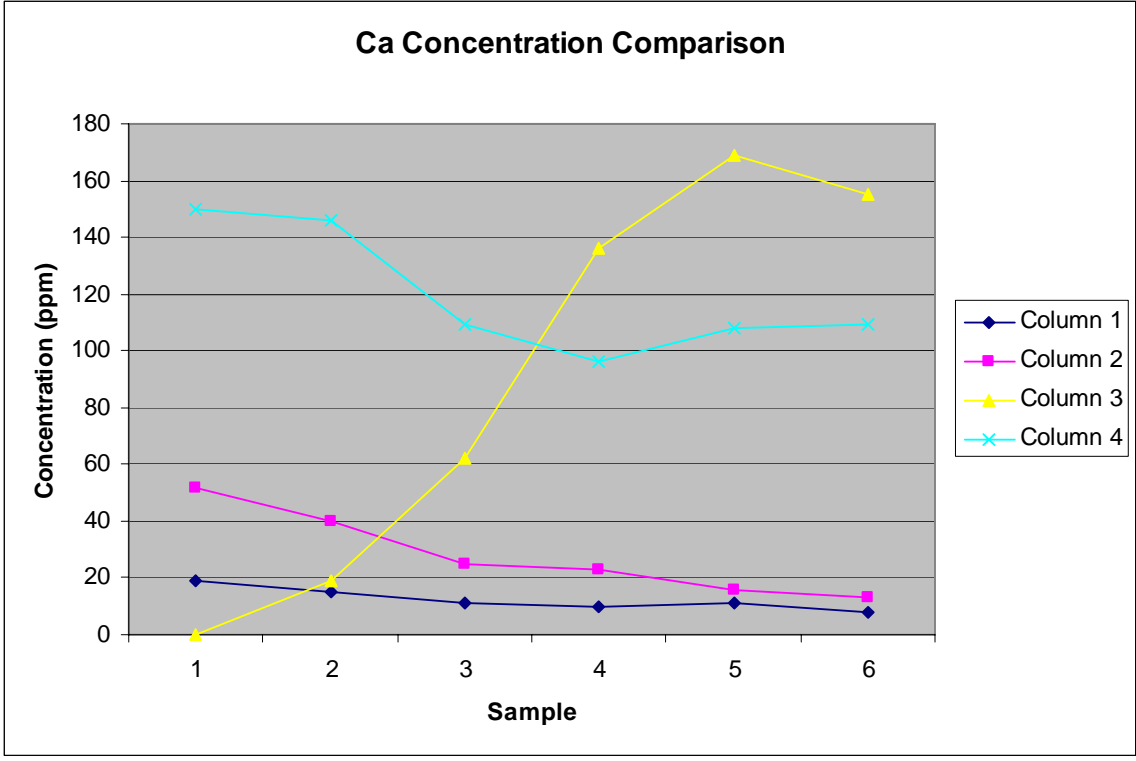


Figure 35 – Graph of the Ca concentrations observed in all six samples from each column.

