REMOVAL AND FATE OF SPECIFIC MICROBIAL PATHOGENS WITHIN ON-SITE WASTEWATER TREATMENT SYSTEMS

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EXECUTIVE SUMMARY

Untreated or improperly treated wastewater has often been cited as the primary contamination source of groundwater. Decentralized wastewater treatment systems have applicability around the world since it obviates the need for extensive infrastructure development and expenditures. The use of a sand filter, a submerged flow constructed wetland and an aerobic treatment unit to remove bacterial and viral pathogens from wastewater streams was evaluated in this study. *Salmonella* sp. and a bacteriophages tracer were used in conjunction with the conservative bromide tracer to understand the fate and transport of these organisms in these treatment systems. Viral transport patterns in the sand filter and constructed wetland had a correlation of 0.8 ($P< 0.05$). In the constructed wetland, the virus exhibited almost a 3-log reduction, while in the sand-filter, the viruses exhibited a 2-log reduction. The bacterial tracers, however, did not exhibit similar reductions. Low numbers of bacteria and viruses were still detectable in the effluent streams suggesting that disinfection of the effluent is critical. The survival of the tracer bacteria and viruses were as expected dependant on the biotic and abiotic conditions existing within the wastewater. The results suggest that the microbial removal characteristics of decentralized wastewater treatment systems can vary and depend on factors such as adsorption, desorption and inactivation which in turn depend on the design specifics such as filter media characteristics and local climatic conditions.

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CHAPTER 1: INTRODUCTION

Literature Review

A wide variety of microbial infections are transmitted through contaminated water supplies and groundwater has been implicated as one of the primary sources of contaminated drinking water (Pillai, 1998). The contamination of groundwater and surface water resources by pathogens such as enteric viruses, *E.coli* 0157:H7 and *Cryptosporidium* are of concern even in developed countries such as the United States. Rotavirus, a major cause for infantile diarrhea in the United States (with a documented fecal-oral route) is responsible for over 3.5 million infections with approximately 75-150 deaths annually (Blacklow and Greensberg, 1991). In developing countries, Rotavirus is responsible for over 125 million infections on an annual basis. Flewett (1982) has reported that human feces contain approximately 10^{10} virus particles/gram. In 1989, in Cabool, Missouri four deaths and 243 cases of infections arose as a result of E.coli 0157:H7 contamination of drinking water (Geldreich et al., 1992). In 1993, 7 deaths and 650 infections resulted from *Salmonella typhimurium* contamination of drinking water. *Cryptosporidium*, a protozoan pathogen is now thought to be one of the third most common enteric pathogens causing diarrheal illnesses worldwide. Monitoring data has shown that *Giardia* and *Cryptosporidium* were both present in 55 wells (12%) out of 463 wells that were sampled (Hancock et al., 1998).

Untreated or improperly treated sewage has often been cited as the primary contamination source. There have been a number of studies documenting groundwater contamination by microbial pathogens from the soil surface. Fecal bacteria from land applied animal manure have been shown to move beyond the root zone whenever there was sufficient rainfall (Stoddard et. al., 1998). Viral tracer studies conducted in Key Largo, Florida have documented the contamination of subsurface and surface marine waters from on-site disposal practices (Paul et al., 1995). Certain sewage disposal practices such septic tanks and sewage treatment plant bore-holes have been cited as being responsible for the presence of fecal indicator bacteria as well in the subsurface aquifer in Key Largo, Florida (Paul et al., 1995). Scandura and Sobsey (1997) have reported on the occurrence of viral and bacterial contamination of groundwater from on-site treatment systems. They studied the survival and transport of a model enterovirus (BE-1)(which was injected) and fecal coliform bacteria in four on-site wastewater treatment systems. The systems included three conventional and one low pressure, small pipe diameter, pumped system in sandy soils. The model enterovirus was detected in groundwater monitoring wells as early as 1 day after seeding and persisted for up to two months. The virus detection in groundwater was greater in winter than in summer and was positively associated with proximity to septic effluent distribution lines, drain field soils with the lowest clay content, elevated groundwater pH and shallower vadose zones. Viruses were not strongly associated with either distance from septic tank or fecal coliform levels in groundwater. Fecal contamination of groundwater can occur by multiple routes. In addition to failed septic systems, groundwater contamination can occur from leaking sewer lines and from land discharge. The availability of proven on-site wastewater treatment technologies could significantly reduce the potential of groundwater contamination in areas where centralized treatment facilities are not an option. Information about the fate and retention of specific microbial pathogens under different treatment technologies in different climatic and seasonal conditions are necessary.

Indicator Organisms and Microbial Pathogens

It is evident that for the majority of infections the specific causative agent is unknown. For those infections for which an agent was identified, bacterial agents are predominant (Table 1). Even though bacterial agents appear to be the predominant causative agent, a majority of the outbreaks are probably viral in origin since it is far more difficult to detect viral agents than bacterial agents.

Table 1: Etiology of groundwater associated waterborne disease outbreaks in the United States between 1971-1996[a]

Causative Agent	Outbreaks	%
Undetermined	232	62
Chemical	22	6
Total Protozoa	26	7
Total Virus	35	9
Total Bacteria	56	15

[a]modified from Craun and Calderon, 1996

For some of the infectious enteric viruses (eg. Norwalk virus) appropriate tissue culture systems are still not available. Additionally, given the low concentrations of pathogens in environmental samples as compared to clinical samples, the detection of human enteric viruses can be extremely problematic. However, the recent availability of molecular methods such as the RT-PCR based detection of enteric viruses has alleviated this problem to some extent. However, for the most part, the detection of specific viral pathogens is still a significant issue facing environmental and public health microbiologists. To overcome the needs to detect specific microbial pathogens, the microbiological quality of water samples is generally assessed based on the levels of fecal coliform organisms eg. *E.coli*. Indicator organisms have been employed to detect fecal pollution as well to monitor the efficiency of treatment processes. Coliform bacteria have been used since the early part of this century (Hazen, 1988). Even though they seem to function as reliable indicators for the presence of bacterial pathogens, they are not useful as indicators for the presence of viral and protozoan pathogens. There have been numerous instances when the enteric viruses were detected in municipal water supplies that were negative for coliform bacteria. In 1991, the International Association of Water Quality's Study Group recommended the use of bacteriomale-specific coliphages (bacterial viruses) as a promising alternative to the detection of enteric viruses (IAWPRC, 1991). Bacteriomale-specific coliphages are physically and chemically more closely related to enteric viruses and are more similar to them in such characteristics such as persistence in the environment and resistance to disinfection and other water treatment regimens (Wentsel, 1982). Thus the use of typical fecal coliform bacteria to assess effluent quality for the presence of human pathogens can be extremely misleading. Thus adequate attention should be paid to choose the right organism to test the efficacy of a treatment system. In a number of situations it would be preferable to detect specific pathogens of interest rather than indicator organisms especially when public health issues are of paramount importance.

Removal of Microbial Pathogens and Indicator Organisms

Maschinski et al (1999) studied the reduction of total and fecal coliforms in a subsurface constructed wetland system using native southwestern plants. They monitored the performance of a small-scale 3-cell (12.2m X 5.4m X 1m) unit.

Table 2: Mean log values of total coliforms within a 3-cell subsurface constructed wetland[a]

Month	Input Cell#1	Output-Cell#1	Output-Cell#2	Output-Cell#3
May	6.61	4.23	2.45	0.3
June	6.79	5.21	4.19	3.66
July	6.9	5.23	4.13	1.5
August	6.59	4.79	3.26	3.14
September	6.82	5.34	4.09	3.58
October	6.38	3.67	3.49	2.75

[a]modified from Maschinski et al., 1999.

Table 3: Mean log values of fecal coliforms within a 3-cell subsurface constructed wetland[a]

Month	Input Cell#1	Output-Cell#1	Output-Cell#2	Output-Cell#3
May	4.65	3.95	2.16	0.45
June	5.38	3.35	2.45	2.17
July	6.49	4.64	3.89	3.24
August	5.84	3.89	2.69	1.48
September	5.58	4.16	3.03	2.05
October	4.33	2.82	1.35	0.77

[a]modified from Maschinski et al., 1999.

They report significant reduction of both total and fecal coliform counts (>99%) in all the months (Table 2 and Table 3). The fecal coliform loads of the effluent leaving the wetland were below the standard for full-body recreational water bodies throughout the year except in July. Chendorain et al (1998) studied the fate and transport of viruses through surface water constructed wetlands using MS2 (a bacteriophage) as an enteric virus surrogate. They compared a one-phase cell and a three-phase cell that received unchlorinated secondary effluent at a constant rate. They observed a 97% reduction in MS2 bacteriophage numbers in both types of wetlands. Converse et al (1994) studied the efficacies of 13 Wisconsin mound systems by sampling from 6 inches to 42 inches beneath the aggregate. The average fecal coliform count was 103 MPN/g soil at the 22-inch depth, which was higher than what is typically found beneath ponded gravity systems but lower than what is normally found at-grade systems.

CHAPTER 2: STUDY OBJECTIVES

The underlying hypothesis of this project was that on-site wastewater treatment systems such as constructed wetlands, sand filtration units and aerobic treatment systems are effective at both retaining the specific microbial pathogens and moreover, the operating conditions of these systems within the context of the natural environment reduces the survivability of the pathogens. The overall objective of the study was to determine the fate and retention of selected microbial pathogens and indicator organisms within on-site wastewater treatment systems that were attached to residential wastewater streams. The specific objectives were:

1. Determine the survivability and retention of Salmonella spp., Cryptosporidium parvum oocysts, fecal coliforms, fecal streptococci and colimale-specific coliphages in a septic tank, aerobic treatment unit, sand filter and constructed wetland that are receiving domestic wastewater.
2. Determine if the survivability and retention of the pathgens can be predicted by the survivability and retention of the indicator organisms.
3. Understand how the seasonal fluctuations can influence the survivability and retention of these organisms within on-site wastewater treatment systems.

A combination of laboratory and field experiments were carried out in the one year period from August 1998 through August 1999 at the research facilities of the Texas A&M University's Agricultural Research and Extension Center at El Paso, Texas.

CHAPTER 3: FIELD SITES

Two treatment systems (previously funded by the Texas On-site Wastewater Treatment Research Council as a component of the International On-site Wastewater Treatment Training Center) constructed at single-family dwellings were available for this project. One of them was a sand filter with a subsurface drip dispersal field, while the other was a constructed wetland placed between a septic tank and an existing conventional drainfield. An aerobic treatment unit was installed during the course of this project at a nearby community center.

Sand filter/Subsurface Drip Application System

The sand filter/subsurface drip application system was constructed at a two- bedroom residence on the property of the Texas A&M University Agricultural Research and Extension Center. The system was constructed to provide hands-on training on the operation and maintenance of sand filter treatment systems and subsurface drip application fields. The system consists of a two compartment 1000-gallon tank serving as a primary treatment tank and a pump tank. The wastewater is pumped on demand to a 40 sq. feet free access sand filter that contains two feet of sand and six inches of pea gravel underdrain. The treated wastewater flows into a 500-gallon pump tank from which the water can be recirculated to the septic tank or dosed to the subsurface drip application field. The sand filter, dosing pump and recirculation pumps were timer controlled for regulating the dosing interval and the recirculation volume. The subsurface drip application field was dosed on the demand by the water in the pump tank (Appendix A).

Submerged Flow Constructed Wetland

The constructed wetland treatment system was located at a two-bedroom home on the Texas A&M University Agricultural Research and Extension Center property. The system was constructed to provide class participants with an operating system for discussion of operation and maintenance requirements for this technology. The system consists of a 1000-gallon septic tank/pump tank for primary treatment of wastewater and dosing of the wastewater to the wetland. The constructed wetland is 10' wide by 25' long with a depth of approximately 12 inches. The water level in the constructed wetland is controlled by an overflow structure in the water level control tank. The water overflows to a 500- gallon pump tank from which the treated wastewater is returned to an existing conventional drainfield (Appendix A).

Aerobic Treatment Unit

An aerobic treatment unit was installed at a local community center in Sparks, Texas. This community center serves the local colonia through the delivery of adult education classes, medical services, counseling and community festivities. The community center is open daily and generally has plate lunches delivered on disposable utensils. The center does conduct festivals several times a year that have food prepared on site.

The system consisted of a 1000-gallon trash tank, 750-gallon per day aerobic treatment unit and a 1000-gallon pump tank. A tablet chlorinator was used for disinfection and located in the inlet of the pump tank. Treated effluent was sprayed on the surface of the soil next to the community center and used to irrigate shrubbery around the perimeter fence. A soil absorption bed was installed as an overflow for the system (Appendix A).

