Desalination & Water Purification Research
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Consequences and Possible Solutions for Small Scale Saline Water Residue Disposal in New Mexico – Proof of Concept

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by

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Disclaimer

Acknowledgements

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Glossary

454-pyrosequencing – An advanced lower cost method of pyrosequencing (see pyrosequencing).

Ascomycota – A division name from the kingdom Fungi.

Biomat – A moist layer of bateria that forms under a septic tank leach field. As leach field water passes through this layer into the soil below, bacteria is removed from the water.

Desalinization – refers to many different processes where varring amounts of salt and or other minerals are removed from saline water.

Dothideomycetes – A large diverse class of fungi.

Gammaproteobacteria – A class of several groups of bacteria. A number of pathogens belong to this class.

Heterotrophic Bacteria – Bacteria that use organic compounds that contain carbon as a source of energy and carbon.

Humidification – The process of adding water.

mothur – An open-source software platform that is used to analyze community sequence data.

Pyrosequencing – is a method of DNA sequencing. It is used to determine the order of nucleotides in DNA.

Reverse osmosis – A water purification process where water is forced through a semipermeable membrane. This process removes many types of molecules and ions. This process leaves a concentrate that must be disposed of.

Spectrophotometry – A method that measures the amount of light that a solution absorbs.

Executive Summary

Large amounts of saline water in New Mexico are not suitable for household, agricultural, or industrial uses. In some areas, extraction and desalination is the only viable way to deliver useable water to locations. As the water is desalinized, disposal of the resulting residue becomes a problem. It is believed that land owners will deposit the majority of this concentrate back into waste water treatment systems such as septic tanks. Very little is known about the consequences of this practice.

This study examines the consequences of depositing the saline concentrate into a septic system. The study was separated into two experiments. First effluent was collected from a septic tank. Various samples of this waste water were mixed with varying concentrations of saline water. Particle dispersion and survivability of various bacterial strains were measured.

Salinity concentrations up to 10 ppt were included. Initial microbial populations were lowest for control and treatments with a range from log 4.5 to 4.8 cfu mL^{-1}. A regrowth period followed at the end of the experiment with log 6 cfu mL^{-1} being most common. UV absorbance was used to quantify dispersion of solids and treatments produced statistically significant differences of absorbance. Diversity of bacteria and fungi were determined using 454-pyrosequencing . Treatments shared a similar distribution and richness of bacterial and fungal diversity. Operational taxonomic units (OTUs) from Gammaproteobacteria were common throughout treatments and WW control, but increased with treatment. Fungi diversity showed all OTUs belonged to Ascomycota with most being represented by Dothideomycetes members. Comparison of the distribution of microbial diversity of wastewater control and salinity treatments showed recovered populations had undergone a slight degree of selection with salinity treatment.

The second experiment was conducted on the leach field. In this experiment, saline water was injected directly into the upstream side of the leach field. Infiltration tests were taken at the beginning and the end of the experiment. These tests indicated that there was a decrease in infiltration over the time that saline water was injected into the leach field.

While the 5% clay content in the leach field soil would likely result in dispersion of clay particles that would influence the reduction in infiltration, it is believed that the soil clay content is not the governing factor. Instead the main contributor is believed to be the dissipation of precipitates from a chemical reaction between the various salts in the saline concentrate and carbon dioxide that is emitted from the bacterial activity in the leach field soil. Biomat formation may also reduce infiltration, but there was not enough time for a significant formation of the biomat.

As reverse osmosis technology is refined and efficiency improves, the concentration of contaminants will increase to levels that have been shown to negatively impact the performance of small flow wastewater systems. As septic systems are permitted and installed using existing standards, a septic system that has been compromised by the disposal of salt-rich reject water presents a risk to the surrounding environment and the public health.

This study tried to address the following questions. What are the impacts of concentrate disposal into small sewage systems? Are existing standards for small flow systems sufficient to allow for disposal of reject flows without presenting a risk to public health?

Background

Many water sources in New Mexico contain varying concentrations of dissolved inorganic salts and are not suitable for human consumption or use. Whittier and Goldstein (1986) estimate that New Mexico has about 200 billion acre feet of saline groundwater. Much of this water has a saline content of 1000 ppm or higher of total dissolved solids (TDS). This water is not suitable for human consumption unless it undergoes a desalinization process.

There are a number of farms, ranches, and small communities throughout New Mexico where the only source of water is saline water. In order to make these water sources useable for human consumption, some form of desalinization must be performed. There are a number of desalinization processes ranging from various distillation processes, reverse osmosis, and humidification (Whitworth and Lee, 2003). Regardless of the process, there is always a residue/byproduct that is left after desalinization (Whitworth and Lee, 2003). While small scale desalinization technologies become cost effective, there is very little information available about economically viable disposal of the residue/byproduct. There is a risk that the desalination technologies may be applied without regard for managing the residual concentrate and in the absence of a proven solution, inappropriate disposal may occur.

As reverse osmosis technology has advanced, the reported efficiency of the systems has increased several fold. The first commercially available residential systems wasted upwards of 80 percent of the source stream. Newer systems are efficient enough to produce a more concentrated reject stream of less than 10%. The technology has outpaced the understanding of its potential impact on a small waste water system treatment system. Where previous systems could be installed based on historical performance, risk that a newer system is installed and plumbed into a septic system without a better understanding of the impact is significant.

