

Nitrogen dynamics in flow-through microcosms of
NITREX[®] reactive media.

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Abstract

There have been many signs of eutrophication of Cape Cod bays and estuaries. Eutrophication or the elevated concentration of nutrients has many negative effects on the organisms living in and around the bays. It is critical to eliminate or at least reduce nitrogen loading. In order to address all sources of nitrogen loading, the Marine Biological Laboratory is currently testing two experimental permeable reactive barriers along the coastlines of Falmouth, MA. Although the NITREX® medium has been used several times to reduce nitrogen, it has never been used along the coastline where perturbations of saltwater are possible.

I assembled barriers in the laboratory using PVC columns, a Marriot bottle drip system, limestone and the NITREX® medium. I tested their ability to remove nitrogen and also the affects exposing saltwater to systems might have. I determined that adding saltwater at 18,000 uM for 48 hours, has no immediate affect on the ability of the NITREX® medium to remove nitrate from the groundwater. Initially there is a relatively short transition time for the barriers microbial communities to develop and drive the system anaerobic. Within one week of assembly, the systems were removing nitrogen by either nitrogen immobilization or denitrification. The inflow groundwater had a concentration of 150 uM nitrate, the nitrate was removed leaving a concentration of approximately 0 uM in the outflow of my barriers.

Introduction

Urbanization of Cape Cod has increased dramatically over the decades. The increased number of households has also increased the input of nutrient concentrations from treated wastewater, residential septic systems, agricultural and residential fertilizer applications, and atmospheric deposition. The increased levels of nutrients result in increased cases of eutrophication of Cape Cod's bays and estuaries. Eutrophication can cause the replacement of eelgrass by macroalgae, low dissolved oxygen, periodic fish kills, and other negative results (Bowen and Valiela 2001).

Several approaches have been suggested to reduce estuarine nitrogen loading, however, the methods only focus on fertilizer and wastewater inputs. The decrease in the amount of agricultural fertilizer used in the Mississippi River basin will diminish anoxia in the Gulf of Mexico (Rabalais *et al.* 2002). However, in urban and suburban areas, it is more difficult to obtain a considerable decrease in fertilizer inputs since this is dependent upon the individual routines and behaviors of several house owners. Individual alterations in fertilizer usage would not provide significant enough results.

Decreasing wastewater inputs to estuaries is similarly an important issue. Individual wastewater treatments have been developed, however are extremely expensive in the installation, operation, and maintenance of the systems. The individual systems might not obtain high rates of nitrogen removal and will not provide significant decreases in nitrogen loading unless used in conjunction with all septic systems.

It is very important to eliminate or at least reduce the amount of nitrogen entering the estuaries from all three sources; wastewater, fertilizers, and atmospheric deposition. The Marine Biological Laboratory (MBL) has begun a project called the *Effectiveness of Reactive Barriers for Reducing N-Loading to the Coastal Zone*. This project is using a NITREX® permeable reactive barrier placed on the groundwater/estuarine interface to intercept the nitrogen-rich water prior to entering the estuary. This method is more effective when placed at the groundwater/estuarine interface because it is less costly than installing the NITREX® system at individual septic systems and therefore the system will address the nitrogen loading from

residential septic systems, as well as wastewater, fertilizer applications and atmospheric deposition.

The NITREX® barrier apparatus is comprised of wood chips, sand, gravel and limestone and is designed to provide a carbon source that will fuel denitrification. The experimental NITREX ® system is being tested in two areas of Cape Cod; Waquoit Bay (WB), and Child's River (CR), both located in Falmouth, MA. Both barriers are on the coastline, however, the WB barrier is located on the Waquoit Bay National Estuarine Research Reserve (WBNERR) and is experiencing moderate nitrogen loading. The CR barrier is located in an area of high housing density and as a result experiences high nitrogen loading (Vallino, 2004). (Fig. 1 and 2).

Since the NITREX ® system has never been tested in coastal regions; the affect of periodic salt water intrusion is unknown, as with the specific mode by which the nitrogen is removed from the groundwater. There is significant evidence that the process is denitrification; however, there are possibilities that the NO_3^- is removed by immobilization or conversion to dissolved organic nitrogen (DON) (Robertson *et al.* 2000).

Pilot-scale barriers resembling those present on WB and CR were used to study the effects of saltwater intrusion, and the fate of the nitrate entering the systems. The production of hydrogen sulfide is possible, and has been shown to inhibit denitrification. This could possibly cause permanent or temporary alteration of the microbial communities responsible for denitrification which would result in an unsuccessful method of denitrifying the nitrate entering the estuaries of Cape Cod. (Joye and Hollibaugh. 1995).

Methods

Assembling Apparatus:

Six miniature NITREX® barriers were assembled in the laboratory. The structures of the barriers were made using six PVC pipes 10 inches in diameter, 2.5 feet long. The ends were sealed using plastic circular disks with a plastic tube and stopcock placed in the center of the base. Attached to the stopcock, there is a plastic tube 1 meter long that leads to a 1 liter graduated cylinder that is used to collect the outflow of liquid. A mesh lining was placed in the bottoms of the columns to prevent small pieces of woodchips from flowing through the bottom tubing. Four of the columns were then filled six inches from the top with dry wood chips and 150 mL of limestone (treatments 1 and 2). The two other columns were filled with dry wood chips, woodchips taken from the CR barrier and limestone (treatment 3). A mesh lining was placed over the woodchips and sand filled the remainder of the column. The sand matrix acted as a distributor so the water flows evenly into the column. The mesh lining also prevented any sand from mixing with the woodchips. To assemble the Marriot bottle apparatus, one foot of 1/10 inch diameter tubing was attached to a 5 inch glass rod that was inserted into a plastic cork. Tubing 1/16 inch diameter was then inserted into the larger tubing until the smaller tubing was extended 3.5 inches past the larger tubing. The cork was then inserted into the glass jug, so that the 1/16th inch tubing extended 2 feet and was able to drip into the column (Burce, 2005). Groundwater was collected in 20 liter carboys from a well 2.5 meters deep at WBNERR. The water was then spiked with 100uM nitrate and then poured into the glass jugs. The Marriot bottle dripped 2.7 liters a day into the column. This 2.7 liters a day corresponds to the assumption that groundwater flows a foot per a day. The columns sat in a wood shelf below the Marriot bottles in the walk in refrigerator set at 18°C in the Semester in Environmental Science (SES) laboratory. The outflow tubing was inserted into a cork and the cork was inserted into 1 L graduated cylinders which sat on milk crates slightly below the top of the columns (Fig. 3).

