2005-07-06

Microbial and Chemical Affects on Leachate from Calcareous Soils Treated with Wastewater Effluent

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MICROBIAL AND CHEMICAL AFFECTS ON LEACHATE FROM CALCAREOUS SOILS TREATED WITH WASTEWATER EFFLUENT

by

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A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Plant and Animal Sciences

Brigham Young University

August 2005

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

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This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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ABSTRACT

MICROBIAL AND CHEMICAL AFFECTS ON LEACHATE FROM CALCAREOUS SOILS TREATED WITH WASTEWATER EFFLUENT

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Increasing human populations are placing greater strain on water resources, prompting the use of treated wastewater effluent for irrigation in some areas, including the desert regions of the Western United States. To determine the potential effects of using secondary effluent for irrigation, we applied wastewater effluent and irrigation waters to natural and artificially constructed calcareous soils in greenhouse and field lysimeters, and in soil columns. The leachate from one field lysimeter contained increased fecal coliform counts than the effluent. Leachate coliform counts were decreased or not significantly changed in two field lysimeters. Electrical conductivity (EC), sodium adsorption ratio (SAR), chloride and nitrate concentrations also increased significantly in the leachate of the three field lysimeters however. Samples collected from the greenhouse lysimeters showed a significant decrease in all categories except EC, was not significantly changed. Soil column drainage samples showed a decrease in coliform counts, and increase in EC and

chloride levels while SAR and nitrate levels varied with clay content. Preferential flow of coliform bacteria and high EC and SAR values could indicate long term effects that may affect the sustainability of the practice.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Professors Terry, Nelson and Breakwell for their assistance in the preparation of this manuscript. In addition, special thanks to Dr. Clinton Williams for the design and original concept of the project. A special thanks also to Dr. Frank Williams for his invaluable support.

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A manuscript submitted to Soil Science

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ABSTRACT

Increasing human populations are placing greater strain on water resources, prompting the use of treated wastewater effluent for irrigation in some areas, including the desert regions of the Western United States. To determine the potential effects of using secondary effluent for irrigation, we applied wastewater effluent and irrigation waters to natural and artificially constructed calcareous soils in greenhouse and field lysimeters, and in soil columns. The leachate from one field lysimeter contained increased fecal coliform counts than the effluent. Leachate coliform counts were decreased or not significantly changed in two field lysimeters. Electrical conductivity (EC), sodium adsorption ratio (SAR), chloride and nitrate concentrations also increased significantly in the leachate of the three field lysimeters however. Samples collected from the greenhouse lysimeters showed a significant decrease in all categories except EC, was not significantly changed. Soil column drainage samples showed a decrease in coliform counts, and increase in EC and chloride levels while SAR and nitrate levels varied with clay content. Preferential flow of coliform bacteria and high EC and SAR values could indicate long term effects that may affect the sustainability of the practice.

INTRODUCTION

Water quality is an issue of great global concern. As human populations increase, the demand for clean, potable water also increases. In an effort to meet the demand for urban and agricultural irrigation water, many countries have begun using treated wastewater effluent (see Shelef, 1991, Mancino and Pepper, 1992, AlSalem, 1996, VazquezMontiel et al, 1996, Nhapi and Hoko, 2004). Unfortunately, research has shown that special challenges exist when effluents are used for irrigation. Some of these issues are of great concern to those who manage agricultural lands. For instance, Pedersen et al. (2003) found that veterinary and human pharmaceuticals and personal care products can be found in the runoff from agricultural fields irrigated with wastewater effluent. Even if these products are not found in an unaltered state, constituents or byproducts may persist in the environment for long periods of time (Celis et al., 1997). Of additional concern is the question of subsurface transport of these substances and the possibility of groundwater pollution (Celis et al., 1997, Nelson, et al., 1998, Williams et al., 1999, Letey et al., 2000, Seol and Lee, 2001).