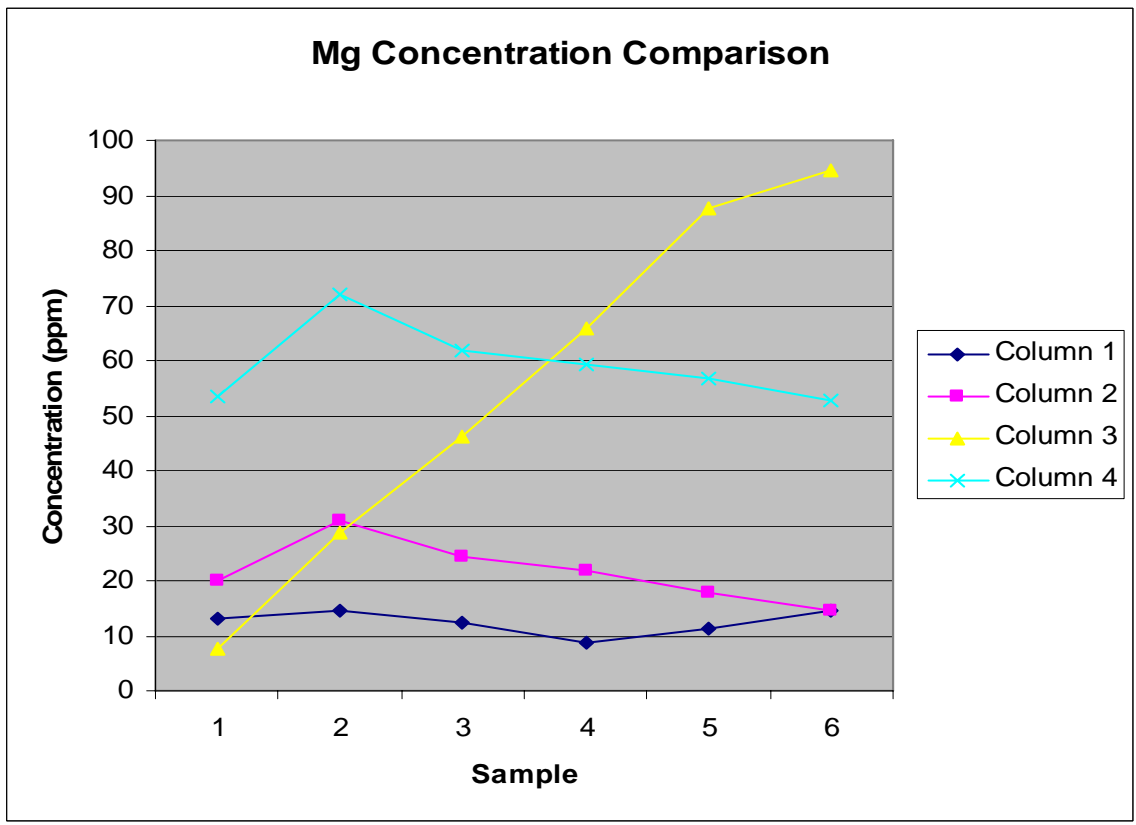


Figure 36 – Graph of the Mg concentrations observed in all six samples from each column.

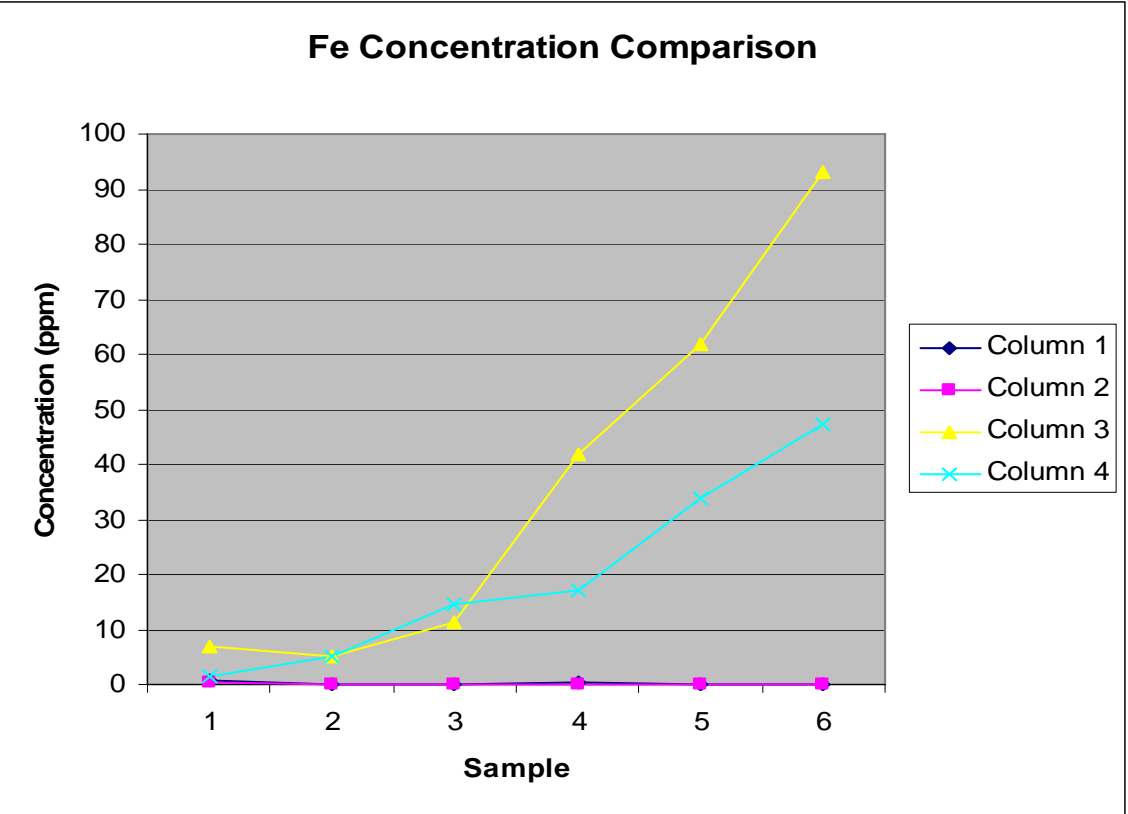


Figure 37 – Graph of the Fe concentrations observed in all six samples from each column.