Additionally, the risk is not limited to the septic system performance itself. It is believed that the concentrations of pollutants may reach a level where the biomat of the leach field distribution piping is compromised. If this is the case, commonly accepted design and implementation practices would no longer apply and the potential for under-treated sewage reaching the groundwater is increased. The potential of pathogens passing through a damaged or compromised leach field biomat present a risk to the public health.

Most of the research directed at the impact of salt-rich discharge into small systems has focused on the use of chemical water softeners. In these cases, the risk is sufficient that most reputable wastewater treatment manufacturers have clauses in their warranties voiding the warranties if water softener brine is discharged to the treating system (Gross and Bounds, 2007). As the efficiency of reverse osmosis systems improve, the resulting concentrations will reach levels that have been shown to impact the ability of a typical bio-system (Gross and Bounds, 2007).

A number of regional water budgeting and planning efforts in New Mexico have used a rule-of-thumb assumption that approximately 50 percent of the water pumped from onsite domestic wells is returned to the aquifer through the septic system (McQuillan and Basset, 2009). However in New Mexico, widespread ground-water contamination has occurred in many rural areas utilizing on-site wells and septic systems (N.M. WQCC, 2002a). Effluent discharged to the subsurface by drainfields often percolates into the same aquifer tapped by wells for domestic supply. In New Mexico, on-site septic systems have contaminated more acre-feet of ground water, and more public and private water supply wells, than all other sources combined (McQuillan, 2004)

It was suspected that additions of concentrate to a septic system can have both immediate and accumulated impacts. In this study two separate sets of experiments were designed to estimate both immediate and accumulated impacts. The sensitivity of a bio-system to increasing levels of concentrate can be accomplished in a laboratory setting using field samples. In other words, field samples will be collected from a septic system and taken to a lab where saline concentrates will be mixed into the sample. The samples will then be analyzed for the effect that the concentrate has on the biological organisms within the sample.

There is a gap in knowledge on the impact to the microbial community of the septic system. Previous work has shown that septic systems and associated leach field biomats are unique at the microbial level (Tomaras, Sahl, et al., 2009). Sudden changes such as increasing the concentration of NaCl can have an impact on the activity of bacteria involved in reduction of organic pollutants (Cortés-Lorenzo, Rodríguez-Díaz, et al., 2012). Here we investigate the impact of saline concentrate application into wastewater by studying microbial diversity and viability.

The original plan for this project was to inject saline water into the septic tank and try to determine the effect on the septic tank water as well as the leach field. Prior to the selection of a research site, the researchers became concerned with the possible results of injecting the saline water into the septic tank. Moore (2001) claimed that organic particles in the septic tank would disperse with the introduction of saline water. If this occurred the septic field would likely see an increase in particle deposits from this dispersion. While this is an important conclusion to draw from this research, it was determined that the same possible outcome could be determined by taking samples from the septic system and conducting experiments in the lab where the saline water is mixed with the septic tank effluent. Conducting these tests in the laboratory would make particle dispersion measurement easier.

In addition, the study wanted to determine the effect that saline water had in the leach field. It would be very difficult to separate the effect that saline water had on the leach field with the addition of particles from the effluent stream. It was concluded that if the saline water was injected into the leach field the effect that this would have on the leach field could be separated from the deposit of effluent particles.

For these reasons, this study was separated into two experiments. One focused on the effect that saline water had on the septic tank effluent and the other examined the effect that saline water had on the leach field. The findings from these two experiments are reported below.

While this study will help to understand the consequences of introducing saline concentrates into the waste streams of small septic systems, there is more that will need to be understood. Additional studies should address what the consequences of adding these concentrates into larger municipal systems and industrial treatment systems. While the data from this study can be used to make assumptions, complete understanding can only be accomplished by studying these larger systems as well.

Conclusions and Recommendations

Saline Concentrate Lab Test Conclusions

Increased salinity selected for bacterial populations that included a subset of the WW sample. Results suggest that increased salinity produced shifts in the microbial diversity. Implementation of this method is likely to reduce some bacterial activity but bacterial viability is not much of a concern. The data suggest an increase in dispersion will be observed with increased salinity. Particle dispersion will likely send particles into the leach field before they are completely digested. As these particles are deposited, there would be a reduction in infiltration.

Leach Field Test Conclusions

The change in permeability rate for the leach field may be attributed to three things. The first may be due to the incorporation of salts into the clay soil below the leach field. This would likely disperse the clay particles and reduce the rate of water movement through the soil. However, considering that the clay content in the soil was about 5%, it is believed that the clay dispersion would have a limited effect. Corey *et al.*, (1977) claims that clay is not a problem unless the soil has a clay content over 15%.

The second thing that could contribute to the lower water movement is possibly the formation of a biomat below the leach field. A properly functioning leach field will have a biomat develop below it, but it takes 3 to 8 years for a biomat to completely form (Farrell-Poe, 2014). Farrell-Poe (2014) indicates that at the most a biomat could only have formed under half of the leach field in one year. If this is the case, the biomat will have little to no effect on the infiltration of water into the leach field.

It is believed that the main contributing factor to the infiltration reduction is the deposition of precipitates in the leach field. The bacterial action in the leach field will produce carbon dioxide. This will likely combine with calcium, magnesium, potassium, and sodium in the saline concentrate. The result would be the formation of carbonate precipitates. These precipitates will also fill soil voids and reduce infiltration.

Final Conclusions

Depositing saline concentrate into a septic system appears to have multiple consequences. While there is limited impact on survivability of the organic particles in the septic system, the particles are dispersed and will likely become part of the stream that flows into the leach field. This will eventually limit the infiltration of the leach field.