Wastewater effluents are known to be high in certain potential contaminants. Chloride and chlorination by-products can greatly affect bacterial and plant populations (Amy et al. 1992). Heavy metals can negatively affect plant growth and groundwater quality (Madyiwa et al. 2002). Coliform bacteria are indicative of fecal contamination that may survive the wastewater treatment process and enter the environment through the use of effluents as irrigation sources (Krejsl et al. 1994, Gerba and Smith, 2005). Phosphorus,

nitrogen, sodium and salinity are commonly monitored when effluents are applied to soils (Vengosh and Keren, 1996, Jnad et al. 2001). Of these, salinity and sodium are the greatest danger to soils, and potentially present the greatest economic damage once the receiving land becomes saline and unsuitable for agriculture (Tillman and Surapaneni, 2002). However, there is evidence that proper management of effluent application to soils can greatly reduce the concentrations of these contaminants (Shelef, 1991, Mancino and Pepper, 1992, AlSalem, 1996, Shahalam et al. 1997, Hoko, 2004). Use of treated effluent can potentially open an abundant new source of irrigation water for use in arid areas.

Soils in many arid regions are calcareous or alkaline and may possess deleterious chemical, physical and microbial properties. Researchers have made significant observations concerning the effects of effluent application to these soils. Cabrera et al. (1996) reported that olive oil mill wastewaters increased organic matter, nitrogen, phosphorus and sodium contents with a concomitant increase in electrical conductivity. Olive oil mill wastewaters have also been shown to increase magnesium, potassium (Gallardo-Lara et al. 2000), and calcium concentrations (Gallardo-Lara et al. 1998) in calcareous soils. Perez and Gallardo-Lara (1993) also reported an increase in zinc after olive oil wastewater application. Assadian et al. (1999) reported similar results with untreated olive oil effluent application, but also demonstrated slightly increased heavy metal concentrations.

General Subsurface Transport

Subsurface particle transport is the result of complex physical and chemical interactions that are only partially understood (DeNovio et al. 2004). There is an interaction of forces attempting to move particles and forces which resist movement

(DeNovio et al. 2004). The primary force of movement under saturated conditions is gravity (Jansons et al. 1989, Powelson et al. 1993). Powelson et al. (1993) and Sirivithayapakorn and Keller (2003) demonstrated that pore size is one of the most important factors involved in the transport of particles. Small pores will not allow large particles to pass easily through a porous medium. Adhesion of charged particles to clay or organic matrices can also impede movement (Bales et al. 1991). In addition, when saturation is achieved, there are often preferential drainage pathways through natural and man-made soils (Powelson et al. 1990, for a discussion on preferential flow, see Jury et al., 1986).

Evidence suggests that dissolved organic matter (DOM) can complex with some pesticides, such as napropamide and atrazine, (Celis et al., 1997, Nelson, et al., 1998, Seol and Lee, 2001) and increase their transport through the soil (Williams et al., 1999, Letey et al., 2000). This increased mobility has been shown to be independent of the presence of preferential flow channels, the traditional mechanism for rapid subsurface transport (Williams et al., 1999, Dunnivant et al., 1992). This may also be true of other particles, besides pesticides. Effluents are known to coat some particles, in the same manner as DOM (Muszkat et al., 1993), which will affect soil-particle interactions and could either impede or facilitate subsurface transport of these particles.

The presence of solid organic matter can impede the flow of chemicals and particles. Ben-Hur et al. (2003) reported that high (>7%) organic matter soils could cause the DOM to adsorb to solid organic matter in the soil. Moorman et al. (2001) showed that increasing organic matter in a soil impeded the flow of atrazine.

Microbial Subsurface Transport

Sewage effluents may contain large numbers of human enteric bacteria, with some studies indicating as much as 10^3 organisms per liter (Aulicino et al., 1996). There is evidence that bacteria would behave much like other charged particles in soils (Sinton et al. 1997, McLeod et al. 2003). Powelson et al. (1993) found that particle size greatly affected transport speed and distance.

Enteric bacteria may not survive in the soil environment long enough to be of concern. Sinton et al. (1997) reported finding viable coliforms 445 m downstream and more than 8 hours after injection into an aquifer. Selvaratnam and Gealt (1992) presented evidence that *Escherichia coli* could survive for up to 6 days in field soils amended with untreated wastewater. Spackman et al. (2003) found that coliform populations decreased 99% after 28 days in a silt loam following polyacrylamide applications. Other researchers showed a decrease in fecal coliforms of 92% over a 2 day period in a muck soil (Tate and Terry, 1980). Zhai et al. (1995) reported similar results after a 2-week period; however Vasseur et al. (1996) found that after 2 years of application, coliform numbers increased, suggesting possible long-term effects not detected by shorter studies. Tanik and Comakoglu (1997) showed that contact distance influenced coliform survival more than time.