CHAPTER 4: METHODOLOGIES

Monitoring Studies

Influent and effluent wastewater samples were routinely collected over a period of 25 weeks at the aerobic treatment unit, the sand filter and the constructed wetland. The samples were assayed for a suite of chemical and microbial parameters. During the course of the study the aerobic treatment unit was disconnected from the waste stream (unknown to the researchers). Thus, only the results that were obtained during the functioning of the aerobic treatment unit is included in this report. Also, during the course of the study, the residence that was connected to the sand filter unit became unoccupied for a period of about 8 weeks. Thus only the data sets that were obtained when the system was functioning is presented in this report.

Chemical Analysis

Two hundred and fifty milliliters of the influent and effluent samples were routinely collected in polypropylene bottles from all three different locations. The samples were maintained under cold (blue-ice) conditions until analysis. The samples were frozen at -20^0C if there was a delay in submitting the samples for analysis. The samples were analyzed at the analytical laboratory of the Texas A&M University's Agricultural Research and Extension Center at El Paso. The samples were analyzed under EPA recommended QA/QC programs for nitrate (NO_3), nitrite (NO_2), phosphate (PO_4), Total phosphorus, Total Nitrogen (TKN), pH, EC, Total Dissolved Solids (TDS), Total Suspended Solids (TSS) and

Biological Oxygen Demand (BOD). The temperature and pH of the samples were also collected at the time of sample collection.

Microbial Analysis

Aliquots of the influent and effluent samples were analyzed for selected microbial parameters such as *Salmonella* spp., fecal coliforms, fecal streptococci, and bacteriomale-specific coliphages (male specific colimale-specific coliphages). Salmonella spp. , fecal coliforms and fecal streptococci were enumerated using the 3 tube Most Probable Number (MPN) technique (APHA, 1992).

Sample Processing

Ten milliliters of each sample was serially diluted in 90 ml of 0.1% peptone to a final dilution of 10^{-7}. One-milliliter aliquots from these dilutions were inoculated into the respective media.

Salmonella spp.

Universal Enrichment Broth (Difco, MI) tubes were initially inoculated with aliquots of the dilutions. The tubes were incubated at 35C for 48 hours. A loopful from each of the Universal Enrichment Broth tubes that exhibited growth were streaked on to Brilliant Green Agar (BGA) plates. The plates were incubated at 35C for 24 hours. The presumptive colonies from the BGA plates were streaked on to XLD agar plates. Characterisitic *Salmonella* spp. colonies (black colored) were used as the basis for enumeration. The MPN was calculated using EPA software

Fecal coliforms

Aliquots from the serial dilutions were inoculated into Lauryl Tryptose Broth (LTB) tubes containing fermention tubes. The tubes were incubated at 35°C for 24 hours. Aliquots (0.1 ml) were removed from tubes that showed characterisitic lactose fermention (turbidity and gas production) and inoculated into EC medium tubes and the tubes incubated for 24 hours at 44.5C. Lactose fermentation (turbidity and gas production) at this elevated temperature was used as the basis of enumerating fecal coliforms.

Fecal streptococci

KF broth was inoculated with aliquots from the original serial dilutions. A loopful of the culture from tubes that exhibited growth in the KF tubes was streaked on KF agar plates. The presence of characterisitic colonies (chocolate brown) on the KF plates was used as the basis of enumeration.

Bacteriomale-specific coliphages

The presence of male specific colimale-specific coliphages was used as the indicator organism for viruses. The host bacterium that was used was E.coli F-amp. This male specific coliphage host was originated by Victor Cabelli and is resistant to ampicillin and streptomycin (15 μg/ml). The doubel agar layer technique was employed for coliphage enumerations.

Preparation of host cells: Overnight log phase cultures of the E.coli Famp host were prepared by shake-incubating a loopful of glycerol stocks of this culture in Tryptic Soy

Broth (TSB) amended with ampicillin and streptomycin (15μg/ml). The cultures were incubated for 18-24 hours at 37C.

Preparation of plates: The bottom layer of Tryptic Soy Agar (TSA) was prepoured (1.5%). The top agar layer was prepared as follows: Ten milliliters of the sample was mixed with 1 ml of the host bacterium (E.coli F-amp) and incubated for 5 minutes at 37C. After the incubation period, 10 ml of Tryptic soy Agar was mixed with this mixture and the contents poured into four plates (5 ml each).

Survival Studies

The Salmonella spp, bacteriomale-specific coliphages and Cryptosporidium parvum oocysts were monitored for survival in the constructed wetland and the sand filter systems. Wastewater contained in 50 ml polypropylene containers was inoculated with defined numbers of these organisms separately. The Salmonella typhimurium strain (NO/NA) (resistant to naldixic acid and novobiocin @ 25 μg/ml) and the male specific bacteriophage, MS2 were employed in these studies. The microcosms were placed randomly within the constructed wetland and the sand filter matrices. A control microcosm was maintained in the laboratory under ambient conditions to compare the survival patterns. At periodic intervals, the samples were retrieved from the field and the surviving numbers of the different organisms was determined. In the case of Cryptosporidium oocysts, the % viability of the organisms was determined using the protocol published by Dowd and Pillai (2000). These survival studies were performed both in the summer and the winter months to determine whether climatic conditions influenced the survival patterns. The temperature of the microcosms was monitored throughout the course of these studies.

Transport Studies

Since one of the primary objectives of this project was to determine the removal of bacterial and viral pathogens within onsite treatment systems, injection studies were performed. Since only the sandfilter unit and the constructed wetland units were self contained units that were physically removed from extensive public contact, injection studies were performed only at these sites. To determine the removal efficiencies of these two onsite systems, defined numbers of Salmonella sp., and MS2 bacteriophage were injected into the waste stream. For comparison purposes, defined amounts of KBr were also injected. Potassium bromide is regarded to be a conservative tracer and thus used as a control. Once the organisms had been injected into the wastewater, samples were collected at regular intervals from the septic tank, the pump tank and the post-treatment effluent. Additionally the flow meter readings at the two sites were regularly monitored.

Prior to the transport studies, wastewater samples were collected from different locations of the CW and SF and analyzed for the presence of nalidixic acid and novobiocin resistant bacteria on BGA and for background levels of male-specific phage. This was done to ensure that no potentially interfering background levels of these organisms were present. A total of 1.0×10^{13} PFU of MS2 virus and 6.4×10^{9} CFU of *S. typhimurium* along with 5 gallons of KBr (final concentration = 0.3g/L) was added to the toilet bowl of the residence to which the sand-filter was attached. A total of 1.1×10^{11} PFU of phage, 6.4×10^{9} CFU of Salmonella sp.and 5 gallons of KBr (final concentration = 0.3g/L) solution were added into the toilet bowl at the residence where the CW was installed. Wastewater samples were collected every 24 hours at the pump tank outlet and the CW outlet. *Salmonella sp.* was enumerated on BGA containing 25 μg ml^{-1} of nalidixic acid and novobiocin. Characteristic colonies (red-pink, opaque colored colonies) after a 24-hour incubation at 35° C were enumerated as *Salmonella*. MS2 bacteriophages were enumerated using the double agar layer method and *E. coli* F-amp as the host (USEPA, 2001). Plaques were enumerated after the plates were incubated at 37° C. for 24 hours. Bromide concentration was determined using ion chromatography.

Survival of Salmonella sp and MS2 phage

The survival of the bacterium and phage under temperature conditions within the CW and SF was studied during the summer and winter. Wastewater samples were collected from the septic tanks that were connected to CW and SF and aliquoted (25 mL) into multiple 50 mL polypropylene conical tubes. Each of the tubes were separately inoculated with the MS2 phage (7.9×10^9 PFU/mL) and *Salmonella* (4.0×10^9 CFU/mL). The 50 mL conical tubes were placed at random throughout the CW and SF. During the winter study, the initial levels of Salmonella and MS2 phage were 3.9×10^7 CFU/mL and $1 \text{ X } 10^9$ PFU/mL respectively. During both the summer and winter studies, triplicate sample tubes, covered in foil, were placed at ambient room temperature in the laboratory as controls. The study was conducted over a total duration of 4 weeks. At weekly intervals, three replicate tubes were collected from the CW, three replicate tubes from the SF, and three replicate tubes from the laboratory controls were assayed for *Salmonella typhimurium* and MS2.

Data and statistical analysis

The tracer transport data were represented as moving average concentrations over the duration of the study to understand the tracer transport patterns. However, the actual concentrations of *Salmonella typhimurium*, MS2 phage, and Bromide were used for calculating the Spearman Rank correlation. For the survival studies, linear regression was used to compare the survival rates for *Salmonella typhimurium* and MS2 phage. SigmaPlot was used for graphical representation while the statistical analyses were performed with SPSS 10.0 for Windows (SPSS Inc, Chicago, Illinois).

CHAPTER 5: RESULTS

Monitoring Studies

The chemical characteristics of the influent and the effluent wastewater samples over the 20 weeks of sampling at the sand filter, the constructed wetland unit and the aerobic treatment unit is shown in Tables 4, 6, 8, 10-12. The average daily flow for the month during the reporting period is shown in Tables 5, 7, and 9. Daily meter and flow rates for each system are shown in Appendix C.

Wastewater quality from the sand filter was relatively good. A dose of dog food was added to the system during the early part of April to increase the organic loading to the system. The concentration of BOD_5 increased dramatically during the next couple of weeks as a result of this organic loading (Appendix B).

Table 4: Wastewater quality for the sand filter study.

	BOD_5 (mg/L)		TSS (mg/L)		EC (dS/m)		pH	
Date	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	41	20	22	6	1.26	1.26	7.58	7.13
3/23/99	18	0	6	4	1.3	1.324	7.54	7.60
3/30/99	38	0	16	2	1.323	1.357	7.59	7.50
4/6/99	34	17	35	25	1.434	1.405	8.06	7.97
4/12/99	667	0	132	26	1.7	1.41	6.38	8.43
4/14/99	791	3	126	38	1.8	1.45	6.43	7.79
4/20/99	1009	75	86	68	1.83	1.54	6.10	7.76
4/22/99	964	72	100	22	1.91	1.61	6.70	7.74
4/27/99	811	144	66	46	1.65	1.79	7.09	7.59
4/29/99	639	102	48	54	1.9	1.67	7.13	7.65
5/4/99	327	75	52	22	1.55	1.6	6.67	7.28
5/6/99	356	67	8	16	1.56	1.53	6.80	7.50
5/11/99	27	4	46	30	1.64	1.65	7.19	7.88
5/18/99	123	11	28	0	1.5	1.53	7.18	7.75
5/25/99	163	64	42	16	1.67	1.6	7.20	7.69
6/2/99	14	8	28	12	1.56	1.62	7.58	7.92
6/8/99	11	4	68	18	1.56	1.63	7.37	7.75

Date	BOD$_5$ (mg/L)		TSS (mg/L)		EC (dS/m)		pH	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
7/21/99	133	6	16	0	1.89	1.75	7.75	8.18
7/27/99	119	35	18	0	1.84	1.77	7.62	7.82
8/3/99	46	33	0	0	1.21	1.36	7.28	7.49
8/10/99	58	26	0	6	1.32	1.38	7.19	7.56
8/17/99	36	26	2	0	1.41	1.24	7.63	7.67
8/24/99	61		14		1.44		7.74	
9/1/99	30	41	0	0	1.30	1.38	7.58	7.54

The sand filter had a relatively low hydraulic loading rate (Table 5). The surface area for the sand filter is 40 square feet. The average loading rate to the sand filter is approximately 1.3 gallons per square foot per day. This loading rate is similar to the loading rate applied to standard sand filters but is considerably lower than the loading to a high rate sand filter. A single person was living in the residence and this could have explained the low loading rate during the study. A bedroom residence is generally expected to have a flow of approximately 180 gallons per day.

Table 5: Average daily flows for the sand filter study.

Month	Average Daily Water Use (gallons/day)	Average Hydraulic Loading Rate (gallons/sq.ft-day)
April	45.6	1.14
May	90.3	2.26
June-July	39.9	1.0
August	32.5	0.81

The constructed wetland system maintained a fairly uniform flow of wastewater through the system. The effluent quality entering and exiting the system was a little lower than desirable for the experiments. Students occupied the facility and their lifestyle limited the quantity of food prepared in the kitchen. The wastewater quality experienced some drops in organic strength and the wetland responded by increasing the effluent organic strength. This is fairly typical with natural treatment systems.

Table 6: Wastewater quality for the wetland study.