It also appears that depositing saline concentrate in the septic system will dissipate clay particles and likely cause chemical reactions that will result in the deposit of precipitates in the soil. Both of these results will reduce leach field infiltration rates.

Presently it appears that there is no elegant solution for how saline concentrate can or should be treated when it is removed from the water supply. This waste stream should not be deposited into the septic system. Instead, this concentrate should be deposited in a properly designed evaporation pond or some other safe place. This would also apply to a small community that may have to remove salts from their water supply. However, a small community may be able to develop an underground injection site that the concentrate can be safely deposited into.

It may be suggested to add chemicals such as acid from time to time to try to free salts from the leach field soil. This would likely damage the biomat and risk polluting the ground water.

Recommendations

This work has only considered the consequences of depositing saline water into the septic tank for a 1 year period. Further study is needed to understand the long term consequences. While this work showed that there is limited negative effects on the bacteria in the septic tank we were not able to determine what would happen to the biomat under a leach field. It takes several years to form a biomat and we do not understand if saline water would limit biomat formation. The biomat helps to purify septic water before it flows into the ground water. Understanding the effects that saline concentrate has on biomat formation needs to be understood.

In addition, the deposition of participates in the leach field over a longer period of time needs to be understood. It is believed that the reaction between carbon dioxide and the salts in the saline water would eventually reduce leach field permeability to zero. However, this needs to be verified.

Methods for Mixing Saline Concentrate with Septic Tank Effluent

Sampling

Saline solution was sampled from a desalination plant located in Alamogordo, NM. A volume of 8 L was collected in plastic containers which were DI washed and sterilized prior to sampling. The solutions were kept at room temperature in the lab until use.

Wastewater was collected from a residential septic tank located near Las Cruces, NM. The septic tank is a one family unit featuring a typical two-chamber system. A wastewater sample was retrieved from the septic tank outlet to leach-field (i.e. tank effluent). The wastewater sample was immediately transported to the lab, stored at 4 °C, and used the same day.

Physico-chemical analyses

EC was tested using a bench top Fisher Scientific conductivity meter. pH was tested using a Beckman Phi 72 pH meter. Dissolved oxygen (DO) was determined at the time of sampling using a multi-parameter Hach field test kit (FF-1A). TSS was determined using gravimetric methods.

A survey of the chemical profile of both wastewater and saline solution was carried out on an ICP/OES Optima 4300 DV system according to the standard EPA method

200.7 (EPA, 1994).

Experimental setup

For most tests, a 100 mL 1:1 saline concentrate to wastewater reaction mixtures were prepared. Four levels of saline concentrate treatments were used including a 10 ppt stock and three dilutions of the stock using DI water yielding saline solutions at 6 ppt, 4 ppt, and 2.6 ppt. A reaction with only wastewater (WW) served as one control and a second reaction with only saline concentrate (SS) served as a second control. A third control included wastewater mixed with deionized water (WW-DI).

In a second experiment, a higher ratio of 3:1 saline concentrate to wastewater volume was prepared while maintaining the same overall volume of 100 mL. The same final salinity concentrations were as above.

All reactions were performed in triplicate in 500 mL Erlenmeyer flasks. All glassware were acid bath washed and autoclaved. Flasks were aluminum sealed and placed on orbital shaking at 30 rpm. Reactions were held at room temperature and in dark conditions.

UV/Vis Spectrophotometry

Absorbance as an indicator of dispersion was detected by UV/Vis spectrophotometry using a Genesys 20 spectrophotometer at 600 nm wavelength and 1 cm path length. Plastic disposable cuvettes were used using 2 mL assay volume. All readings were performed in triplicate.

Bacteria viability

Heterotrophic bacterial counts were determined by incubation on Mueller-Hinton Agar (MHA). High motility/swarming was observed on pre-test, therefore the filter plate method was performed instead of the agar sweep method. Dilutions ranging from 10^{-3} to 10^{-5} mL were prepared. Incubation was carried out for 36 hours at 37 °C.

DNA extraction and sequencing

Several centrifugation steps were performed to pellet and wash the bacteria from the 80 mL samples. DNA was extracted using a MoBio PowerSoil DNA isolation kit according to the manufacturer’s protocol. The final dilution volume was 100 µL. All extractions were performed in duplicate and were composited for downstream application.

DNA was quantified on a NanoDrop 1000. For most samples DNA yields were 20 ng µL^{-1} range. Only 2 ng of DNA were measured per µL of the saline concentrate (SS). Samples were analyzed by 454 pyrosequencing at Molecular Research MRDNA in Shallowater, TX. Bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) was performed using primers 28F (GAGTTTGATCNTGGCTCAG) and 519R (GTNTTACNGCGGCKGCTG) covering the V1 to V3 region of the 16S rRNA (Erb-Downward et al., 2011). Fungal primers EndoITSF (AAGGTCTCCGTAGGTGAAC) and EndoITSR (GTATCCCTACCTGATCCGAG) were used which sequence the internal transcribed spacer region (Lucero, Unc, et al., 2011). As described previously (Dowd et al., 2008) a 30-cycle single amplification with 1 U HotStarTaq Plus Master Mix kit (Qiagen) was used to amplify sequences prior to sequencing on Roche 454 Titanium instrument using recommended guidelines and reagents.

For bacteria, mothur 1.32.0 (Schloss et al., 2011) was used for analysis of sequencing results using a standard analysis approach. Sequences 200 bp or greater of high quality (qwindowaverage criteria in mothur) and with homopolymer not greater than 6 were kept. Clustering was calculated at 0.03 and OTUs were classified using the RDP taxonomy in mothur. A taxonomic approach was used for fungi pyrosequencing results. A similar workflow to bacteria was implemented for fungi to eliminate bad quality sequences. BLASTn was performed using a custom curated GreenGenes database at MRDNA (Dowd, Callaway, et al., 2008).