Sampling:

After the NITREX® barriers were assembled; the microbial communities on the woodchips were enabled to develop. The columns needed to fill up with water prior to any sample exiting the columns. Once liquid was flowing into the cylinders, the liquid was filtered using 25 mm GF/F filters and then sampled for nitrate, ammonium (NH_4^+), and total dissolved nitrogen (TDN). Approximately 20 mL was put into each scintillation vial. The NH_4^+ was preserved with 5 M hydrogen chloride (HCl). The NH_4^+ and TDN samples were stored in the refrigerator, and the NO_3^- was stored in the freezer until further analysis. After running groundwater through the systems for two weeks, the water jug of two of the barriers (Treatment Two) was filled 3/4th with saltwater and 1/4 with nitrate-spiked groundwater. The salted groundwater was then allowed to flow through the two columns for 48 hours. In addition to NO_3^- , NH_4^+ , and TDN, the columns were sampled for hydrogen sulfide (H_2S) and sulfate (SO_4^{2-}). To sample for H_2S , 250 μL was put into a scintillation vial containing 6 mL 2% zinc acetate (ZnAc) and stored in the refrigerator until further analysis. To sample for SO_4^{2-} , 15 mL was put into a scintillation vial and stored in the freezer until further analysis.

Analysis:

Analyses were performed twice during the experiment. For NH_4^+ analysis, 3 mL of sample was pipette into pre-reacted glass test tubes. To the 3 mL of sample, 0.12 mL of Phenol Solution, 0.12 mL Sodium Nitroprusside Solution, and 0.3 mL of Oxidizing Solution was added to each test tube. The test tube was mixed using the vortex mixer after each reagent was added. The samples were allowed to stand at room temperature, in the dark, for at least 1 hour. The tubes were covered in parafilm to lessen the contamination by atmospheric ammonia. The absorption was read on Shimadzu 1601 spectrophotometer at 640 nanometers (nm) (Solarzano, L. (1969).

For TDN analysis, 1 mL Potassium Persulfate Oxidizing Reagent and 1.8 mL of sample and 7.2 mL DI water was added to each test tube. The tubes were capped tightly and inverted to mix several times. The samples were then autoclaved by placing the tightly capped tubes into a metal autoclavable rack. The rack was placed in a 1 inch deep water bath. The tubes were allowed to autoclave for 1 hour. Upon completion, the test tubes were cooled to room temperature and stored in the refrigerator until samples were run (Burce, 2005).

To do analysis on the NO_3^- samples, the sample was poured into glass test tubes, approximately 3/4 full and tested for NO_3^- using a Lachat autoanalyzer. (Staff, 2005).

For H_2S analysis, 5 mL dye solution was added to the scintillation vials containing the sample and ZnAc. The vials were immediately capped after each dilution to prevent any H_2S gas from escaping. The vials were briefly shaken and allowed to stand in dark for at least 30 min. to allow the color to develop. The absorbance was then read on the spectrophotometer set at 670 nm (Gilboa-Garber, 1971).

For SO_4^{2-} analysis, the 15 mL of sample was tested for salinity using a refractometer. Once the salinity was known, the sample was diluted so the concentration of chloride was less than 1000 μM . The samples were then run on an ion chromatography (Staff, 2005).

Results

The MBL's experimental Nitrex® barriers are located in Falmouth, MA. One of the barriers is on a private beach on CR, which is indicated by the red '2'. There is high nitrogen loading due to the high housing density. The second barrier is in WBNERR, a research reserve, and experiences moderate nitrogen loadings. This barrier is indicated by the red '1' (Fig. 1).

The permeable reactive barriers are composed of the NITREX® medium and are set at the groundwater/estuarine interface so that groundwater must enter the barrier, prior to entering the ocean. There are two up gradient wells, three wells inside the barrier, and two wells downstream of the barrier (Fig. 2).

In the laboratory, the miniature barriers closely resemble the actual barriers. Using a Marriot bottle, groundwater drips at a steady rate, into the top of the column. In the column, there is a layer of sand sitting in a mesh sheet on top of the NITREX® medium. Samples are collected into a graduated cylinder that is sitting slightly below the top of the column (Fig. 3).

The groundwater collected at WBNEER from a well 2.5 m into the ground was sampled for NO_3^- , NH_4^+ , and TDN. The NH_4^+ concentration is consistently about 0 uM during the 3 weeks experiment. The NO_3^- concentration varies throughout the weeks. The groundwater was collected at different times throughout the experiment. Each time, the groundwater was spiked with 100 uM NO_3^- . During days 16 and 17, 3 parts saltwater and 1 part groundwater was mixed together. The concentration of NO_3^- during these days was only 50 uM. The concentration of TDN varies significantly through the weeks; as can be seen through the concentrations of DON. The concentration of DON was right around 100 uM throughout the three weeks (Fig. 4).

The different treatments had similar trends throughout the 3 week experiment. The NH_4^+ concentration in all three treatments peaked at day 5. Treatments 1 and 3 had NH_4^+ concentrations about 10 uM, where treatment 2 had a NH_4^+ concentration of 32 uM. The NH_4^+ concentration then leveled off at about 0 uM throughout the remainder of the experiment. In Treatment 1 and, the NO_3^- concentration followed a similar trend as the NH_4^+ . The NO_3^- peaked early on in the experiment and then declined and leveled off to about 0 uM. In Treatment 3, there was a slight peak in NO_3^- concentration in the beginning of the experiment, however, it peaked at day 12 and then leveled off to 0 uM (Fig. 5, 6, 7).

In all three treatments, the DON inflow was less than the DON outflow for about the first two weeks. Then the DON outflow became less than the DON inflow. In Treatments 1 and 3, the DON leveled off at about 75 uM, where in Treatment 2; it did not quite level off (Fig. 8, 9, 10).

In Treatments 1 and 3, the salinity is about 0 ppt. In Treatment 2, the salinity of the inflow of days 15 – 17 was 21 parts per a thousand (ppt). The outflow gradually increased in salinity to about 7 ppt. The concentration coming out is dramatically lower than the concentration coming into the microcosms (Fig. 11).

In Treatments 1 and 3, the sulfate concentration is 0 uM. In Treatment 2, the sulfate concentration of the inflow was about 18,000 uM and the outflow is only about 5,000 uM. The sulfate concentration of the outflow is dramatically lower than the concentration coming into the microcosms (Fig. 12).