	BOD_5 (mg/L)		TSS (mg/L)		EC (dS/m)		pH	
Date	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	52	25	14	28	1.29	1.28	7.38	7.51
3/23/99	84	18	30	-4	1.408	1.451	7.33	7.43
3/30/99	0	9	14	38	1.105	1.1	7.42	7.50
4/6/99	86	19	36	32	1.461	1.325	7.50	7.83
4/12/99	56	0	44	46	1.53	1.53	7.95	6.97
4/14/99	7	10	52	34	1.23	1.42	7.49	7.09
4/20/99	80	28	46	20	1.41	1.39	7.44	7.59
4/22/99	45	11.1	18	0	1.4	1.59	7.59	7.67
4/27/99	105	24	20	2	1.56	1.57	7.56	7.69
4/29/99	57	7	4	0	1.35	1.37	7.22	7.52
5/4/99	13	4	4	14	1.42	1.32	7.26	7.39
5/6/99	104	21	10	62	1.44	1.52	7.22	7.13
5/11/99	34	10	6	22	1.31	1.44	7.19	7.41
5/18/99	31	8	8	4	1.31	1.52	7.37	7.62
5/25/99	47	59	16	16	1.19	1.27	7.62	7.71
6/2/99	8	19	18	26	1.4	2.39	7.67	7.16
6/4/99	7	0	0	18	1.42	4.07	7.54	7.09
6/8/99	5	16	24	24	1.29	2.26	6.98	6.98
6/15/99	59	56	0	0	1.39	3.92	7.12	6.98
6/22/99	64	77	0	0	2.25	1.27	7.22	7.43
6/30/99	3	9	0	6	1.28	2.74	7.59	7.49
7/14/99	0	9	0	0	1.23	1.86	7.23	7.49
7/21/99	25	5	0	0	1.17	1.98	7.58	7.58
7/27/99	35	40	0	0	1.05	1.36	7.24	7.21
8/3/99	26	33	0	0	1.11	1.39	7.34	7.37
8/10/99	34	29	0	20	1.20	1.52	7.36	7.15
8/17/99	73	41	0	0	1.27	2.51	7.19	6.95
9/1/99	40	34	2	0	1.28	2.41	7.50	7.30

The flow rate to the constructed wetland was greater than the expected flow of 180 gallons per day (Table 7). The wetland had a surface area of 250 square feet and an average hydraulic loading of approximately 0.84 gallons per surface square foot per day.

Table 7: Average daily inflow and outflow for the wetland study.

Month	Average Daily Inflow	Average Daily Outflow	Average Hydraulic Loading Rate (Gallons / surface sq. ft – day)
November	268.5	253.4	1.07
December	172.1	341.9	0.69
January	234.2	48.7	0.94
February	220.2	228.2	0.88
March	223.8	233.2	0.90
April	214.3	200.2	0.86
May	327.6	249.2	1.31
June	85.2	144.0	0.34
July	146.5	99.9	0.59

The aerobic treatment unit system was located on a community center serving a colonia. The wastewater quality and subsequent effluent quality were quite variable. This type of facility generates wastewater officially characterized as high strength wastewater. Wastewater BOD_5 concentration ranged from 96 to 508 mg/l. An airline was broken going into the aeration chamber between the April 20th and May 18th sampling events. Therefore, effluent quality during that time period was poor.

Table 8: Wastewater quality for the aerobic treatment unit study.

	BOD_5 (mg/L)		TSS (mg/L)		EC (dS/m)		pH	
Date	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	426	67	806	66	1.73	1.49	6.7	7.4
3/23/99	120	29	216	100	1.564	1.544	7.04	7.65
3/30/99	144	13	96	100	1.758	1.606	7.57	8.02
4/6/99	96	31	156	32	1.868	1.754	7.6	8.36
4/12/99	100	102	408	142	1.71	1.62	6.97	7.9
4/14/99	133	17	146	98	1.9	1.7	7.43	7.83
4/20/99	225	208	172	100	1.81	1.58	6.75	7.34
4/22/99	169	284	312	46	1.79	1.58	6.65	7.31
4/27/99	199	82	74	42	1.49	1.86	6.59	7.31
4/29/99	175	128	154	82	1.84	1.51	7.33	6.76
5/4/99	235	188	220	68	1.59	1.89	7.15	6.69
5/6/99	256	227	352	72	1.96	1.5	7.22	7.02

Date	BOD$_5$ (mg/L)		TSS (mg/L)		EC (dS/m)		pH	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
5/11/99	335	296	310	84	2.11	1.61	7.17	6.72
5/18/99	341	267	262	110	2.07	1.64	7.23	6.97
5/25/99	113	39	96	56	2.05	1.7	7.25	6.54
6/2/99	117	18	116	88	1.9	1.56	6.95	7.02
6/4/99	304	174	30	8	2.03	1.97	7.32	7.45
6/8/99	117	7	162	44	2	1.61	7.15	6.6
6/15/99	508	36	612	4	2.03	1.71	6.44	5.63
6/22/99	336	51	254	0	1.94	1.68		
6/30/99					1.96	1.78		

The aerobic treatment unit had a fairly low average hydraulic loading rate (Table 9), however some peak flows were large (Table C3). This variation in flow may have caused some challenges with the operation of the system.

Table 9: Average daily flow for the aerobic treatment unit study.

Month	Average Daily Flow
March	234.7
April	150.7
May	120.6

Nitrogen concentrations in the sand filter wastewater did exhibit a reduction in concentration. Nitrate was present in the effluent at greater concentrations than the influent. This demonstrated an aerobic condition present following passage through the sand filter. Nitrite was present in the effluent during part of the study. Nitrite and Nitrate signify the presence of aerobic conditions in the sand filter and the occurrence of nitrification. After the addition of Dog food (approximately April 7th), the nitrate and nitrite concentrations were reduced in the effluent. This would signify the presence of an anoxic environment, thus fostering the denitrification of the nitrified forms of nitrogen. Once the effluent BOD$_5$

concentration decreased, denitrification apparently stopped and the nitrate and nitrite concentrations increased.

Table 10: Wastewater nutrient concentrations for the sand filter study.

Date	Nitrate (mg/L)		Nitrite (mg/L)		TKN (mg/L)		PO_4 (mg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	0	79.05	0	58.4	35.2	10.8	8.40	6.09
3/23/99	2.14	53.7	0	32.4	30.6	15.4	8.46	6.66
3/30/99	1.58	134	0	48.7	34.1	9.21	11.7	7.39
4/6/99	1.96	119	0	32.3	49.7	7.85	14.2	9.06
4/12/99	0	1.96	0	0	58.9	5.33	43.9	9.33
4/14/99	0	2.12	0	0	60.2	5.03	31.0	9.81
4/20/99	2.12	2	0	0	64.1	11.1	69.5	16.1
4/22/99	1.88	0	0	0	75.5	8.97	63.3	12.7
4/27/99	0	0	0	0	77.4	14.1	64.8	12.5
4/29/99	1.84	0	0	0	74.9	11.6	65.3	25.6
5/4/99	4.16	4.08	0	0	42.9	12.3	22.6	21.2
5/6/99	0	4.4	0	0	46.9	18.9	14.9	9.41
5/11/99	4.51	79.7	0	14.9	49.6	26.5	24.6	8.57
5/18/99	0	2.77	0	36.3	20.5	39.5	16.5	8.89
5/25/99	2.3	116	0	55.1	54.4	14.7	20.0	18.5
6/2/99	0	46.7	0	57.9	49.8	16	17.0	14.7
6/8/99	1.98	108	0	50.8	60.9	22.2	16.9	13.1
7/21/99	0	240	0	48.1	77.3	21.6	15.4	7.43
7/27/99	0	277	0	46.9	82.2	15.6	15.3	8.13
8/3/99	0.141	289	0	6.99	21.1	11.9	6.37	8.22
8/10/99	0.291	251	0	3.31	25.6	11.9	8.07	5.16
8/17/99	0.186	214	0	0.94	49.9	12.2	10.4	4.78
8/24/99	0.817		0		63.0		11.0	
9/1/99	0.226	229	0	1.66	48.4	10.8	11.8	7.45

Nitrogen series data demonstrates a nitrogen reduction in organic nitrogen through the system. The oxygen state in the wetland was not sufficiently aerobic to support presence of nitrate.

Table 11: Wastewater nutrient concentrations for the wetland study.

	Nitrate (mg/L)		Nitrite (mg/L)		TKN (mg/L)		PO_4 (mg/L)	
Date	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	0	1.311	0	0	26.8	22.1	6.55	5.79
3/23/99	2.21	2.16	0	0	39.9	21.3	12.3	9.28
3/30/99	1.72	2.24	0	0	23	19.3	6.98	4.46
4/6/99	0	2	0	0	47.6	23.8	12.6	8.15
4/12/99	2.04	1.93	0	0	48.7	19.9	15.5	14.8
4/14/99	1.62	1.81	0	0	19.4	28.3	5.45	12.6
4/20/99	2.12	2.37	0	0	33.8	20.9	9.10	7.18
4/22/99	2.36	2.32	0	0	31.8	22.9	8.76	12.2
4/27/99	1.9	1.83	0	0	48.4	24.1	14.2	8.76
4/29/99	2.37	2.24	0	0	14.9	18.5	5.53	4.42
5/4/99	4.25	4.31	0	0	15	15.6	7.05	6.71
5/6/99	4.16	4.58	0	0	26.4	18.2	9.48	4.55
5/11/99	4.32	4.11	0	0	19.1	13.7	11.2	4.46
5/18/99	2.23	2.23	0	0	21.8	22.6	9.78	6.51
5/25/99	2.33	2.38	0	0.417	13	13.2	4.30	3.86
6/2/99	0	5.39	0	1.44	50.8	19.4	11.9	2.86
6/4/99	0	0	33.1	0	31.7	7.30	10.7	0
6/8/99	1.98	2.58	0	3.15	30.8	15.8	7.73	2.92
6/15/99	0	5.92	112	10.8	33.9	14.9	10.94	0
6/22/99	5.76	1.81	0.804	0	8.08	33.1	0	7.87
6/30/99	0	2.2	0	0	31	5.52	7.71	0
7/14/99	0	7.21	20.7	12.1	24.9	8.40	7.04	3.17
7/21/99	0	2.25	0	1.27	22.9	4.38	5.35	1.69
7/27/99	0	2.88	0.39	1.06	9.49	4.85	3.28	1.82
8/3/99	0.118	11	0	0.94	18.1	0	5.89	2.07
8/10/99	0.122	6.31	0	0.40	18	0	6.43	1.19
8/17/99	0.236	1.35	0	0.64	27.3	5.77	8.37	0
9/1/99	0.452	4.74	0	0.31	29.8	7.57	9.81	0.54

Nitrogen data for the aerobic treatment unit demonstrated some nitrogen removal through the system. The organic nitrogen was generally reduced. The nitrate concentration increased in the effluent toward the end of the study. Initially, the nitrifying bacteria population would have been developing in the unit. Then the airline broke which decreased the airflow and limited the nitrate concentration. After the aeration unit was operational again, the effluent nitrate concentration increased.

Table 12: Wastewater nutrient concentrations for the aerobic study.

	Nitrate (mg/L)		Nitrite (mg/L)		TKN (mg/L)		PO_4 (mg/L)	
Date	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/15/99	0.288	0.582	0	0	75.5	51.9	10.6	5.73
3/23/99	2.22	2.14	0	0	39.4	35	9.69	9.31
3/30/99	1.77	1.72	0	0	74.2	60.1	17.3	13.8
4/6/99	1.7	2.18	0	0	89.4	87.1	16.9	17.9
4/12/99	1.91	2.21	0	0	55.5	81.9	14.7	15.5
4/14/99	1.67	2.21	0	0	84.7	65.9	17.0	15.8
4/20/99	0	3.58	0	0	61.5	45	14.4	13.6
4/22/99	2.35	3.55	0	0	67	62.3	13.7	14.5
4/27/99	8.25	1.83	0	0	66.4	19.3	17.4	19.3
4/29/99	2.44	8.23	0	111	75.7	28.2	20.4	20.7
5/4/99	0	34.3	0	100	54.8	25.9	16.8	23.1
5/6/99	4.12	10.7	0	76.1	66.7	27.1	18.2	13.2
5/11/99	4.32	14.8	0	109	94.1	43.3	20.6	24.7
5/18/99	0	13.9	0	104	45.8	68.7	27.6	25.9
5/25/99	2.25	37.1	0	100	70.9	20.3	27.2	26.5
6/2/99	0	31.3	0	90	95.2	31	20.9	24.1
6/4/99	0	0	0	0	102	88.3	28.5	25.8
6/8/99	2.07	55.6	0	101	128	23.6	24.5	29.7
6/15/99	0	288.3	0	4.37	128	17.4	29.3	58.7
6/22/99	0	237			104	28.4		
6/30/99	1.85	1.91			72.2	85.8		

Microbial Monitoring Studies

Aerobic Treatment Unit

The levels of the indicator organisms and the specific bacterial pathogen (Salmonella spp.) in the influent and the effluent of the aerobic treatment unit is shown in Fig 1A and 1B respectively.