Methods for Addition of Saline Concentrate in the Leach Field Test

The goal of this portion of the project was to determine the impact that concentrated saline water would have on the leach field of a septic system. Richards et al. (1954) and Brady and Weil (2010) report that if saline water is allowed to flow over and through a soil that contains clay, there will be a dispersal of clay particles. As clay particles disperse, they will fill soil voids and reduce infiltration. In order to see if this occurred in a septic tank leach field, the researchers tried to find a septic system that had a leach field located in a clay soil.

A functioning septic system that was in good condition was needed for this project. Saline concentrate was originally going to be injected into the waste stream that went into the septic tank, but that was determined to be risky considering that Moore (2001) indicates that saline water would disperse the particles in the septic tank. If this occurred, the particles would likely flow with the septic water into the leach field. This would likely reduce infiltration in the leach field. If this occurred, the experiment may not be able to determine if changes in septic field infiltration where from saline concentrate interacting with soil particles or from organic particles in the waste stream.

In case the study had a long lasting negative effect on the septic leach field, it was determined that a second leach field would be installed on a septic system. Using an actual septic system that served a household would give the most realistic results. Several sites were examined to determine their suitability for the study. One sight showed promise, but as the addition of a second leach field was considered, New Mexico State representatives informed us that all other septic tanks on the property would have to be brought to state standard requirements. The particular property had 5 septic systems. All of these sites would have to be upgraded and this requirement exceeded the funding that was available for the project.

A second site was inspected for use as a possible research site. However, the water table was high and the site would have required the addition of a soil mound and pumping system to operate the leach field. Again the cost would have exhausted the funds that were available. The high water table may also have given none typical results.

Multiple other sites were inspected for use as a research site but the area that would be required for the leach field was made up of sand and it was suspected that a leach field that was placed over a sandy soil would not give any measureable results. It was determined that the most significant results would occur if the soil below the septic tank leach field contained some clay. However, the sites that were available for this study had only sand in the leach field area.

A site was finally located where a septic system had been installed 2 years prior to this research project. An additional leach field could be connected to the original septic system with no negative effects to the system. The soil was sampled for the leach field area and sand was the primary component of the soil located in this area. As mentioned earlier, it was desired to have clay soil in the soil mix. It was suggested that a clay soil be incorporated/mixed into the sandy soil below where the leach field would be located. A trench was excavated for the leach field and clay soil was spread out over the leach field area. The clay soil was then mixed into the sandy soil. This created a clay-sand soil layer below the leach field that was about 8 to 10 inches thick. After mixing, the soil was analysed for proportions of sand, silt, and clay. It was determined that the soil was 87.8% sand, 7.23 % silt, and 4.96 % clay.

Once the soil was in place, the leach field was installed and connected to the septic system. The septic system was operated with the new leach field for approximately one month. After a month, tests were conducted to determine the leach field permeability rate. This was accomplished by pouring water down into the leach field through an observation tube. The amount of water that was poured into the leach field and the time that was required for the water to infiltrate were measured

After the permeability tests were completed, a salinity metering injection system was connected into the observation tube so that saline water could be injected into the system. A saline solution with an approximate EC of 2700 µS/cm was injected into leach field. The chemical analysis for this saline solution is shown in table 1. The metering pump was incorporated into the saline injection system with a timer so that saline water would be injected into the leach field in the morning and evening. This was done to simulate the removal of salts using an IO system at key times of the day when there would be high water use. During these high use periods, the waste concentrate would be dropped back into the house waste stream. However as mentioned earlier, the concentrate was injected into the leach field.

Injection of the saline water into the leach field continued for a period of about one year. At the end of a year the leach field was again tested to determine the permeability rate.

Results and Discussion

Chemical and Physical Parameter

Stock saline solution had a salinity of about 10,000 mg L^{-1} or 1% (w/v) solution (10 ppt). Physical and chemical parameters of both saline and wastewater solution are shown in table 1.