In Treatment 1 and 3, there is no H_2S present in the barriers. However, after day 15, there is H_2S present in the barriers of Treatment 2. The concentration of H_2S fluctuates throughout the remainder of the treatment, peaking at 0.07 uM (Fig. 13).

Discussion

I was expecting to see consistent concentrations of NH_4^+ , NO_3^- , and TDN in the groundwater over the three weeks. To further analyze the results from the TDN analysis, the DON was determined taking into consideration that $\text{TDN} - \text{DIN} = \text{DON}$. In the groundwater there was not consistency over the three collection points. The NO_3^- and DON varied. I assumed the groundwater contained approximately 50 uM NO_3^- , however, the groundwater

ended up containing about 25 μM on two occasions and on the third contained 75 μM prior to being spiked (Fig. 4).

The outflow trends seen with the NH_4^+ are expected. There should be little NH_4^+ present in the system to begin with. Once the microbial communities responsible for denitrification develop over time, they drive the system anaerobic and eliminate all the NH_4^+ . In the three different treatments, the overall trends were consistent. During the first few days, there are small concentrations of NH_4^+ present in the barriers. This is due to the possible leaching of NH_4^+ from the woodchips. Once the systems are driven anaerobic, the concentration of NH_4^+ levels off to about 0 μM . The trends seen with the NO_3^- are similar to the NH_4^+ results. The systems undergo a transition period and then level off. However, the NO_3^- is clearly being reduced in all three systems because the NO_3^- inflow concentration ranges from about 125 – 175 μM , and the outflow concentration is very close to 0 μM after just 5 days in all three treatments (Fig. 5, 6, 7).

The DON in all three treatments were larger in the outflow than in the inflow for the first two weeks. This is presumably due to DON removal. Once the barriers stabilized and passed the overall transition period, the DON of the inflow fell below the DON of the outflow. The DON of the inflow leveled off in Treatments 1 and 3 to about 75 μM . In Treatment 2, the DON of the outflow never completely leveled off. The DON ‘scooped’ and could be due to the saltwater perturbation. However, this is a definite reason for the ‘scoop’ since the difference in concentration is very minimal (Fig. 8, 9, 10).

In order to determine whether or not saltwater would affect the barriers ability to remove nitrogen, after two weeks, Treatment 2 was exposed to 3 parts saltwater and 1 part NO_3^- spiked well water for 2 days. This was done to simulate a storm entering the barrier. The miniature barriers had 5.4 L, 21 ppt saltwater passed through them. Because sulfate-reducers and nitrate reducers live amongst each other, it was not certain whether or not the competition between the two might alter the nitrate-reducers’ ability to remove the NO_3^- in the groundwater. The saltwater was added as one great sum over a 48-hour time period. However, there is a residence time, and the saltwater did not exit the barrier in one great sum. This was determined from both the concentrations of salinity and hydrogen sulfate. The salinity concentration of the inflow was 21 ppt, but the outflow was 7 ppt at the peak. This indicated that the saltwater gradually exited the barrier. Some of the saltwater got stuck in the pores and therefore did not exit the barriers at 21 ppt (Fig. 11). The SO_4^{2-} followed the exact pattern seen with the salinity. The SO_4^{2-} went into the barrier at about 18,000 μM but exited the barrier at about 5,000 μM . The SO_4^{2-} showed the same gradual pattern as the salinity, which supported that there is a residence time enabling the saltwater to exit the barriers gradually rather than in one great sum (Fig. 12). Sulfate reduction takes sulfate and reduces it to hydrogen sulfide. If sulfate-reduction was not taking place, then there would be no hydrogen sulfide present in the outflow of the barriers. In the two saltwater perturbed barriers there was H_2S produced. The concentration of H_2S was not a substantial amount; however, enough to indicate that sulfate-reduction was in fact taking place (Fig. 3).

Adding saltwater in the concentration I did, for as long as I did had no immediate affects on the ability of NO_3^- to be removed. When comparing the concentrations of NO_3^- in all three treatments, there are no distinct differences. In treatment 2, when the saltwater was added for 2 days, there still was 0 μM NO_3^- , therefore based on my results, bringing saltwater into the barriers by a storm would not result in the inability to remove NO_3^- from the groundwater (Fig. 6).

Recent unpublished studies indicate that the barrier is being exposed to saltwater with every high tide. This being the case, the amount of saltwater added to my experimental barriers is very small relative to what the barriers are actually getting. Future studies might focus on exposing the barrier to a much larger amount of saltwater and then determining if the ability to remove nitrogen is altered. More saltwater will result in larger amounts of hydrogen sulfide produced, which might result in sulfate reduction to ammonia (Brunet, 1996).

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Literature Cited

- Blowes, D.W. Ptacek, C.J. Benner, S.G. McRae, C.W.T, Bennett, T.A. and Puls, R.W. (2000) Treatment of inorganic contaminant using permeable reactive barriers. *Journal of Contaminant Hydrology* **45**, 123 – 137.
- Brunet, R.C. and Garcia-Gil, L.J. (1996) Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiology Ecology*. **21**: 131 - 138
- Bowen, J.L. and Valiela, I. (2001) The ecological effects of urbanization of coastal watersheds historical increase in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Can.J.Fish.Aquat.Sci.* **58**, 1489-1500.
- Burce, A. (2005) Marriot Bottle Procedure. *Semester in Environmental Science*, Marine Biological Laboratory.
- Burce, A. (2005) TDN procedure modified. *Laboratory Manual for Semester in Environmental Science*, Marine Biological Laboratory.
- Gilboa-Garber, N. (1971). Direct spectrophotometric determination of inorganic sulfide in biological materials and in other complex mixtures. *Analytical Biochemistry* **43**: 129 – 133.
- Joye, S.B. and Hollibaughm J.T. (1995) Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* **270**, 623 – 625.
- Rabalais, N.N., Turner, R.E., and Wiseman, W.J. (2002) Gulf of Mexico Hypoxia, a.k.a. “The dead zone”. *Annu.Rev.Ecol.Syst.* **33**, 235 – 263.
- Robertson, W.D. Blowes, D.W., Ptacek, C.J., and Cherry, J.A. (2000) Long-term performance of