As expected the levels of Salmonella spp. was lower than the microbial indicators. The male specific bacteriomale-specific coliphages averaged 2.41 log PFU/ml in the influent over the 2-week period, while the fecal coliforms were the highest averaging 4 log MPN/ml. There were fluctuations in the numbers of the different organisms with fecal streptococci fluctuating between a maximum and a minimum of 4.17 and 1.5 log MPN/ml.

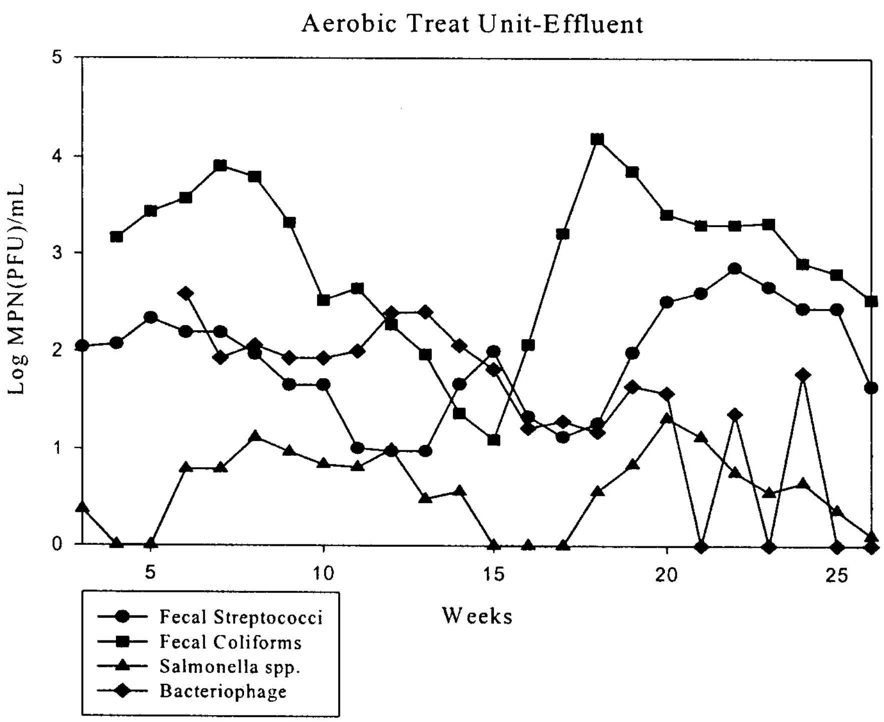

Figure 1A: Influent concentrations of selected microbial groups at the aerobic treatment unit.

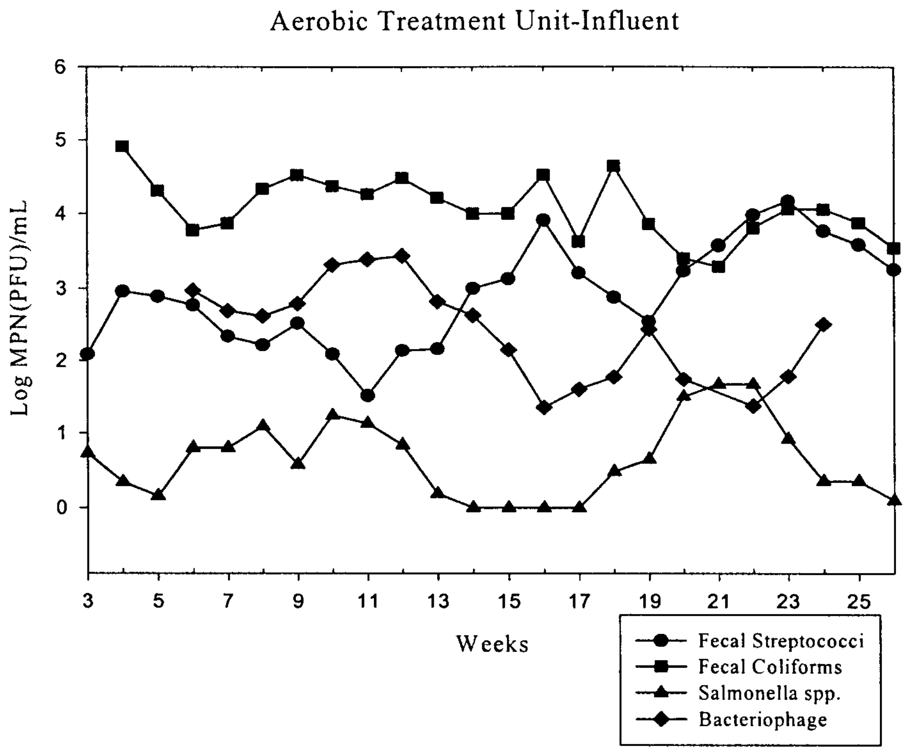

Figure 1B: Effluent concentrations of selected microbial groups at the aerobic unit. Salmonella spp ranged between below detection and 1.6 log MPN/ml. In the effluent, the levels of the indicator organisms and the bacterial pathogen were at least 0.5-2 log units lower than that in the influent.

The fecal coliforms showed a significant reduction in numbers (from 4.07 log MPN/ml in the influent to 2.95 log MPN/ml in the effluent) while surprisingly Salmonella spp. did not exhibit similarly large decreases. However, the numbers fluctuated over the 25-week monitoring. During the course of the monitoring it was observed that the aerobic treatment unit was not functioning as designed during a series of technical glitches. The relatively low reduction efficiency could be attributed to these technical malfunctions.

Sand-filtration Unit

The levels of the selected microbial groups at the influent and the effluent at the sand-filtration unit over a 25 week monitoring period are shown in Figures 2A and 2B.

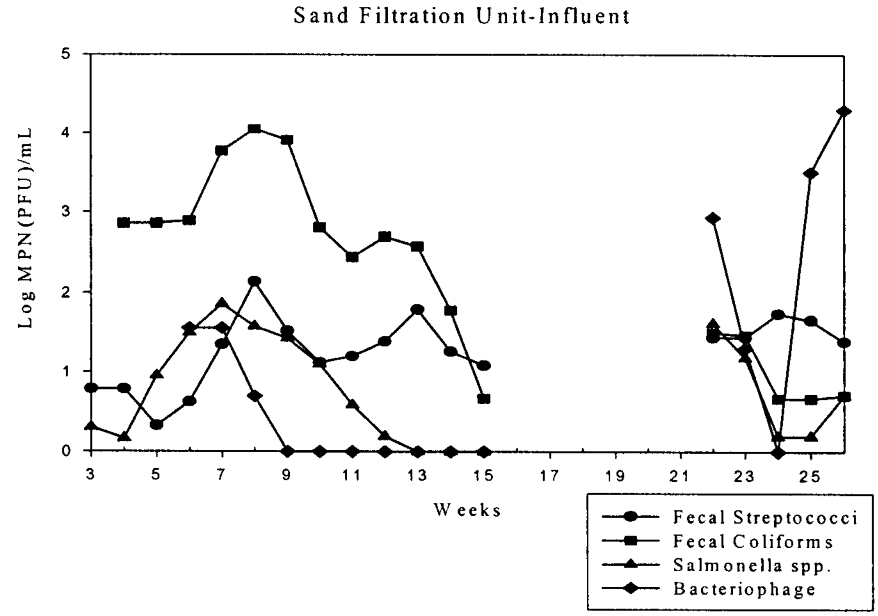

Figure 2A: Influent concentrations of microbial groups at the sand-filtration unit.

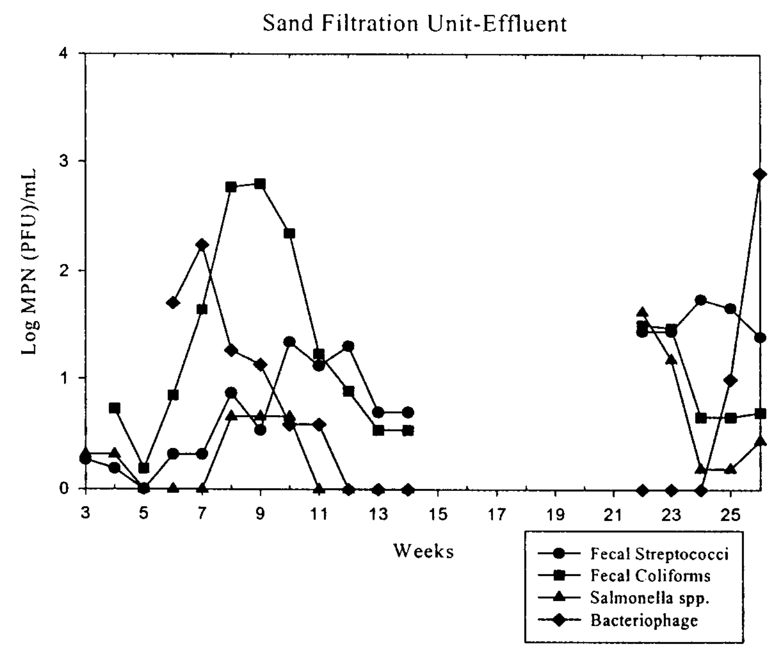

Figure 2B: Effluent concentrations of microbial groups at the sand-filtration unit.

During the course of the monitoring program, the residence to which this unit was attached became vacant and thus no waste stream was available for sampling purposes. The bacteriophage levels showed the greatest amount of fluctuation at this site with their numbers ranging from below detection limit to 4.3 log PFU/ml. As was seen in the aerobic treatment unit, the levels of Salmonella spp were lower than the other microbial groups. Overall, there was a 1-2 log unit decrease in the microbial groups between the influent and the effluent concentrations. As would be expected in any wastewater system such as this, the numbers fluctuated over the 25 weeks that the study was conducted. The fecal coliforms as in the aerobic treatment unit were the predominant microbial group in both the influent and the effluent.

Constructed Wetland

The levels of the selected microbial groups at the influent and the effluent at the constructed wetland unit over a 25 week monitoring period are shown in Figures 3A and 3B.

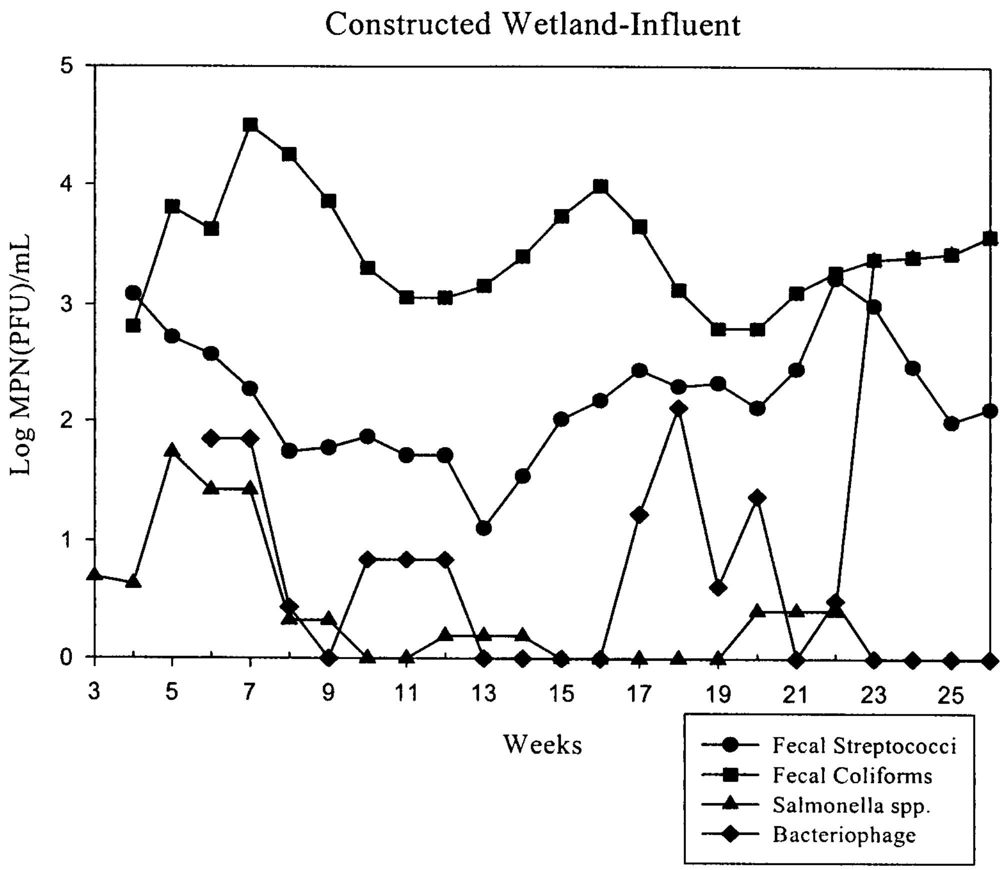

Figure 3A: Influent concentrations of the microbial groups at the constructed wetland.