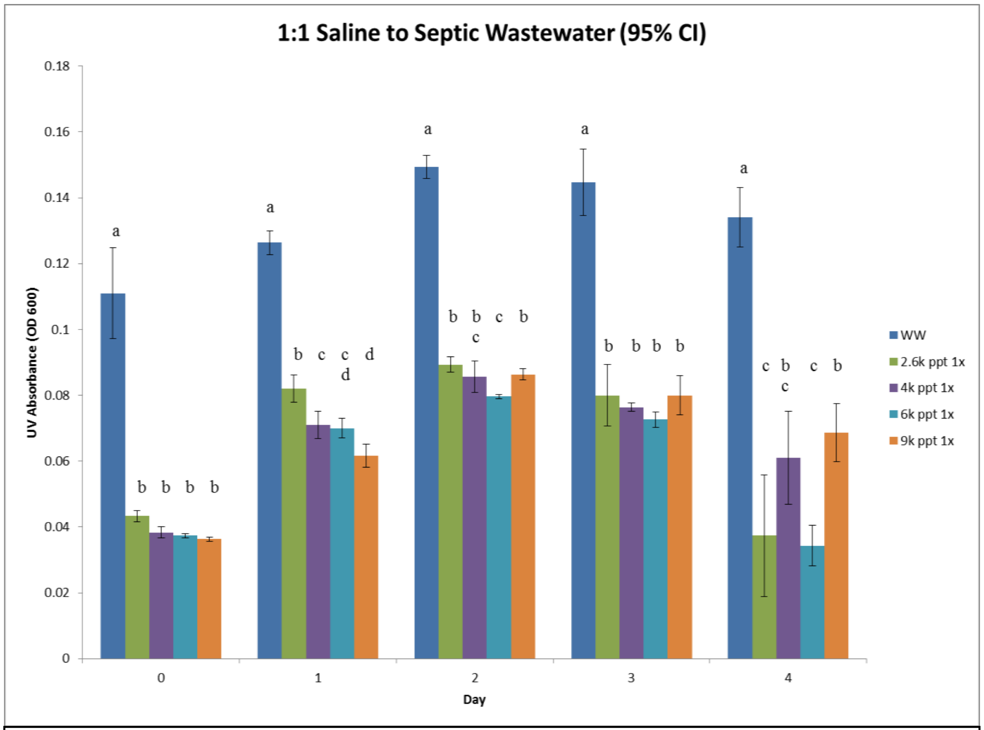
In the first experiment, solutions of 1:1 saline concentrate to wastewater were prepared with varying concentrations of salinity. UV absorbance results were measured daily during the experiment. Absorbance of experimental controls WW and SS at initial t_0 were statistically different (figure 1). Throughout the experiment, both controls had statistically significant differences in absorbance between each other and compared to the treatments. Among treatments, assays from t_0 and t_3 yielded non-significant differences in absorbance. At day t_4, two treatments, 10 ppt and 4 ppt had the highest mean absorbance; this difference was statistically significant between 10 ppt and both 2.6 and 6 ppt.

Table 1. Physico-chemical parameters of t_0 wastewater and saline concentrate samples.

	Wastewater	Saline Solution
pH	6.85	6.89
DO (mg/L)	< 0.2	1.6
EC (mS/cm)	1.04	8.98
SAR	2.47	6.98
TSS (mg/L)	34	10,600
Mg	14.46	459.27
Ca	71.74	593.18

Na	87.86	931.5
K	18	6.737
Al	0.2167	Nd
As	0.1042	0.5425
B	0.3733	0.8825
Ba	0.057	0.0369
Be	Nd	Nd
Cd	0.0018	Nd
Co	0.0023	Nd
Cr	Nd	Nd
Fe	0.274	Nd
Mn	0.0474	Nd
Mo	Nd	Nd
Ni	0.0052	0.0188
Pb	Nd	Nd
Se	0.0515	Nd
Tl	Nd	Nd
V	Nd	Nd
Zn	0.055	0.1033
Bi	Nd	Nd
Li	0.067	0.0973
P	6.006	Nd
Sr	0.6683	14.14
Si (as SiO2)	31.33	47.64
S	11.74	1271
Cu	Nd	0.6135

Note: Elemental concentration in mg/L

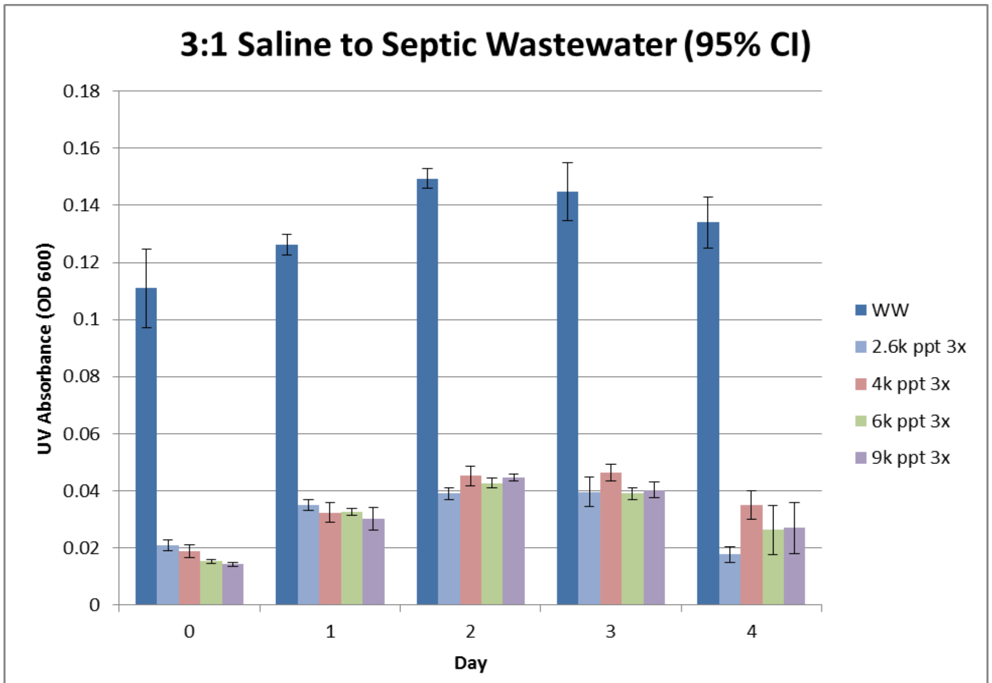


These results show that WW control and treatments increase in absorbance temporarily with a peak at t_2 followed by a decrease. The same trend was observed in the experiments with 3:1 ratio of saline to wastewater.

In the 3:1 ratio experiments, differences in the absorbance among treatments were mostly non-significant (Fig S1). At t_4, only the 4 ppt treatment had statistically higher absorbance compared to 2.6 ppt, while it was higher but not statistically significant than the absorbance measured for the 6 and 10 ppt treatments.