- in situ reactive barriers for nitrate remediation. *Ground Water* **38**, 689 - 695.
- Robertson, W.D. and Cherry, J.A. (1995) In situ denitrification of septic-system nitrate using reactive porous media barriers: field trials. *Ground Water* **33**, 99 - 111.
- Robertson, W.D. Ford, G.I, and Lombardo, P.S. (2004) Wood-based filter for nitrate removal in septic systems. *American Society of Agricultural Engineers*. **48(1)**, 121 -128.
- Robertson, W.D. Yeung, N. vanDriel, P.W. and Lombardo, P.S. (2004) High permeability layers for remediation of ground water; go wide, not deep. *Ground Water*.
- Schipper, L.A. Barkle, G.F. Hadfield, J.C., Vojvodic-Vukovic, and M. Burgess, C.P. (2004) Hydraulic constraints on the performance of a groundwater denitrification wall for nitrate removal from shallow groundwater. *Journal of Contaminant Hydrology* **69**, 263 – 279.
- Solarzano, L. (1969). Determination of ammonium in natural waters by phenol hypochlorite method. *Limnology Oceanography*, **14**: 799 – 800.
- Staff, SES. (2005) LCHAT Startup. Laboratory Manual for Semester in Environmental Science, Marine Biological Laboratory.
- Staff, SES. (2005) Dionex-120 Manual. Laboratory Manual for Semester in Environmental Science, Marine Biological Laboratory.
- Vallino, J. (2004) Effectiveness of Reactive Barriers for Reducing N-loading to the Coastal Zone. *Project Proposal*, 1 – 11.

Figures and Tables

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- Figure 11: Salinity of inflow and outflow of Treatment Two.
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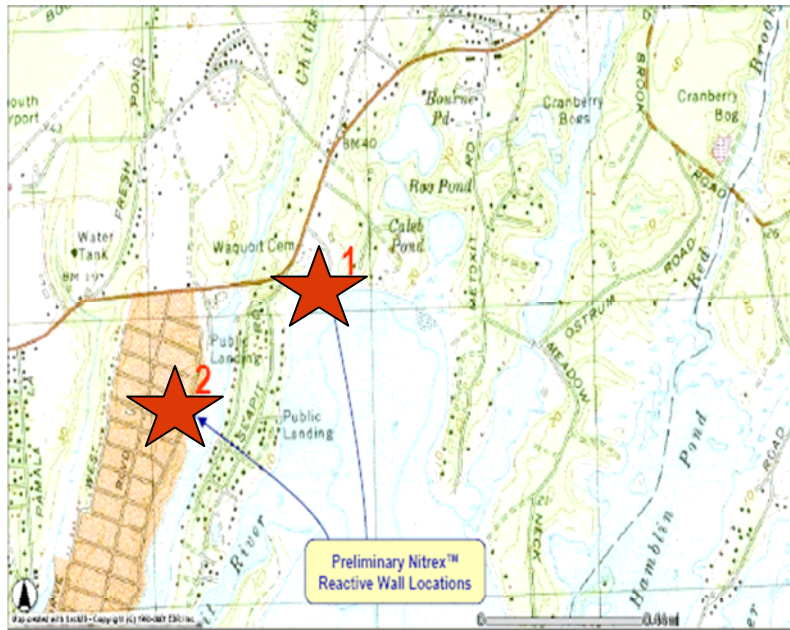


Figure 1: Locations of experimental NITREX ® barriers in Falmouth, MA.

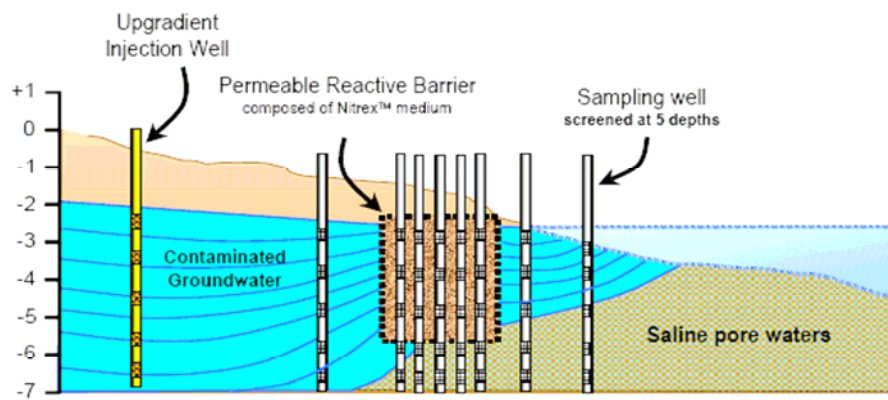


Figure 2: Setup of permeable reactive barrier placed at Child's River and Waquiot Bay.