DOM has been shown to increase the subsurface mobility of various charged particles, and it is likely that microbial particles would act similarly. Many enteric bacteria are adsorbed onto colloids already present in the wastewater effluent before they reach the soil environment (Jin et al., 2000). This allows these microbes to move much further and

faster than naturally occurring soil-borne microbes, and possibly as fast and as far as the effluent itself (Sinton et al., 1997 and Nelson et al., 2000).

Soil environmental factors other than the organic matter content also play a role in the movement of microbes. Gerba (1984) found that pH, soil type, soil water content, temperature and the ionic strength of the soil solution have great affects on the adsorption of bacteria.

Wastewater effluent use will increase as population growth places greater demands on the finite water resources of the planet (Shelef, 1991, Mancino and Pepper, 1992, AlSalem, 1996, Nhapi and Hoko, 2004). This study was designed to determine some of the potential microbial and chemical affects of irrigation with secondary wastewater effluent on calcareous soils. The primary goal was to measure the effect of subsurface transport on coliform counts. Selected chemical contaminants were measured to determine the possibility of groundwater contamination from these sources.

MATERIALS AND METHODS

We designed an experiment involving lysimeters and soil columns, under both field and greenhouse conditions. Three lysimeters were installed at the Central Davis Water Reclamation Facility, located in Kaysville, Utah while two lysimeters and 27 soil columns were constructed and installed in the greenhouse at Brigham Young University, Provo, Utah.

Secondary wastewater effluent was procured from the Central Davis Water Reclamation Facility and the Provo Water Reclamation Facility. Both treat wastes solely from municipal sources. Effluent from Central Davis undergoes a treatment process consisting of trickling filters and oxidation trenches. The Provo effluent is treated with trickling filters.

The lysimeters constructed and placed at the Central Davis Water Reclamation Facility (Davis County lysimeters), were 1.2m x 1.2m x 1.2m. The soil in the area is loamy sand and all the lysimeters were planted with Kentucky bluegrass (*Poa pratensis*). Two were placed in areas that have received effluent for more than 10 years, while the third was placed in a park area adjacent to the treatment plant. They were installed by excavating a hole of sufficient size for the lysimeter, filling the bottom with gravel and placing in the lysimeter in the hole. The sides were then packed with soil from the hole and the lysimeter filled with roughly 5 cm of gravel and repacked with the soil which was removed from the excavation. All water collected in the lysimeters drained to a collection basin located below the lysimeter. Collection of samples was achieved through two PVC pipes, one of which served as access for the vacuum pump and the other allowed for ventilation during

pumping. These collection tubes were protected by a larger piece of PVC piping covered with a heavy metal cover. In addition, each tube was capped with a piece of flexible tubing which was taped over to create a barrier to insects and water.

One lysimeter was installed in an area of lawn next to a control building, and labeled lysimeter 1. Another was installed in an area which is used for turf farming and labeled lysimeter 2. This area also received applications of sewage sludge before planting each new crop of turf. The final lysimeter was installed in a small park area adjacent to the turf farm and watered with Weber Basin Canal water. This lysimeter, labeled lysimeter 3, served as a control for the other two lysimeters.

The two greenhouse lysimeters were constructed of clear plastic covered with a layer of black plastic to minimize subsurface growth of bacteria and fungi. They were 0.9m x 0.9m x 0.9m. They were placed on a greenhouse bench top and filled with a layer of gravel approximately 3 cm thick. They were then filled with approximately 0.62m^3 of either a loamy sand or sandy loam soil mix and planted in Kentucky bluegrass (*Poa pratensis*). Sample collection occurred through a grid of PVC pipe buried in the gravel that led to a length of flexible tubing and a collection bottle beneath the apparatus.