Bacterial viability, i.e. heterotrophic counts, were determined by MHA agar plate incubation (figure 2). The MHA agar plate incubation confirmed the lower count of recoverable bacteria at the start of the experiment with log 4.5 to 4.8 cfu mL^{-1}. The bacterial population steadily increased with a peak at t_2 associated with short-term regrowth. One treatment at 10 ppt, had a second peak in late phase monitoring (time = 6). A steady bacterial population in SS control could be detected with an average population of log 5.0 cfu mL^{-1}.

Long term bacterial abundance was monitored using spectrophotometry as described (Fig S2). These were performed in parallel to the heterotrophic counts. Results show that for most treatments and controls a baseline was reached at day 6. The one exception was the WW control for which more variability in the absorbance profiles were observed after day 10.



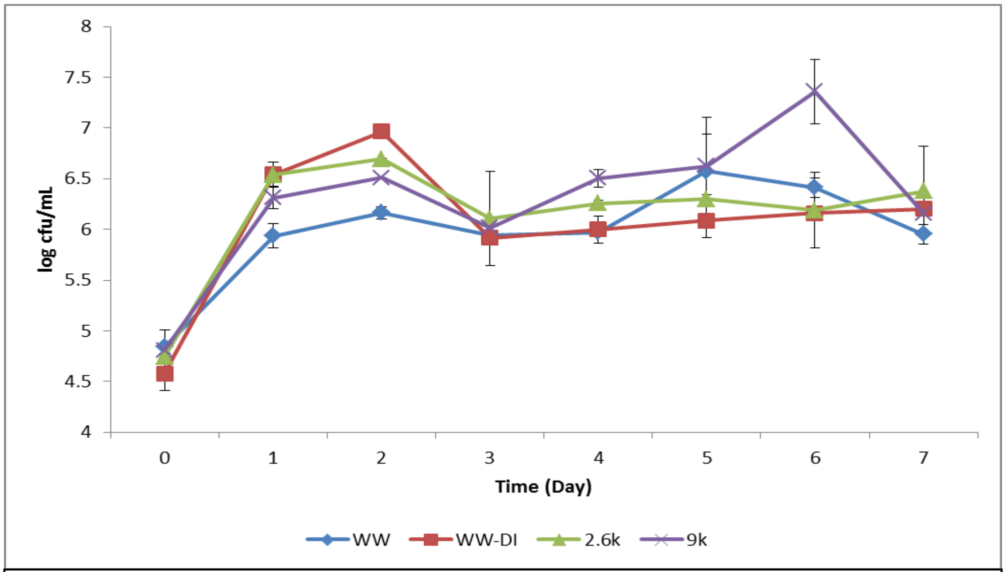


FIGURE 2. Heterotrophic plate count of cultured bacteria on MHA with statistical inference (95% CI)

The data showed that there was a significant difference among treatments when examining the UV/Vis absorbance. This would be in agreement with several studies showing selection of bacteria with salt increase (Ríos et al., 2010). Salt stress affects the microbial cell reducing the amount of solutes in the cell membrane. These effects are counteracted by different mechanisms that have been explored in salt tolerant bacteria (Tsuzuki, Moskvin, et al., 2011; Steil, Hoffmann, et al., 2003). In addition to selection of bacteria, salts are known to disperse dissolved organic carbon in soils, effects that were however related to increases in soil pH (Green et al., 2008).

Varying concentrations of salts are known to influence microbial parameters in several ways (Wong et al., 2008). Some of these are dispersion of soils and organic matter related to increase of bacterial biomass with increasing salinity. The results point to dispersion increasing at a statistically significant difference for greater concentrations of salt. Ng et al. (2005) found that wastewater in SBR encountered problems of high turbidity with increasing concentrations of NaCl up to 60 g L^{-1}. Yet, the sludge volume index was low at higher NaCl, indicating better compaction properties of sludge. In septic tanks, longer retention time have to be taken into account, which is likely to see both greater sludge compaction in both chambers and increased dispersion of small particles which will be introduced to the leach field.

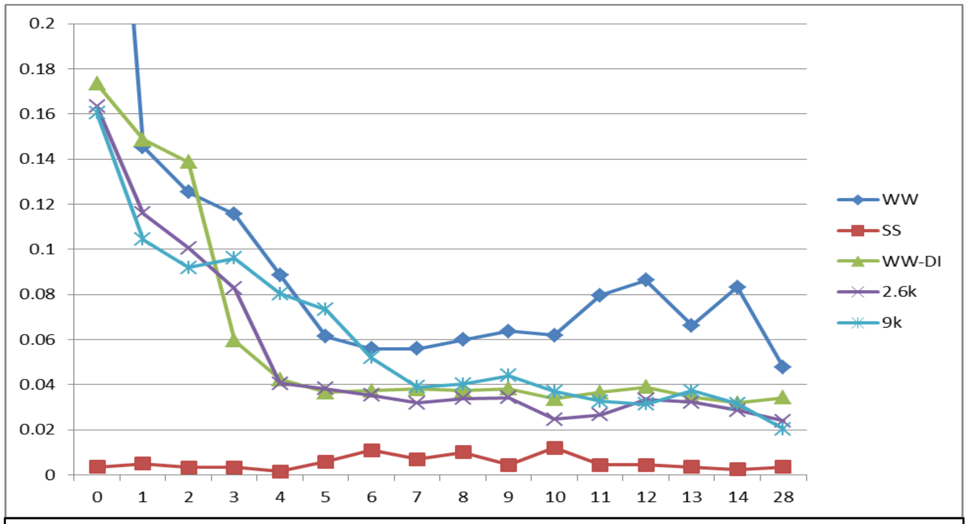


FIGURE S2. UV/Vis Absorbance of 1:1 saline to wastewater of control and treatments

Bacterial Diversity

454-pyrosequencing was performed to analyze the bacterial diversity related to treatments and controls of analyzed samples. Coverage and estimators of richness and abundance are shown in table 2. Results indicate high sequence (>98%) coverage for all samples. Control SS showed the lowest bacterial richness with 105 OTUs (Ace and Chao estimators). WW and 4 ppt samples had the greatest richness at 200 OTUs.