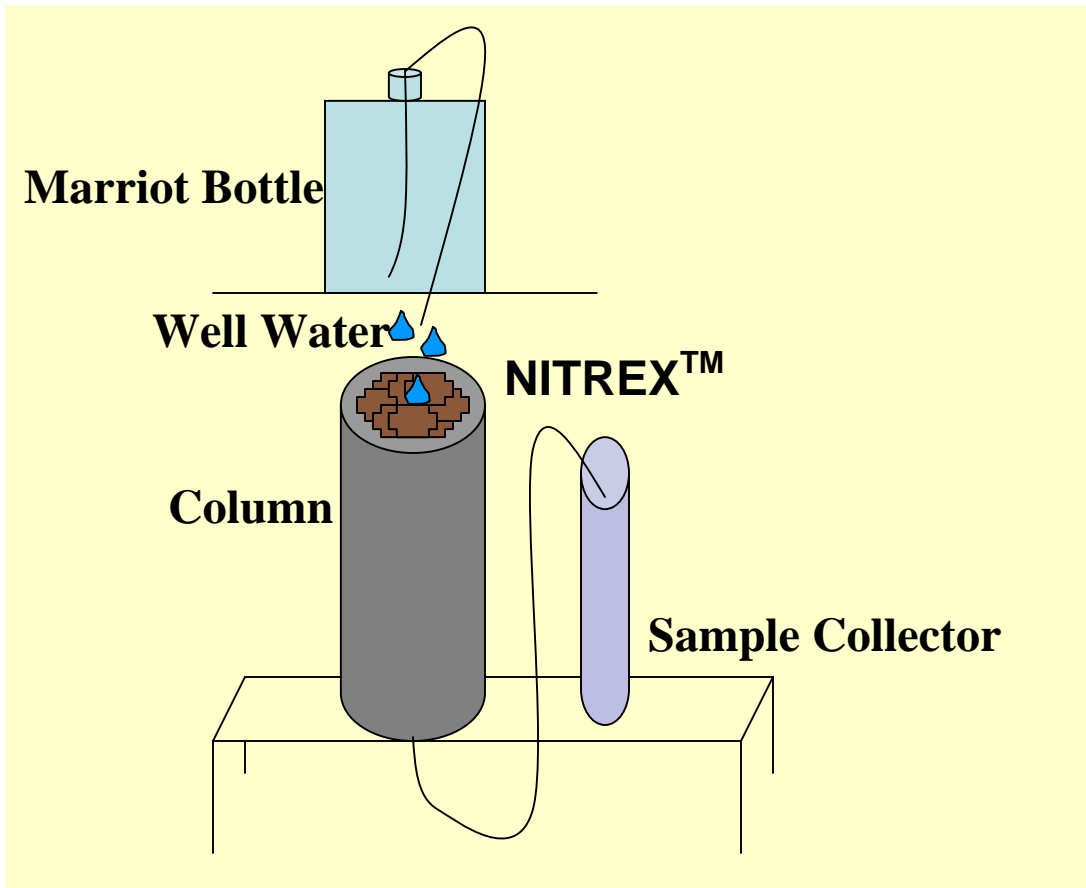


Figure 3: Set-up of miniature NITREX® barriers in the laboratory.

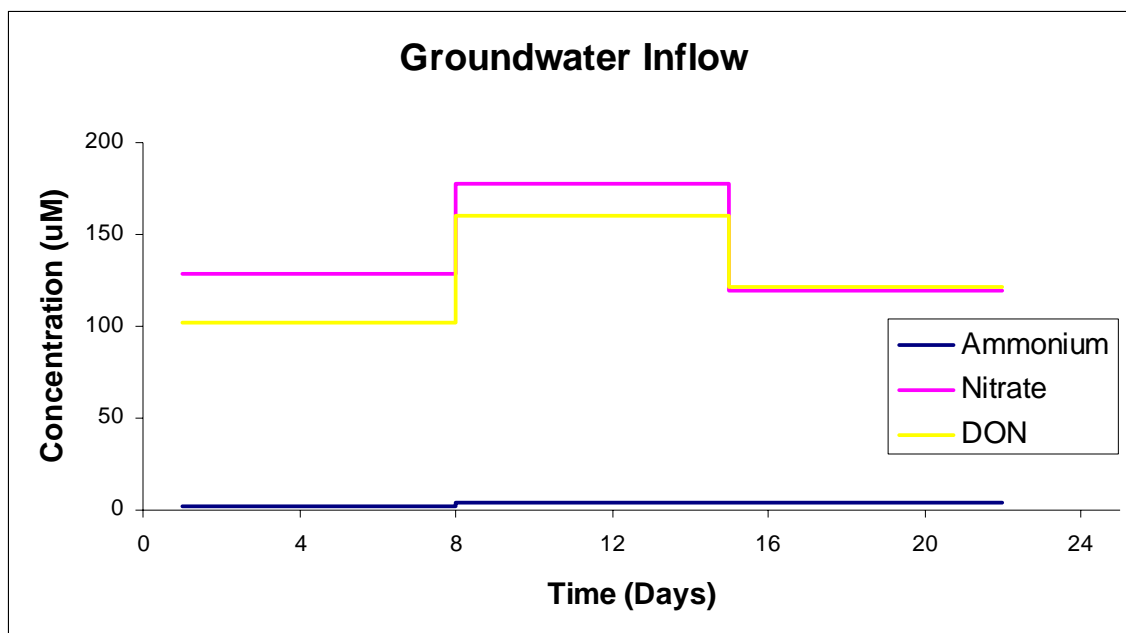


Figure 4: Concentrations (uM) of ammonium, nitrate, and dissolved organic nitrogen in the spiked groundwater obtained from a 2.5 m well at Waquiot Bay National Estuarine Research Reserve.

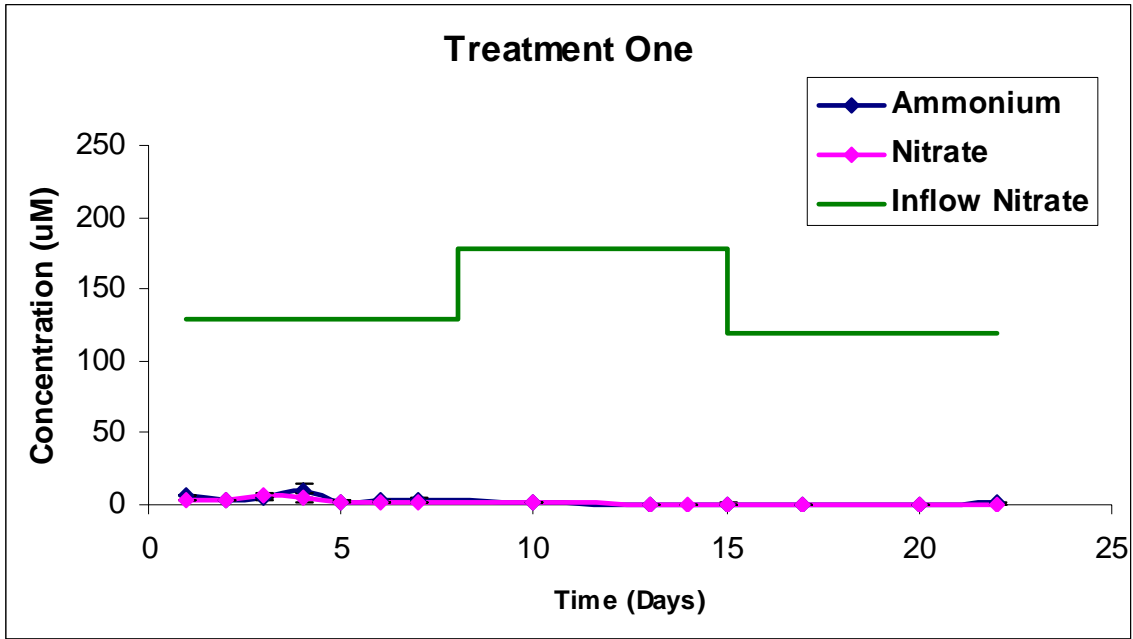


Figure 5: Concentrations (uM) of inflow nitrate and ammonium and nitrate in outflow of Treatment One.