The 27 soil columns were constructed of 15 cm diameter PVC cut into 25.5 cm segments. A metal screen was fastened to the bottom to retain the soil in the column, and then each column was filled with approximately 3888 cm^3 of a soil mix. Each soil column rested above a funnel under which a collection bottle was placed. The columns were randomly assigned into treatments, including a control group of 9 columns, watered with 1.5L of tap water, or a treatment group of 18 columns, watered with 1.5L of effluent. Wastewater effluent or tap water was applied weekly for six weeks and the leachate was

collected. Initially, the soil columns were uniformly packed with a loamy sand. To test the affect of texture on the eluent, they were then emptied, cleaned and refilled with a sandy loam soil mix.

Effluent for the greenhouse lysimeters and soil columns was collected from the Provo Wastewater Treatment plant. Effluent was collected in a bleach-sterilized 18.9 liter HDPE carboy and immediately transported to the greenhouse and applied to the soil. Leachate samples were then stored in a refrigerator at 4°C.

All leachate samples were collected in 1 liter polypropylene bottles previously sterilized by autoclaving. Samples requiring long transportation distances were kept on ice until delivered to the laboratory. All samples were stored at 4°C until analyzed. All samples were analyzed for coliform counts within 24 hours of collection. Samples that were analyzed chemically were kept under refrigeration until just before they were analyzed, at which time they were allowed to warm at room temperature for 2-3 hours and then analyzed. Generally, chemical analysis took place within 72 hours of sample collection.

Microbial Analyses

All samples were collected and analyzed following established procedures outlined in Standard Techniques for the Examination of Water and Wastewater, 20th Ed. (Clesceri et al., 1998). Coliform enumeration was performed using the membrane filtration method. After samples were filtered, the filters were placed on appropriate agar plates and incubated. Fecal coliform enumeration took place on m FC agar (Difco) plates, incubated at 44.5°C for 24 hours. Total coliforms were enumerated using mEndo LES agar (Difco),

incubated at 35°C for 24 hours. The filtered leachate was saved in 500 mL polyethylene bottles for use in the chemical analysis.

A MIDI or fatty acid methyl-ester (FAME) analysis was performed on the last batch of samples collected from the soil columns. To identify the types of bacteria found eluting from the soil columns, colonies growing on both the m FC and mEndo LES agar plates were streaked onto Nutrient agar (Difco) plates and incubated at 35°C for 24 hours. They were then transferred to Sheep's Blood Agar plates (Agilent Technologies) and again incubated at 35°C for 24 hours. At that point they were prepared for analysis on the gas chromatograph and analyzed, according to established procedures (Onderdonk and Sasser, 1995).

Chemical Analyses

Chemical assays were performed on saved filtered leachate and unfiltered leachate samples. Chloride (Cl^-) was determined on unfiltered samples using Hach methods (Hach, 1999) and a Hach DR/3000 spectrophotometer. The nitrate-N (NO_3^--N) concentration in unfiltered samples was ascertained with the chromotropic acid procedure (Sims and Jackson, 1971). Soluble phosphate-P (PO_4^{3-}-P) in filtered samples was analyzed by the ammonium persulfate method (Gales et al., 1966).

Soluble salts were measured on unfiltered samples using an electrical conductivity bridge. Sodium adsorption ratio (SAR) was determined using inductively coupled plasma (ICP) to determine calcium, magnesium and sodium ion concentrations in filtered samples and using the established formula. Procedures for these assays are described by Rhodes (1982).

RESULTS AND DISCUSSION

Coliforms

The mean Davis effluent fecal coliform count was 2.6±2.1 CFU/100mL (Table 2). The leachates from both lysimeters 1 and 2, located in Davis County and receiving effluent, had initial fecal coliform counts of 0 CFU/100mL. In comparison, the final counts, taken more than 3 months later, were 49 CFU/100mL and 7 CFU/100mL, respectively. Mean leachate fecal coliform counts were 17.2±2.0 for samples collected from lysimeter 1. This represents a significant increase over the mean fecal coliform counts recorded from the Davis effluent. Lysimeter 2 leachate had a mean fecal coliform count of 2.2±1.0, which is not significantly different from the results obtained from the applied effluent. Lysimeter 3 leachates, which received canal water, also showed an increase in fecal coliforms over the duration of the experiment, from 18 CFU/100mL to 43 CFU/100mL, although the mean value of 23.2±2.4 shows that passage through the soil decreased fecal coliform counts, when compared to the applied canal water. There was a significant (at the 0.05 level) reduction in fecal coliforms in samples collected from the greenhouse when compared to the Provo effluent (Table 2).