TABLE 2. Summary of sequences results as performed in mothur

Sample	Number of Sequences	Coverage	Ace	Chao1	Inverse Simpson
2k	4730	0.992389	155.18	148	5.02553
4k	16337	0.997062	206.9441	220.7143	3.920377

6k	7960	0.994347	192.1834	197	6.863835
9k	10008	0.996303	166.5482	170.625	3.863572
SS	1616	0.983292	106.0041	104.0714	7.374596
WW	8904	0.99562	204.6678	176.1667	7.321451

Rarefaction curves for the sequencing efforts on these samples were produced (figure S3). Results showed samples did not asymptote, but most featured very similar shapes and lengths. Treatment 4 ppt showed the most richness but this was likely a result of a higher number of tags sequenced from this sample.

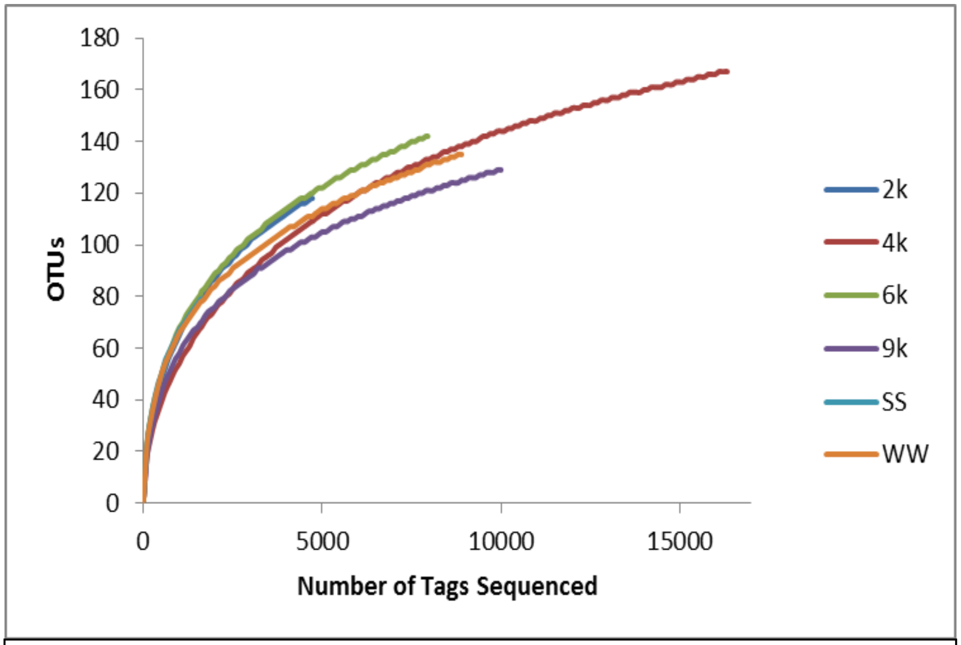


FIGURE S3. Rarefaction curves for sequenced samples. Analysis performed in mothur.

Sample similarity is graphically presented in figure 4. Results indicate that treatments are similar with each other and dissimilar from the SS control. An examination of phyla (figure 5) showed two bacterial classes in SS were Alphaproteobacteria and Actinobacteria. These were among the most common for that sample. Alternatively, Actinobacteria were not detected for WW and treatments.

For fungi, pyrosequencing efforts showed very clearly control SS to be dominated by Ascomycota members belonging to Eurotiomycetes (figure 6). Of which, all sequences were assigned putatively to the genus *Penicillium*. Sequences from this genus have been observed previously in a high salt environment (Smolyanyuk and Bilanenko, 2011).

Selection of bacteria did take place among treatments compared to the native WW microbial population. One possibility is that introduced saline offers different mechanisms and substrates which in turn offer different strategies of survival. Another possibility is that osmotic stress effects drove changes in survival by selection. For wastewater, this is a concern because of the attribution of some species to important wastewater processes. One study found that salinity concentrations of 0.5% and higher decreased the removal of nutrients from sequencing batch reactors (Intrasungkha, Keller, et al., 1999). Ammonia oxidizing bacteria, an important group of bacteria for wastewater treatment, are also known to be affected negatively by increasing salt concentration (Cantera, Jordan, et al., 2006).