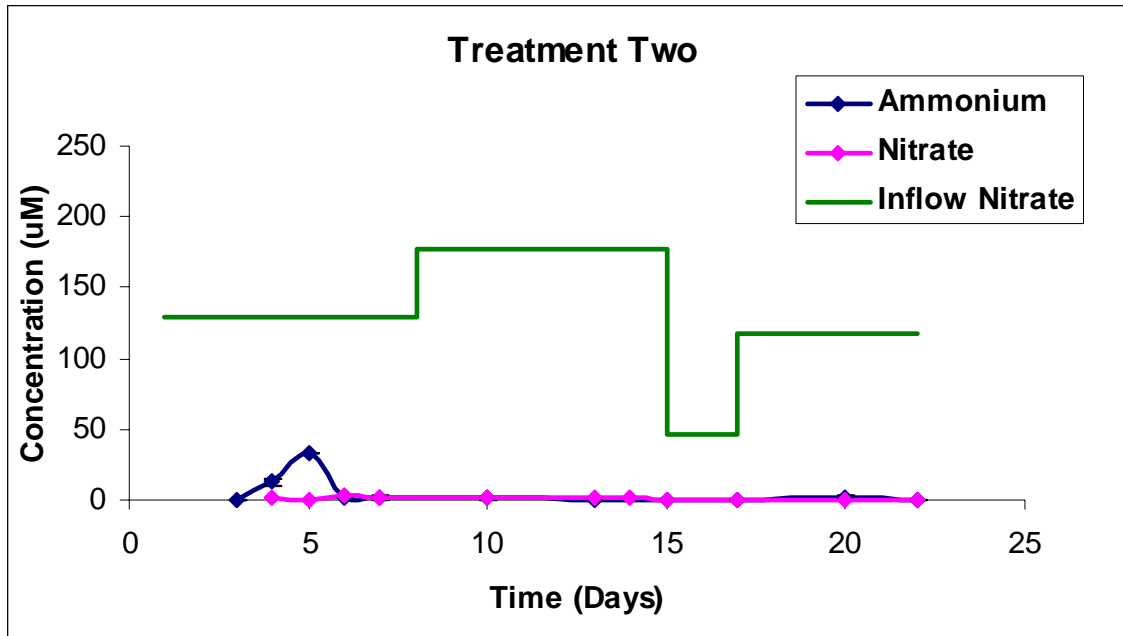


Figure 6: Concentrations (uM) of inflow nitrate and ammonium and nitrate in outflow of Treatment Two.

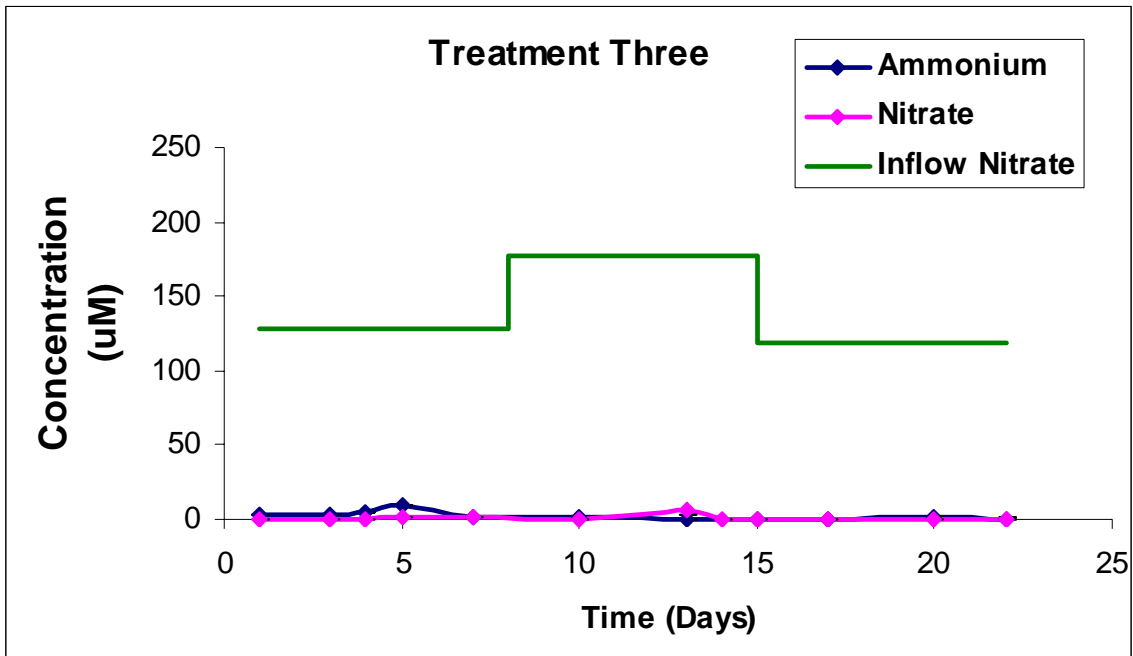


Figure 7: Concentrations (uM) of inflow nitrate and ammonium and nitrate in outflow of Treatment Three.

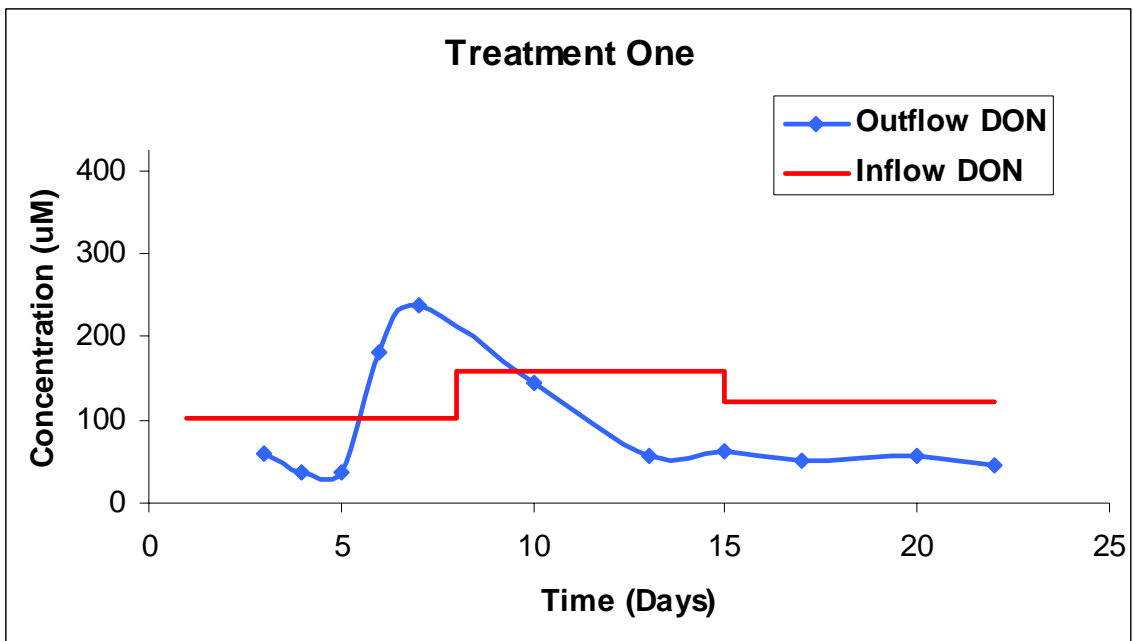


Figure 8: Concentrations (uM) of inflow dissolved organic nitrogen and outflow dissolved organic nitrogen in Treatment One.