Fecal coliforms were the predominant organisms in both the influent and the effluent waste streams. Their numbers ranged from 28 log MPN/ml to 4.5 log MPN/ml with a mean of 3.44 log MPN/ml. Surprisingly, the levels of Salmonella spp. in both the influent and the effluent were higher than the coliphage levels. In no other treatment unit was this noticed. The higher numbers of Salmonella spp (than colimale-specific coliphages) in the effluent stream is interesting considering that it is commonly believed that bacterial cells would tend to get "filtered" by the gravel matrix as they pass through the treatment unit.

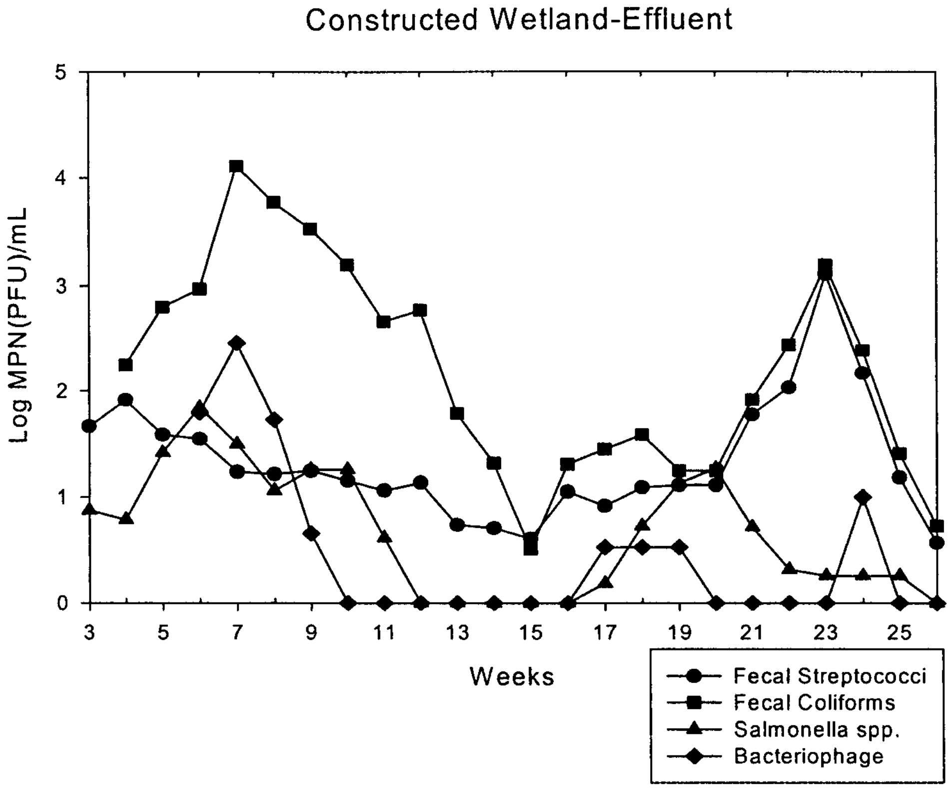

Figure 3B: Effluent concentrations of microbial groups at the constructed wetland.

Pathogen Survival

Pathogen survival studies were performed in the sand-filter and the constructed wetland to determine the survival kinetics of Salmonella spp and a coliphage under cool and warm temperature conditions. For these studies, known numbers of the organisms were incubated in aliquots of the wastewater collected from these sites contained in polypropylene tubes. The tubes were placed within the matrix of the sand-filter and the constructed wetland. At periodic intervals, the samples were enumerated for the numbers of suriviving organisms. An aliquot of the sample was also incubated under constant laboratory conditions as a control.

Sand-Filter Unit

Figures 4A and 4B represent the survival pattern of Samonella spp. within the sand-filter unit during the summer and winter months. It must be emphasized however that the influence of surrounding sand matrix and its associated microbial populations are not taken into consideration here since the survival studies were performed within microcosms and not directly within the sand matrix. The bacterial pathogen showed a relatively rapid decline in numbers over 4 weeks during the study conducted in the summer months. In the study conducted over the winter months, the numbers remained relatively stable after an initial

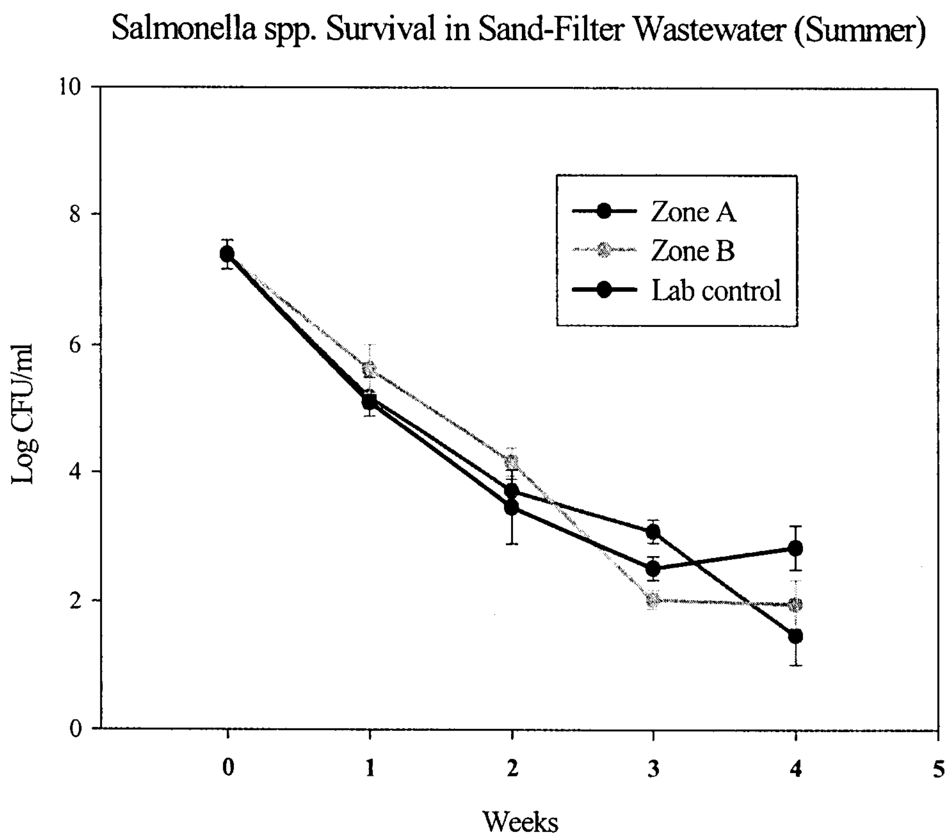

Figure 4A: Survival of Salmonella spp. in the sand filter during warmer months. Zone A and Zone B represent two locations within the sand filter.

rapid decline. There was about a 6 log unit decline in numbers in the summer months as compared to the winter months. In winter after 4 weeks there was about a 4.5 log unit decline. However, it is not possible to conclusively state that only temperature is playing a major role in this decline. Even the laboratory controls showed the same decline in numbers as compared to the field samples.

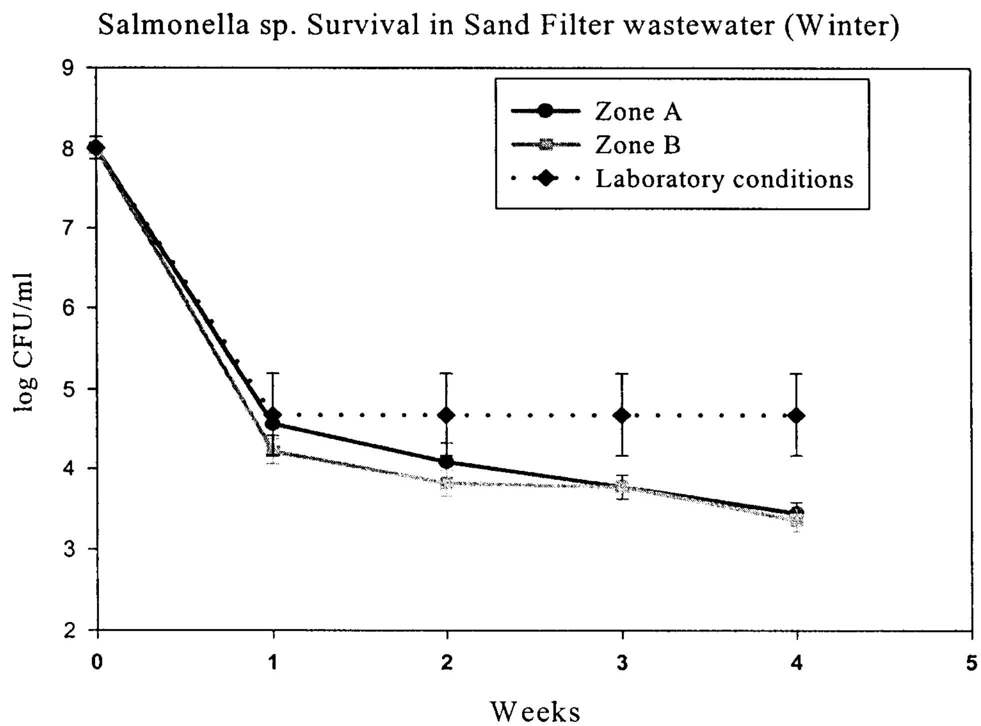

Figure 4B: Survival of Salmonella spp. within the sand filter during the winter months. Zone A and Zone B represents two locations within the sand filter.

The colimale-specific coliphages declined by over 4 log units over a 4-week period in the summer months (Fig 5). The laboratory control showed a similar decline in numbers. It does not appear that the different locations within the sand filter make a difference in the survival pattern. These results are similar to what was observed with the bacterial indicators.

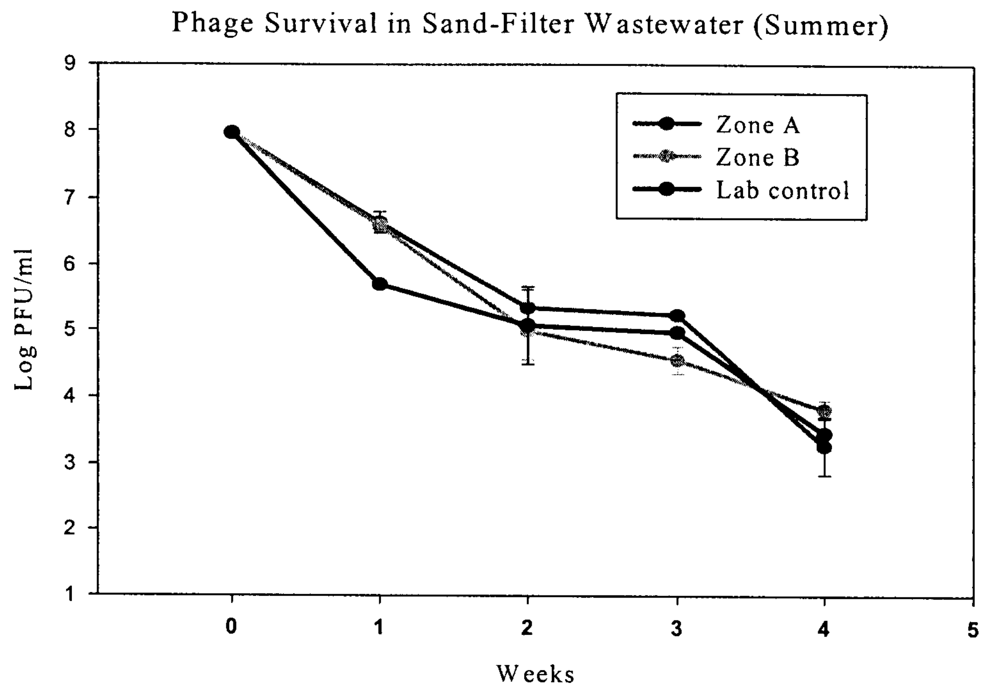

Figure 5: Survival of male specific colimale-specific coliphages in the sand filter wastewater in the summer months.