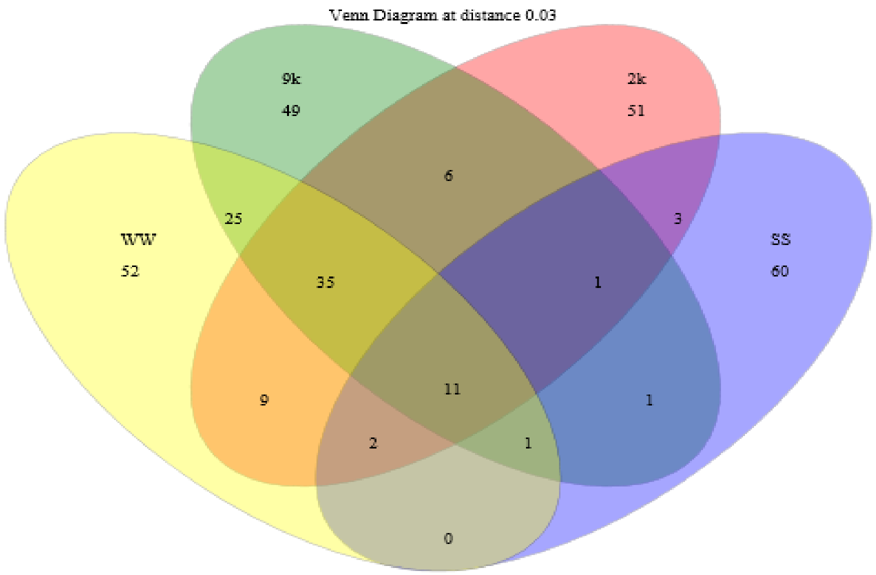


FIGURE 4. Venn diagram produced in mothur at 0.03 distances for clustering

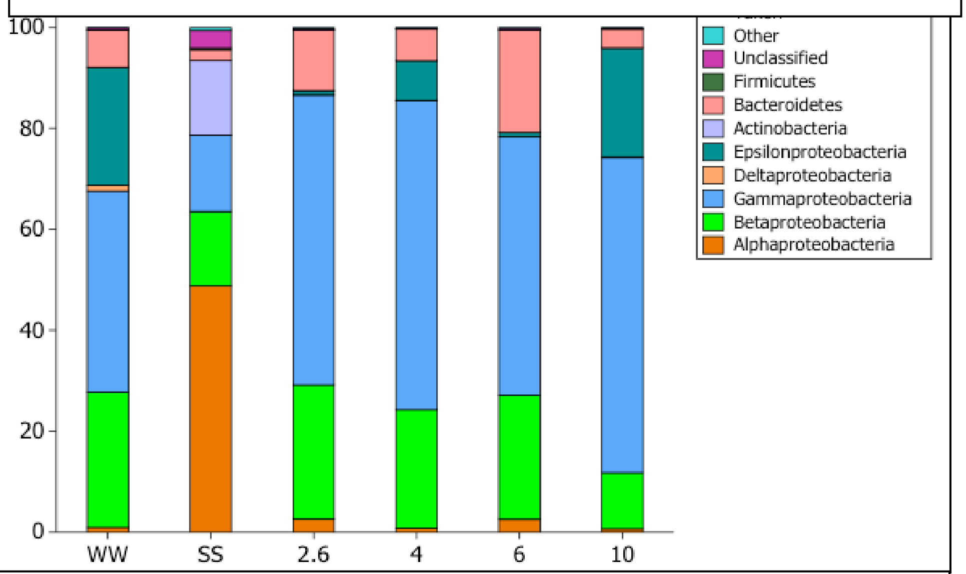


FIGURE 5. Percent of OTUs as belonging to most common bacterial phyla from sequencing efforts

The survival of indicator bacteria as well as pathogenic species are a valid concern. Increasing salt reduces wastewater bacterial activity in most aspects that are measured such as DOC and ammonia removal (Johir, Vigneswaran, et al., 2013) for very high salinity (60 g L^{-1}). At a moderate salinity concentration (10 g L^{-1}) acclimation has been reported (Linarić, Markić, et al., 2013). With longer retention time of waste in a septic tank system, acclimation of bacterial population is likely to take place with Gammaproteobacteria being abundant.

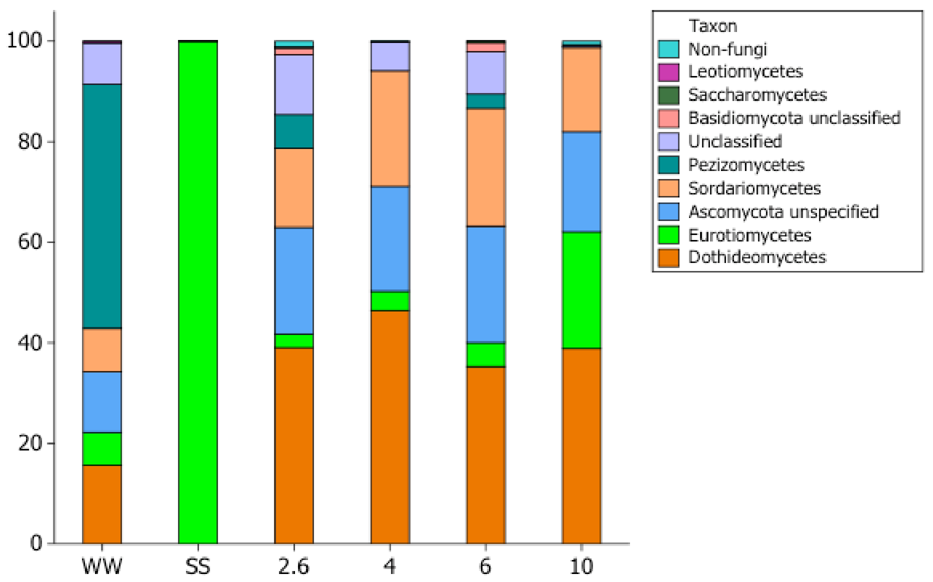


FIGURE 6. Summary of fungal populations as determined through 454-pyrosequencing

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