Total coliform populations also increased over time in leachate samples collected from lysimeter 1 (Table 2). Total coliforms measured in samples collected from Lysimeter 1 increased from 0 CFU/100mL to 36 CFU/100mL. Total coliform counts in the leachate of lysimeter 2 however peaked at 16 CFU/100mL approximately 1 month after the experiment began, and then again at the conclusion of the experiment. Leachates from both lysimeters 1 and 2 showed an increase in mean total coliform counts when compared

to the applied effluent. Leachate samples collected from lysimeter 3 increased from 36 CFU/100mL to 56 CFU/100mL, which represents a decrease in mean total coliform counts, compared to the canal water used for irrigation. Leachate samples collected from the greenhouse showed a decrease in the mean total coliform counts, when compared to the Provo effluent (Table 2).

Initially, coliforms did not easily remain viable following passage through a soil, which is consistent with the results reported by others, who also reported significant decreases in coliform counts after exposure to the soil (Tate and Terry, 1980, Zhai et al. 1995, Spackman et al. 2003). Observed reductions compare favorably with other methods of disposal, such as wetlands (Coleman et al. 2001, Mbuligwe, 2005). Data collected from lysimeters located in Davis County, with soils that had been exposed to coliforms for an extended period of time, may demonstrate that coliforms can adapt to the soil environment after long-term exposure, as suggested by Vasseur et al. (1996). It could also indicate the presence of preferential flow pathways, such as along the sides of the lysimeter, or that the bacteria can survive and grow in the collection basin, thereby artificially inflating the results. These final possibilities could not be tested without removing the lysimeter, which is not currently possible.

MIDI analysis

The MIDI (FAME) analysis for bacteria has become a very useful tool for characterizing microbial populations using an automated gas chromatograph procedure (Sundaram, et al. 2001). There is some evidence to suggest that this analysis can give false identifications (Amy et al. 1992, Oka et al. 2000) when used as the sole identification method. However, none of the genera identified here were among those mentioned as

problematic. For the isolates taken in this study, all the bacterial colonies tested were obtained from selective media and gave the expected results (Table 4). A total of 12 different genera were identified, the majority of which were coliforms, including *Escherichia*, *Enterobacter* and *Shigella*.

Electrical conductivity

A soil is considered saline when the electrical conductivity of the leachate reaches 4 dS/m. Though neither the Davis effluent nor the Provo effluent was saline (1.420 dS/m±129 and 0.793 dS/m±16, respectively), the leachate samples collected from the lysimeters in Davis County were approaching that level, 3.710 dS/m±309 for lysimeter 1 and 3.620 dS/m±781 for lysimeter 2, again possibly due to the long history of effluent application or to concentration following evapotranspiration. Soluble salts, measured as electrical conductivity, were higher in samples collected from the lysimeters located in Davis County, as well as in the soil columns in the greenhouse (Table 3) than in the effluents. The greenhouse lysimeter leachates showed no significant change in electrical conductivities (Table 3), though it should be noted that they had been significantly leached prior to effluent application.

These results are consistent with those reported by Mancino and Pepper (1992) and by Qian and Mecham (2005) who also reported increasing salt concentrations following effluent irrigation. Though the applied effluent was not saline, it would appear that application increased the salt concentrations in drainage waters from the Davis County lysimeters.

Sodium adsorption ratio

The sodium adsorption ratio (SAR) was determined as a measure of sodium content. Samples collected from lysimeters 1 and 2 showed an increase in SAR, while all other leachates showed a decrease or no significant effect in the case of the soil columns filled with loamy sand, when compared to the applied effluent (Table 3). All of the observed results were significantly below the level required to be considered sodic, which is an SAR value of 13. Tillman and Surapaneni (2002) and Cabrera et al. (1996) also found higher sodium concentrations in soils after effluent applications.