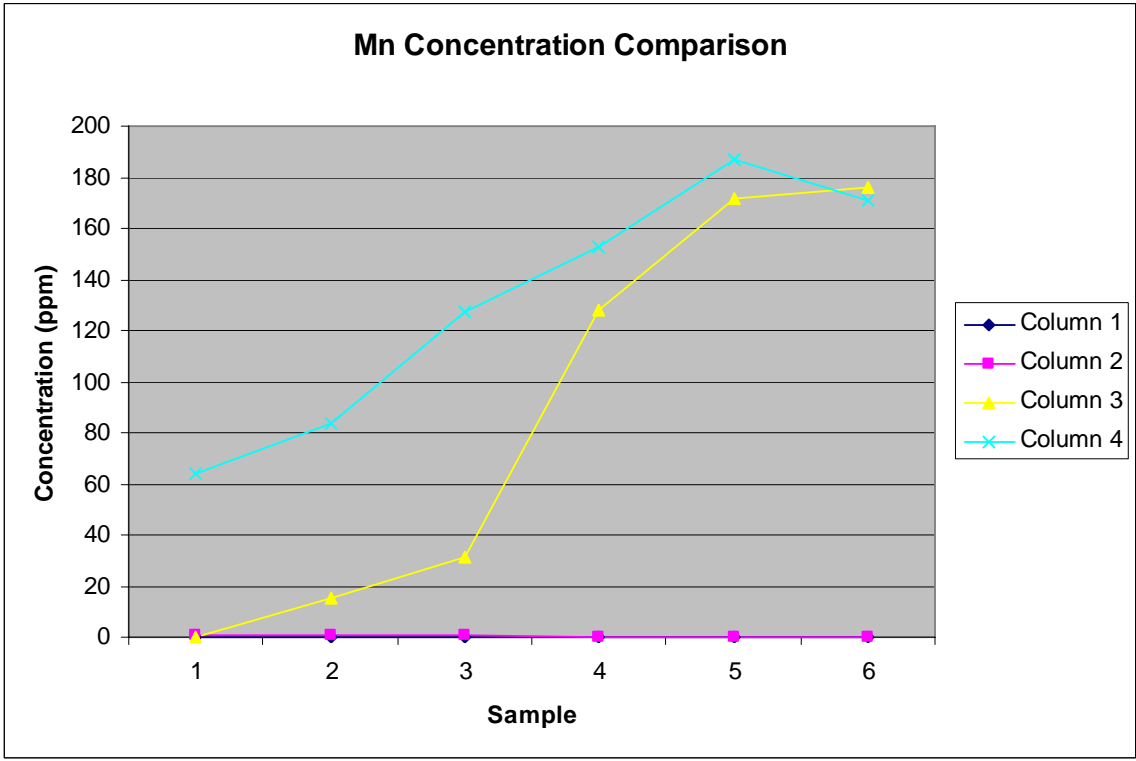


Figure 38 – Graph of the Mn concentrations observed in all six samples from each column.

Sample	Vol (mL)	Ca (ppm)	Ca Drained (mg)	Fe (ppm)	Fe Drained (mg)	Mg (ppm)	Mg Drained (mg)	Mn (ppm)	Mn Drained (mg)
1.1		19	0	0.83	0	13	0	0.04	0
1.2	55	15	0.825	0.09	0.00495	14.6	0.803	0.02	0.0011
1.3	175	11	1.925	0.12	0.021	12.36	2.163	0.02	0.0035
1.4	125	10	1.25	0.44	0.055	8.6	1.075	0.02	0.0025
1.5	130	11	1.43	0.17	0.0221	11.4	1.482	0.08	0.0104
1.6	55	8	0.44	0.08	0.0044	14.4	0.792	0.03	0.00165
2.1		52	0	0.52	0	20.1	0	0.55	0
2.2		40	0	0.08	0	31	0	1.01	0
2.3	120	25	3	0.06	0.0072	24.4	2.928	0.69	0.0828
2.4	110	23	2.53	0.12	0.0132	21.9	2.409	0.27	0.0297
2.5	120	16	1.92	0.18	0.0216	17.7	2.124	0.08	0.0096
2.6	125	13	1.625	0.15	0.01875	14.6	1.825	0.08	0.01
3.1	65	0	0	7	0.455	7.7	0.5005	0.15	0.00975
3.2	135	19	2.565	4.96	0.6696	28.6	3.861	15	2.025
3.3	130	62	8.06	11.1	1.443	46.1	5.993	31.2	4.056
3.4	105	136	14.28	41.7	4.3785	65.8	6.909	128	13.44
3.5	110	169	18.59	61.8	6.798	87.8	9.658	171.6	18.876
3.6	120	155	18.6	93	11.16	94.6	11.352	175.8	21.096
4.1		150	0	1.5	0	53.4	0	63.8	0
4.2		146	0	5.1	0	72	0	84	0
4.3	110	109	11.99	14.5	1.595	61.8	6.798	127.2	13.992
4.4	110	96	10.56	17.2	1.892	59.2	6.512	152.8	16.808
4.5	120	108	12.96	33.9	4.068	56.8	6.816	186.8	22.416
4.6	125	109	13.625	47.4	5.925	52.6	6.575	171	21.375

Figure 39 – Table of data collected by atomic absorption spectrometry. Values of drained cation amounts were obtained by the equation $m_x = [x] \cdot (\text{volume of sample}) / 1000$.

Sample Collected	1	2	3	4	5	6
Time Into Experiment	12 hrs	24 hrs	36 hrs	51 hrs	60 hrs	72 hrs

Figure 40 – Times of sample collections after starting the experiment.