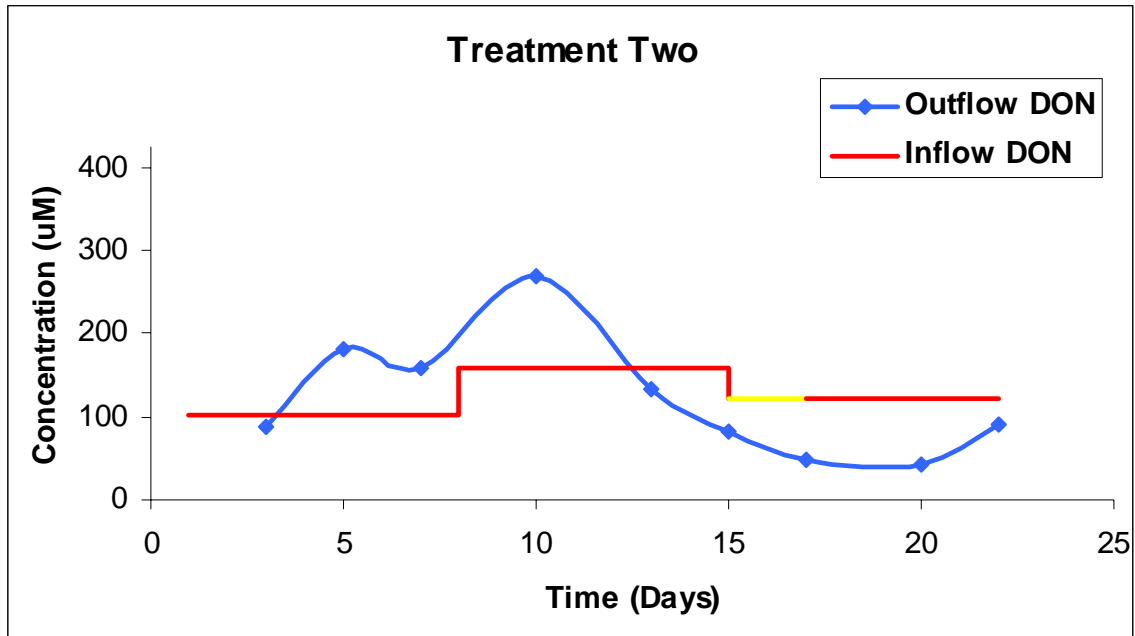


Figure 9: Concentrations (uM) of outflow dissolved organic nitrogen and inflow dissolved organic nitrogen in Treatment Two.

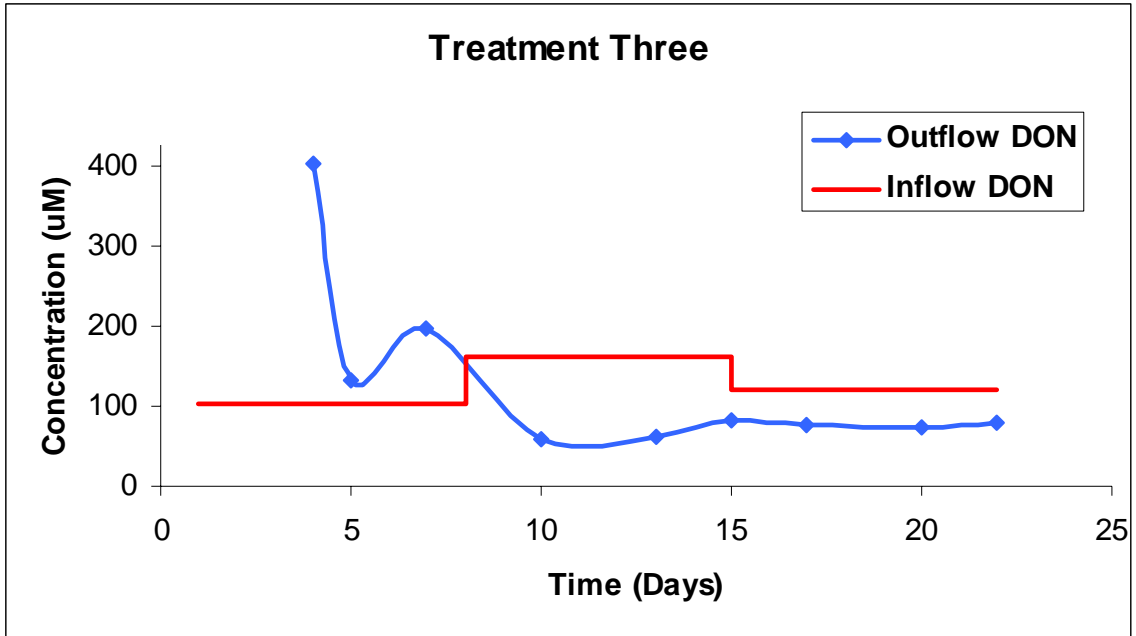


Figure 10: Concentrations (uM) of outflow dissolved organic nitrogen and inflow dissolved organic nitrogen in Treatment Three.