Ion concentrations

The concentrations of Cl^-, NO_3^--N and PO_4^{3-}-P in the effluents and lysimeter and column leachates are presented in Table 3. Chloride ion concentrations in the collected samples were higher than the applied effluent in lysimeters 1 and 3, as well as in the soil columns. Concentrations were decreased, when compared to the applied effluent, in leachates collected from lysimeter 2 and the greenhouse lysimeter filled with sandy loam soil, and showed no significant change in the loamy sand-filled greenhouse lysimeter, which could indicate preferential flow. It should be noted that passage through the soil also increased Cl^- concentrations in the leachate samples from soil columns which only received tap water. Nitrate-N concentrations also yielded mixed results, as with the chloride ion concentrations above. When compared to the concentrations measured in the applied effluent, concentrations of NO_3^--N were increased in leachates from lysimeters 2 and 3, as well as in the soil columns filled with the loamy sand mix; however they were reduced in the greenhouse lysimeter and soil columns filled with the sandy loam mix. The elevated NO_3^--N in lysimeter 2 leachate (348±43 mg/kg), was possibly due to the applications of

sewage sludge combined with the effluent irrigation which occurred there before the lysimeters were installed. Others have also reported increased nitrate levels after effluent application (Schipper et al. 1996). The differences in effluent and leachate nitrate levels were not significantly different in lysimeters 1 and the greenhouse lysimeter filled with loamy sand, again, possibly due to preferential flow. Soluble PO_4^{3-}-P concentrations decreased significantly with leaching through the soil in all lysimeters and soil columns except lysimeter 3. Lysimeter 3 was placed in a park area which was formerly a pasture, and horses are still kept adjacent to the area. This may explain the high nitrate and phosphate levels found in the leachate of lysimeter 3. Though less pronounced than nitrate, there was some evidence of elevated phosphate levels at all of the lysimeters and soil columns. Monnett et al. (1996) also demonstrated elevated phosphate concentrations following effluent applications though Mancino and Pepper (1992) did not.

The results of this study indicate that with proper management, wastewater effluent may be safely used, without endangering the groundwater supply. However, the possibility of water contaminants reaching groundwater through preferential flow remains a concern. With increasing human populations in dry areas driving the use of reclaimed wastewater effluent, it would appear that more studies are needed to determine the long-term affects of effluent use, particularly for microbial transport and salinity.

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TABLES

Table 1. Initial chemical and physical properties of soils.

Source	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Texture	pH	$CaCO_3$ equivalent (%)	NO_3^- (mg/kg)	Infiltration rate (cm/hr)
Lysimeter 1	702	138	160	Loamy sand	7.5	25	40.6	5.51
Lysimeter 2	684	159	157	Loamy sand	7.6	24	55.7	5.08
Lysimeter 3	696	142	162	Loamy sand	7.5	26	60.1	4.93
Loamy sand mix	801	129	70	Loamy sand	7.8	23	33.2	5.38
Sandy loam mix	620	228	152	Sandy loam	7.9	21	60.4	3.79

Table 2. The mean fecal and total coliform counts of the effluents and leachates with the 95% confidence intervals. The large discrepancy between values may reflect the scale difference between the soil columns and lysimeters along with the influence of source material.

	Fecal Coliforms (CFU/100mL)	Total Coliforms (CFU/100mL)
Davis Effluent	2.6±2.1	2±2.0
Lysimeter 1	17.2±2.0	24.2±2.0
Lysimeter 2	2.2±1.0	6.8±1.0
Weber Basin	44.6±12.2	80.4±3.1
Lysimeter 3	23.2±2.4	55±4.7
Provo Effluent	157.143±37.8	689.1±111.1
Columns, loamy sand	56.61±7.2	433.3±44.0
Lysimeter, loamy sand	19.7±7.4	65.6±15.6
Columns, sandy loam	29.7±4.5	47.9±5.5
Lysimeter, sandy loam	0.6±0.8	1.9±2.7

Table 3. The mean electrical conductivity, sodium adsorption ratio, and selected element concentrations with the 95% confidence intervals. The large discrepancy between values may reflect the scale difference between the soil columns and lysimeters along with the influence of source material.