Constructed Wetland Unit

There was a difference in the survival patterns of the bacterial pathogen (Salmonella spp) in the constructed wetland unit's wastewater in the summer and winter months. In the summer months, there was an almost 2.5 log unit decline while in the cooler winter months, there was no appreciable decline in numbers even after 4 weeks. It appears that the cooler temperature may have permitted the organisms to proliferate in the sample. Surprisingly, in the laboratory control, the numbers declined by almost 2.5 log units. These results suggest that in the cooler temperatures the pathogen may survive for extended periods of time within the constructed wetland, provided there is no bacterial predation by the indigenous microbial populations.

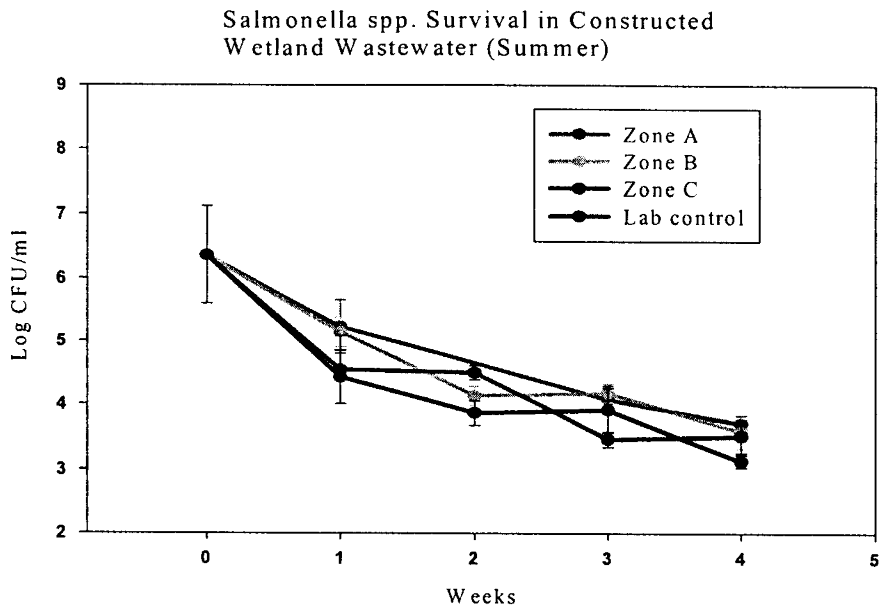

Figure 6A: Survival of Salmonella spp. during the summer months in the constructed wetland unit wastewater.

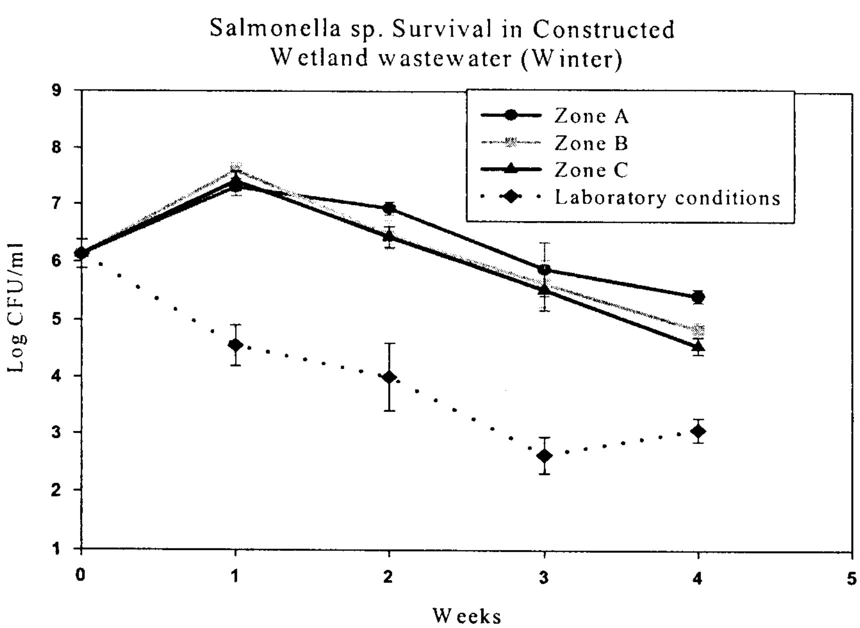

Figure 6B: Survival of Salmonella spp. during the winter months in the constructed wetland unit wastewater.

The survival of male specific colimale-specific coliphages in the constructed wetland wastewater is shown in Fig. 7A and 7B. In contrast to the bacterial pathogen, the colimale-specific coliphages exhibit a somewhat different survival pattern. In summer the decline is only about 3 log units while in winter the decline is significantly different with a reduction of approximately 8 log units occurring over 3 weeks.

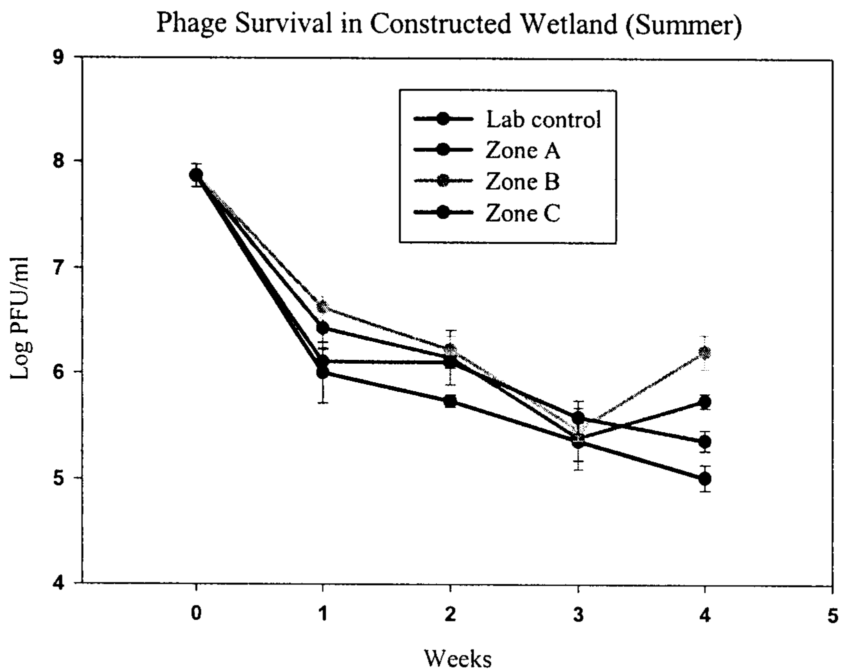

Figure 7A: Survival of male-specific coliphages in the summer months in the constructed wetland unit. Zones A, B, and C refer to different locations within the gravel matrix.

There does not appear to be any significant difference in the survival patterns of the coliphages within different regions of the sand matrix suggesting that the temperature differences within the unit (as a result of the shading created by the plant growth) did not influence the survival of the viruses. Also, the similarity of the survival pattern of the phages in the field in the wetland unit and in the laboratory conditions suggest that the survival in these experiments were a result of the interaction of the virus particles and the wastewater components rather than the temperature. This is significant considering that temperature is considered to be a critical factor controlling virus persistence in the environment.

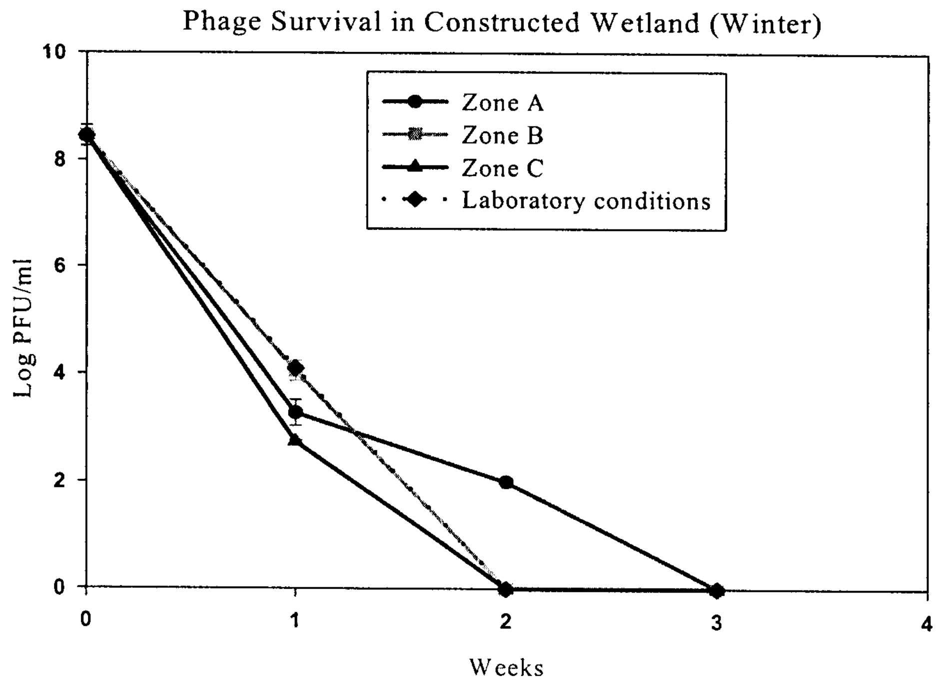

Figure 7B: Survival of male-specific coliphages during cooler months in the constructed wetland. Zones A, B, and C refer different locations within the gravel matrix.

Interestingly, there was an 8 log-unit decline for the majority of the viru sparticles. In on emicrocosm alone, the coliphages survived for an extended period of time. It is not clear whether this difference can be regarded as an ecologically significant difference especially since the phages in the laboratory control behaved similarly to the other phages. It appears that the rapid decline in both the laboratory control and the experimental microcosms is a function of the interaction between the phages and the wastewater components. Theoretically, the phages should have exhibited a faster decline in the warmer summer months rather than the cooler winter months.

Transport Studies

Sand filter pump tank

The sand filtration pump tank served as the primary reservoir for the tracers spiked into the sand filter system. All 3 tracers remained at relatively constant levels for the first 7 days of the study (Figure 8A). Subsequently, the phage concentrated declined rapidly in contrast to the *Salmonella* concentrations that increased to slightly above 10^1 CFU/ml. By the end of the study, *Salmonella* numbers remained constant at around 10^1 CFU/ml . Even at the end of 30 days, all 3 tracers were detectable.

Sand filter effluent

Salmonella was not detected in the sand filter effluent until around Day 18. This is in contrast to the phage and bromide tracers that were detectable even on Day 1 (Figure 8B). Even though the bacterial tracer was not detectable as early as the phage and bromide tracers, bacterial numbers in the effluent actually increased and remained constant between 1-100 CFU/mL in the sand-filter effluent. Bromide concentration decreased steadily over the course of 30 days after an initial increase. Phage numbers declined almost 2-log units over 30 days.