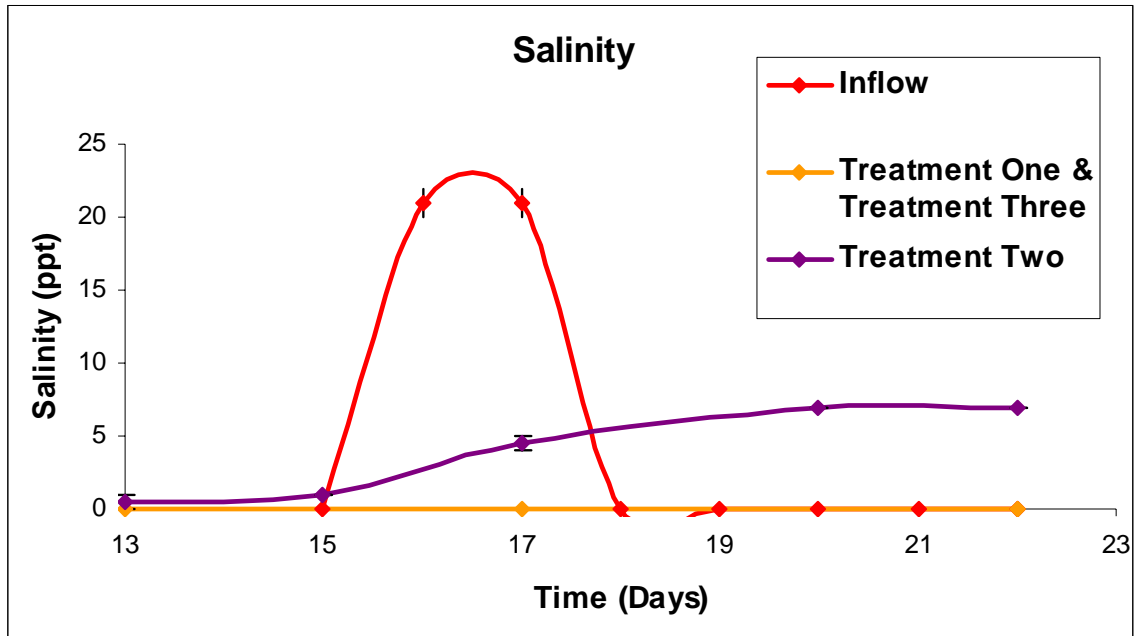


Figure 11: Salinity (ppt) of inflow and outflow of Treatment One, Two and Three.

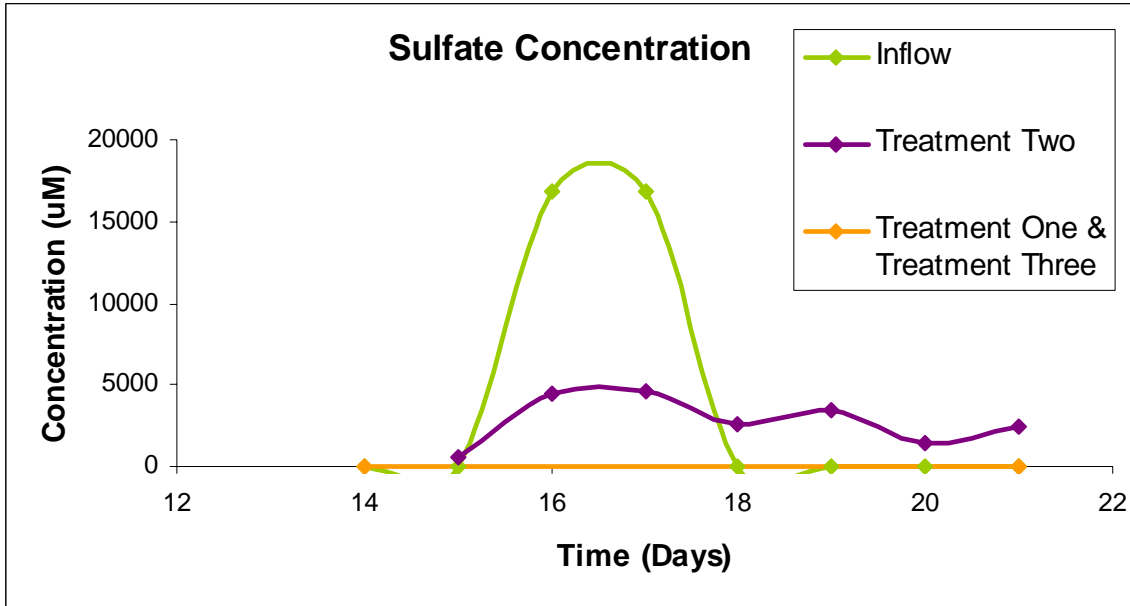


Figure 12: Sulfate concentrations (uM) in inflow and outflow of Treatment One, Two, and Three.

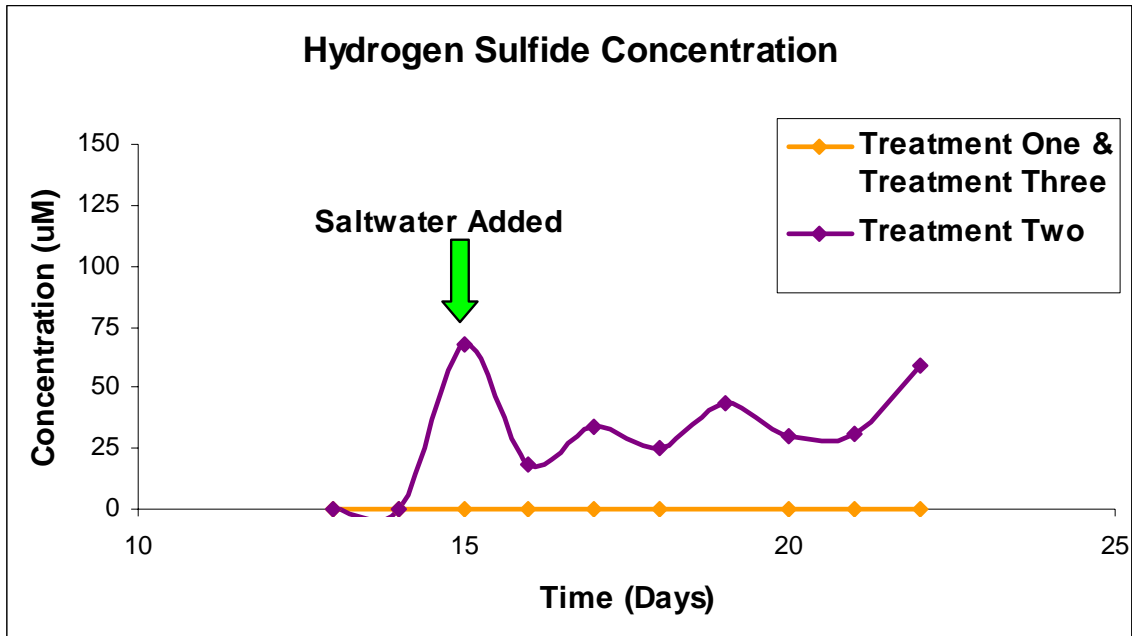


Figure 13: Concentrations (uM) of hydrogen sulfide in outflow of Treatment One, Two and Three.