	EC (dS/m)	SAR	PO_4^{3-}-P (mg/kg)	Cl^- (mg/kg)	NO_3^--N (mg/kg)
Davis Effluent	1.420±0.129	2.9±0.1	3.6±0.1	540±54.5	10.6±0.4
Lysimeter 1	3.710±0.309	5.9±0.5	0.3±0.0	623±19.9	11.2±0.7
Lysimeter 2	3.620±0.781	3.2±0.0	0.4±0.1	283±95.5	34.8±4.3
Weber Basin	0.442±0.042	2.1±0.1	0.2±0.0	15±0.6	0.2±0.0
Lysimeter 3	0.941±0.018	1.3±0.1	2.6±0.0	111±5.4	11.3±0.6
Provo Effluent	0.793±0.016	2.7±0.2	3.6±0.3	54±18.9	95.1±9.3
Columns, loamy sand	1.854±0.041	2.7±0.6	1.7±0.1	320±15.5	169.3±10.9
Lysimeter, loamy sand	0.775±0.188	1.7±0.6	2.4±0.7	65±24.0	79.7±26.2
Columns, sandy loam	1.174±0.544	7.4±0.7	0.3±0.0	182±8.1	76.4±3.9
Lysimeter, sandy loam	0.650±3.012	1.8±0.7	0.3±0.1	12±2.2	46.8±1.9

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Table 4. Microbial species in Provo effluent and column leachate identified by MIDI (FAME) analysis.

Source	Similarity Index	Genus and Species
Column 2	0.677	*Salmonella enteritidi*
Column 2	0.738	*Aeromonas sobria*
Column 2	0.703	*Ralstonia pickettii*
Column 4	0.965	*Pseudomonas aeruginosa*
Column 4	0.668	*Citrobacter braakii*
Column 4	0.842	*Kluyvera ascorbata*
Column 4	0.588	*Citrobacter braakii*
Column 7	0.907	*Shigella boydii*
Column 7	0.169	*Kluyvera cryocrescens*
Column 7	0.695	*Klebsiella oxytoca*
Column 10	0.759	*Pseudomonas aeruginosa*
Column 10	0.879	*Escherichia coli*
Column 10	0.670	*Aeromonas hydrophila*
Column 13	0.426	*Salmonella enteritidis*
Column 13	0.862	*Pseudomonas aeruginosa*
Column 13	0.778	*Shigella sonnei*
Column 13	0.723	*Enterobacter intermedius*
Column 15	0.869	*Escherichia coli*
Column 15	0.537	*Pseudomonas aeruginosa*
Column 15	0.689	*Shigella sonnei*
Column 18	0.806	*Citrobacter braakii*
Column 18	0.787	*Aeromonas veronii*
Column 18	0.837	*Serratia marcescens*
Column 22	0.942	*Escherichia coli*
Column 22	0.926	*Escherichia coli*
Column 22	0.896	*Shigella sonnei*
Column 22	0.750	*Citrobacter freundii*
Column 26	0.941	*Pseudomonas aeruginosa*
Column 26	0.846	*Proteus mirabilis*
Column 26	0.832	*Aeromonas veronii*
Column 26	0.787	*Kluyvera ascorbata*
Column 28	0.801	*Proteus vulgaris*
Effluent	0.960	*Pseudomonas aeruginosa*
Effluent	0.739	*Escherichia coli*
Effluent	0.616	*Salmonella enteritidis*
Effluent	0.810	*Pseudomonas aeruginosa*

Effluent	0.959	*Pseudomonas aeruginosa*
Effluent	0.928	*Pseudomonas aeruginosa*
Effluent	0.800	*Escherichia coli*
Effluent	0.758	*Aeromonas sobria*
Effluent	0.791	*Shigella sonnei*
Effluent	0.821	*Shigella sonnei*
Effluent	0.832	*Aeromonas veronii*
Effluent	0.777	*Aeromonas veronii*
Effluent	0.733	*Shigella sonnei*
Effluent	0.847	*Aeromonas hydrophila*
Effluent	0.836	*Aeromonas caviae*
Effluent	0.773	*Kluyvera cryocrescens*
Effluent	0.850	*Aeromonas veronii*

MICROBIAL AND CHEMICAL AFFECTS ON LEACHATE FROM CALCAREOUS SOILS TREATED WITH WASTEWATER EFFLUENT

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Keywords: Fecal coliforms, wastewater percolation, nutrient leaching, water quality, lysimeters