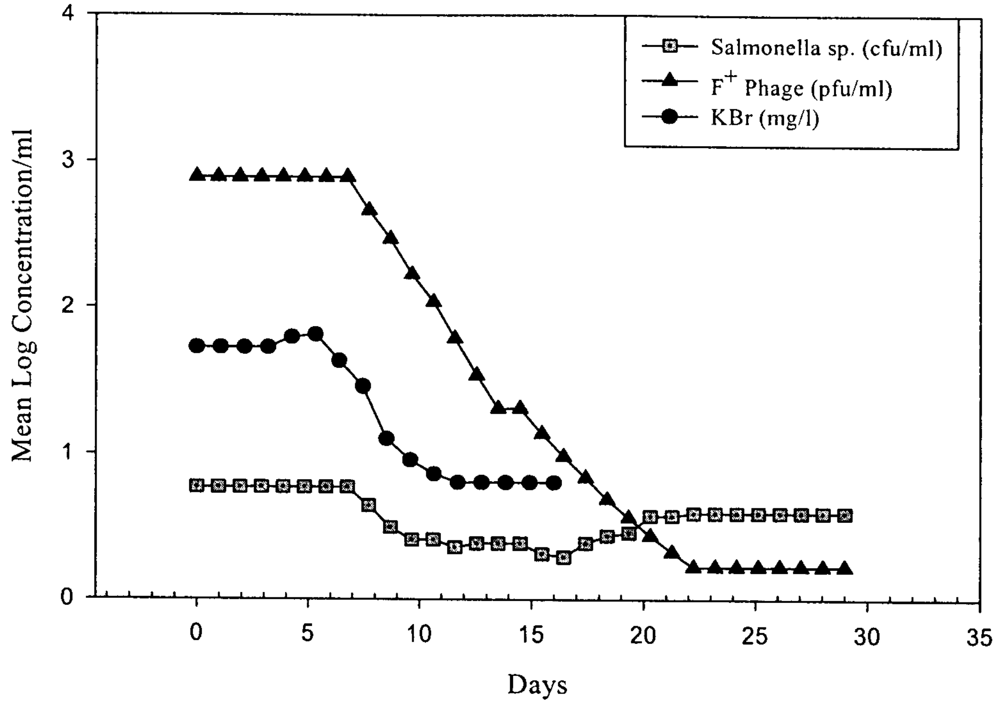

Figure 8A: Concentration (geometric mean) of microbial tracers and bromide in the pump tank prior to entering the sand filter

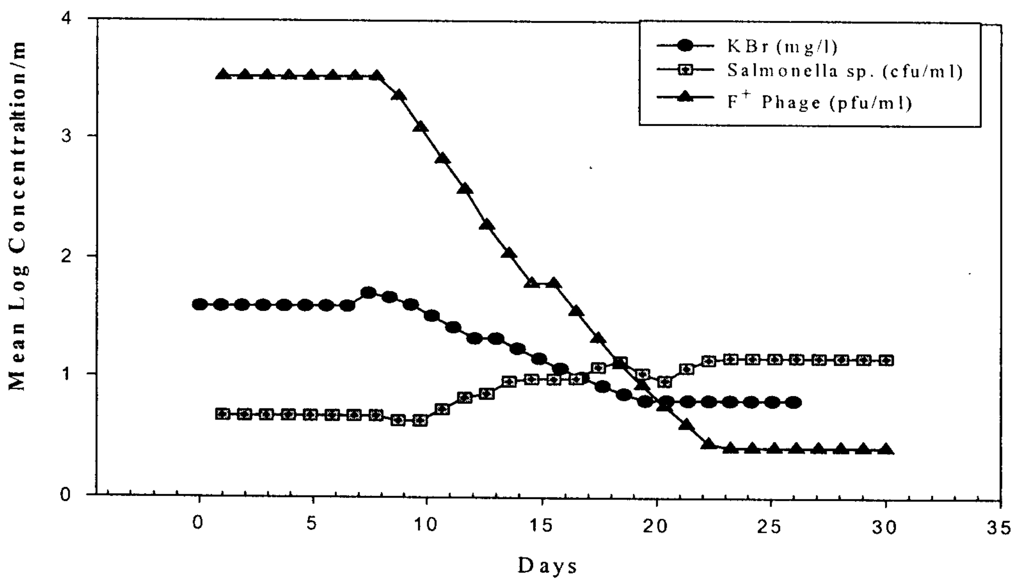

Figure 8B: Concentration (geometric mean) of microbial tracers and bromide in the sand filter effluent.

Constructed wetland pump tank

All 3 tracers were detected in the pump tank soon after their addition into the toilet bowl and remained constant for up to 5 days (Figure 9A). There was, however, a significant reduction in concentration of the tracers compared to the injection concentration. There was about a 2-log difference in the maximum phage concentration as compared to *Salmonella*. After 5 days, all 3 tracers showed a decline in concentration. *Salmonella* remained relatively constant throughout the 30-day study other than for a moderate decline between Day 7 and Day 17. The numbers averaged between 1-10 CFU/mL. The phages were also detected within 10 minutes of the injection. MS2 phage levels, however, decreased by 3-log orders of magnitude from approximately Day 8 until Day 22. The levels of surviving phages were

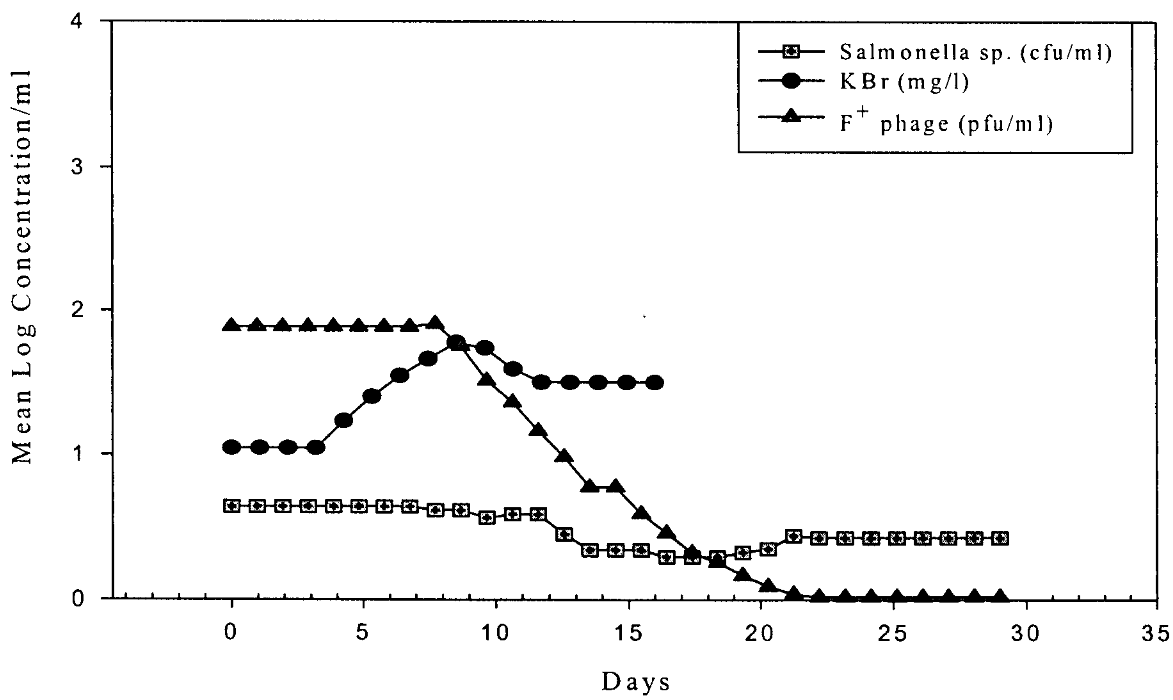

Figure 9A: Concentrations (geometric mean) of microbial tracers and bromide in the pump tank prior to entering the constructed wetland.

lower than that of *Salmonella* towards the end of the study. The chemical tracer (bromide) also showed a decrease that mimicked that of the phage. Even at the end of 30 days, there were detectable levels of phages, *Salmonella* and bromide.

Constructed wetland effluent

The constructed wetland effluent showed a similar pattern as compared to the pump tank effluent in terms of the microbial tracers (Figure 9B). While the levels of phages and *Salmonella* showed a decreasing trend over the 30-day period, the bromide tracer concentrations increased between Day 5 and Day 10. Bromide was detectable even at the end of 30 days. There was a greater decline in phage numbers than that of the bacterial pathogen, which remained relatively constant even at the end of 30 days.

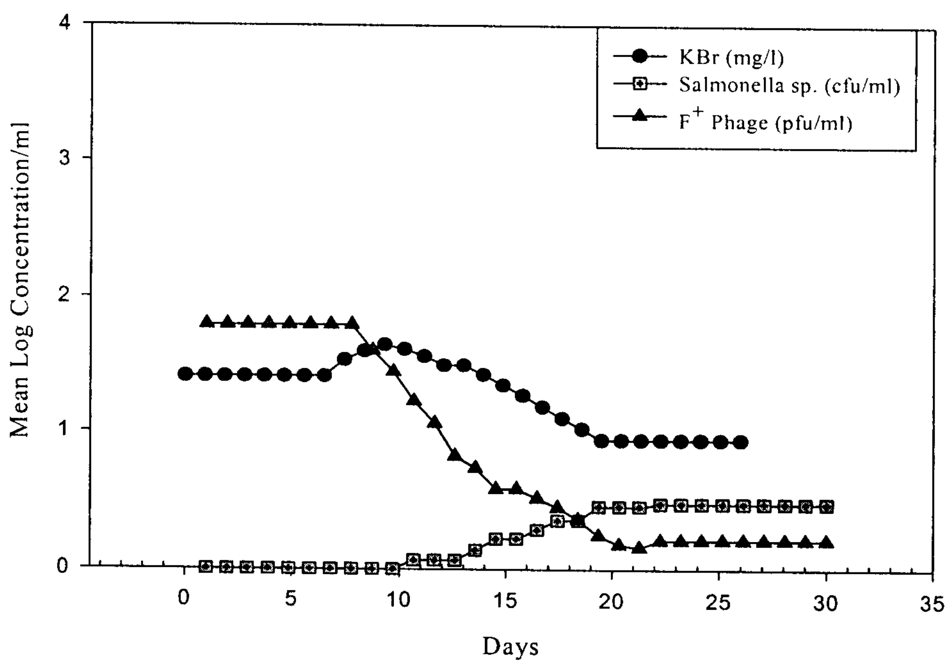

Figure 9B: Concentrations (geometric mean) of microbial tracers and bromide in the constructed wetland effluent.

CHAPTER 6: DISCUSSION

Spearman rank statistics were used to analyze the chemical and biological tracers in the Sand Filter and the Constructed Wetland. Bromide transport in the CW and SF had a strong correlation (0.9) ($P < 0.05$) suggesting that the behavior of the chemical tracer was similar in both the treatment systems.

Phage transport patterns through the CW and SF were also similar yielding a correlation of 0.8 ($P < 0.05$). The difference in the starting concentrations of the phages in the SF can be attributed to the higher inoculum levels of phages that were introduced into the system. It is interesting, however, that within 20 days, both the CW and SF had only trace levels of phages suggesting that in both these systems, a 3-log reduction can be expected by attenuation processes. Greater than 99% removal of MS2 bacteriophage was observed in sand-based lysimeters (Van Cuyk et al., 2001). Chendorain et al (1998) observed a 97% reduction in MS2 bacteriophage numbers in single celled and multi-celled surface water constructed wetlands. Adsorption and inactivation can be considered to be the primary factors controlling virus attenuation within submerged flow systems and sand filter systems. The concept of critical pH has recently been proposed as a key factor controlling viral adsorption onto sediments (Huade et al., 2002). It is also evident that virus particles can be expected to be present in the effluent (possibly due to desorption) until the numbers of viable phage particles decrease below the detection limit. Such prolonged low-level detection has been previously reported (Dowd and Pillai, 1997). Meschke and Sobsey (1998) have shown using Norwalk virus, poliovirus, and MS2 bacteriophages that viruses can exhibit different adsorption characteristics with different soil textures. In this study, within the sand filter there was a very weak negative, but statistically significant correlation

(-0.4) (P <0.05), between *Salmonella* and phage. It is evident that phages are removed in greater numbers than the bacterial pathogen.

The gradual increase in the bacterial concentration in the SF could be due to increased desorption as the study progressed. A similar increase though appearing later in the study was also noted in the CW. It must be emphasized that neither the constructed wetland nor the sand filter totally eliminated the bacterial or the viral tracer. While the bacterial reduction in the wetland and the sand filter were negligible, there was a marked reduction of the viral tracer. There was almost a 3 –log reduction of viruses in the wetland as compared to more than a 3-log reduction in the sand filter. Other studies have reported on the reduction of microbial tracers under wetland conditions (Gersberg et al., 1987; Neralla et al., 2000; Hill and Sobsey, 2001). The detection of low numbers in the effluent indicates that both bacteria and virus particles can migrate through the CW and SF. Studies conducted at seven onsite constructed wetlands in Alabama and North Carolina suggest that microbial removal efficiencies can vary significantly (Barrett et al., 2001). Effluent disinfection may therefore be required to provide an additional barrier against potential environmental contamination. Bromide and phage transport in the SF exhibited a weak correlation of 0.5 ($P < 0.05$). The differences in migration pattern between the bromide and virus tracers was reported previously (Bales et al., 1995; Schijven book). This supports the findings that bromide transport patterns cannot be used to model microbial tracers especially since viruses are reactive with their surrounding matrices. Iqbal and Krothe (1996) have reported on the greater mobility of conservative tracers such as Br^- and Cl^- compared to reactive tracers.

The survival of the target organisms was dependant primarily on the factors in the septic effluent and the ambient temperature. Since the organisms were not in contact with the gravel/sand material, adsorption was not a factor in these studies. The reduced survival or persistence of phages in contrast to the bacterium in these septic tanks agrees with previous results. We have previously shown that in the arid southwest regions of Texas, the high cation content of the water is detrimental to phage survival (Dowd and Pillai, 1997). Studies have shown that wastewater associated bacteria could be harbored directly on the root surfaces of plants within the constructed wetlands (Vymazal et al., 2001a and 2001b). The decline in bacterial numbers could be attributed to biotic and abiotic factors. Davies and Bavor (2000) have reported that bacterial numbers tend to decrease rapidly in constructed wetlands than in ponds and that bacterial predation can be responsible for the decline.

CHAPTER 7: CONCLUSIONS & RECOMMENDATIONS

This project was to evaluate the treatment performance of several on-site wastewater treatment technologies relative to pathogen reduction. The constructed wetland system and sand filter system were treating wastewater from a residence while the aerobic treatment unit was receiving wastewater from a commercial facility. These are the main points for consideration.

1) The aerobic treatment unit was not effectively evaluated.
2) The subsurface flow constructed wetland and sand-filter systems are effective at reducing the viral concentration of waste effluent streams. However, the reduction of bacterial concentrations (when *Salmonella* sp was used a tracer) was not significant. This result indicates that disinfection of effluent must be a component of the treatment process for surface distribution of effluent. Wastewater can be distributed below the ground surface following advanced treatment without disinfection provided sufficient soil and appropriate conditions are present for pathogen removal.
3) The influent and effluent BOD_5 and TSS concentrations vary substantially on these three systems. This study did not focus on evaluation of the organic removal rates associated with the treatment processes.

REFERENCES

Ayaz, S. Ç. and L. Akça. 2001. Treatment of wastewater by natural systems. Environment International. 26: 189-195.

Bales, R.C., S.Li, K.M. Maguire, M. T. Yahya, C.P. Gerba and R.W. Harvey. 1995. Virus and bacteria transport in a sandy aquifer, Cape Cod, MA Groundwater. 33: 653-661.

Chendorain, M., M Yates, and F. Villegas. 1998. The fate and transport of viruses through surface water constructed wetland. J. Env. Qual. 27: 1451-1458.

Craun, G.F., and R. Calderon. 1996. Microbial risks in groundwater systems. Epidemiology of waterborne outbreaks. In: Under the microscope, Proceedings of the Groundwater Foundations, 12th Annual Fall Symposium, Sept 5th and 6th, 1996, Boston, MA. American Water Works Assn, Denver, CO.

Davies C.M., and Bavor, H.J. The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. J. Appl. Microbiol. 89: 349-360

Dowd, S.E., S.D. Pillai, S. Wang and M. Y. Corapcioglu. 1998. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. Appl. Environ. Microbiol. 64: 405-410.

Gersberg, R.M., S.R. Lyon, R. Brenner, and B.V.Elkins. 1987. Fate of viruses in artificial wetlands. Appl. Environ. Microbiol. 53: 731-736.

Hill, V.R., and M.D. Sobsey. 2001. Removal of Salmonella and microbial indicators in constructed wetlands treating swine wastewater. Wat. Sci. Technol. 44: 215-222.

Iqbal, M.Z., and N.C. Krothe. 1996. Transport of bromide and other inorganic ions by infiltrating storm water beneath a farmland plot. Groundwater. 34:972-978.

Meschke, J.S., and M.D. Sobsey. 1998. Comparative adsorption of Norwalk virus, poliovirus 1 and F+ RNA coliphages MS2 to soils suspended in treated wastewater. Wat. Sci. Tech. 38: 187-189.

Neralla, S., R.W. Weaver, B.J. Lesikar and R.A. Persyn. 2000. Improvement of domestic wastewater quality by subsurface flow constructed wetlands. Bioresource Technol. 75: 19-25.

Scandura, J.E., and M.D. Sobsey. 1997. Viral and bacterial contamination of groundwater from on-site sewage treatment systems. Wat. Sci. Tech. 35: 141-146.

Schijven, J.F. 2001. Virus removal from groundwater by soil passage: modeling, field and laboratory experiments. Doctoral dissertation. Technische Universiteit, Delft.

USEPA, 1980. Design manual - on-site wastewater treatment and disposal systems. USEPA Rteport 625/1-80-012. Washington, D.C.

USEPA, 2000. National Primary Drinking Water Regulations:Ground Water Rule; Proposed Rules. Vol 65: No 91. May 10.

USEPA, 2001. Method 1602. Male-specific (F+) and somatic coliphages in water by single agar layer (SAL) procedure. Office of Water, Washington, DC.

Van Cuyk, S., R. Siegrist, A. Logan, S. Masson, E. Fischer and L. Figueroa. 2001. Hydraulic and purification behaviors and their interactions during wastewater treatment in soil infiltration systems. Wat Res. 35: 953-964.

Vymazal, J., J. Balcarova and H. Dousova. 2001. Bacterial dynamics in the sub-surface constructed wetland. Wat. Sci. Technol. 44: 207-209.

Vymazal, J., V. Sladecek and J. Stach. 2001. Biota participating in wastewater treatment in a horizontal flow constructed wetland. Wat. Sci. Technol. 44: 211-214.

APPENDIX A: DRAWINGS

Research systems were constructed at three locations to facilitate evaluation of the three different technologies. Site and construction drawings are presented for the sand filter system in Figures A1-A4. Site and construction drawings are presented for the constructed wetland system in Figures A5 – A8. A site drawing is presented for the aerobic treatment system in Figure A9.

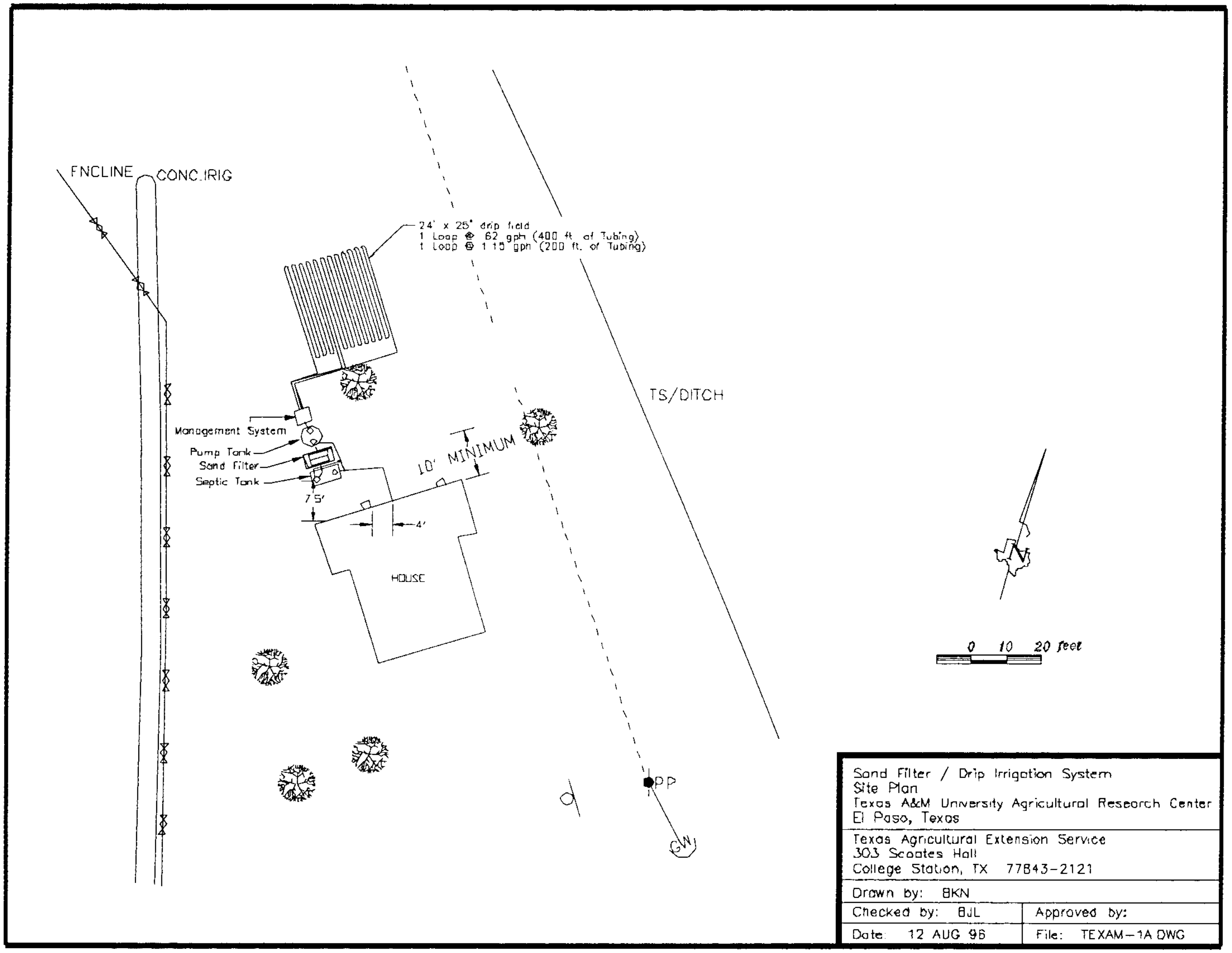

Figure A1: Sand filter treatment system components.

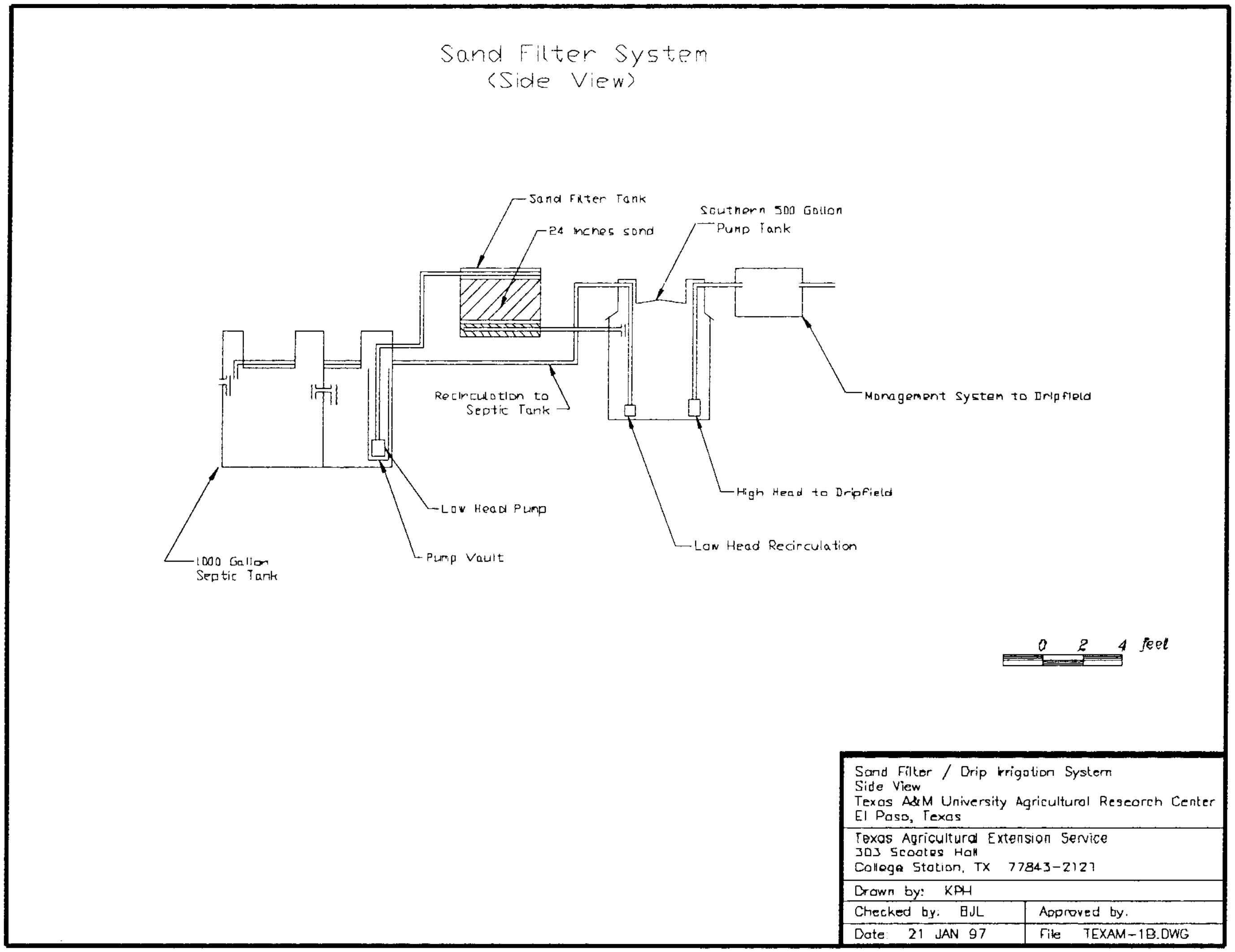

Figure A2: Side view of sand filter treatment system.

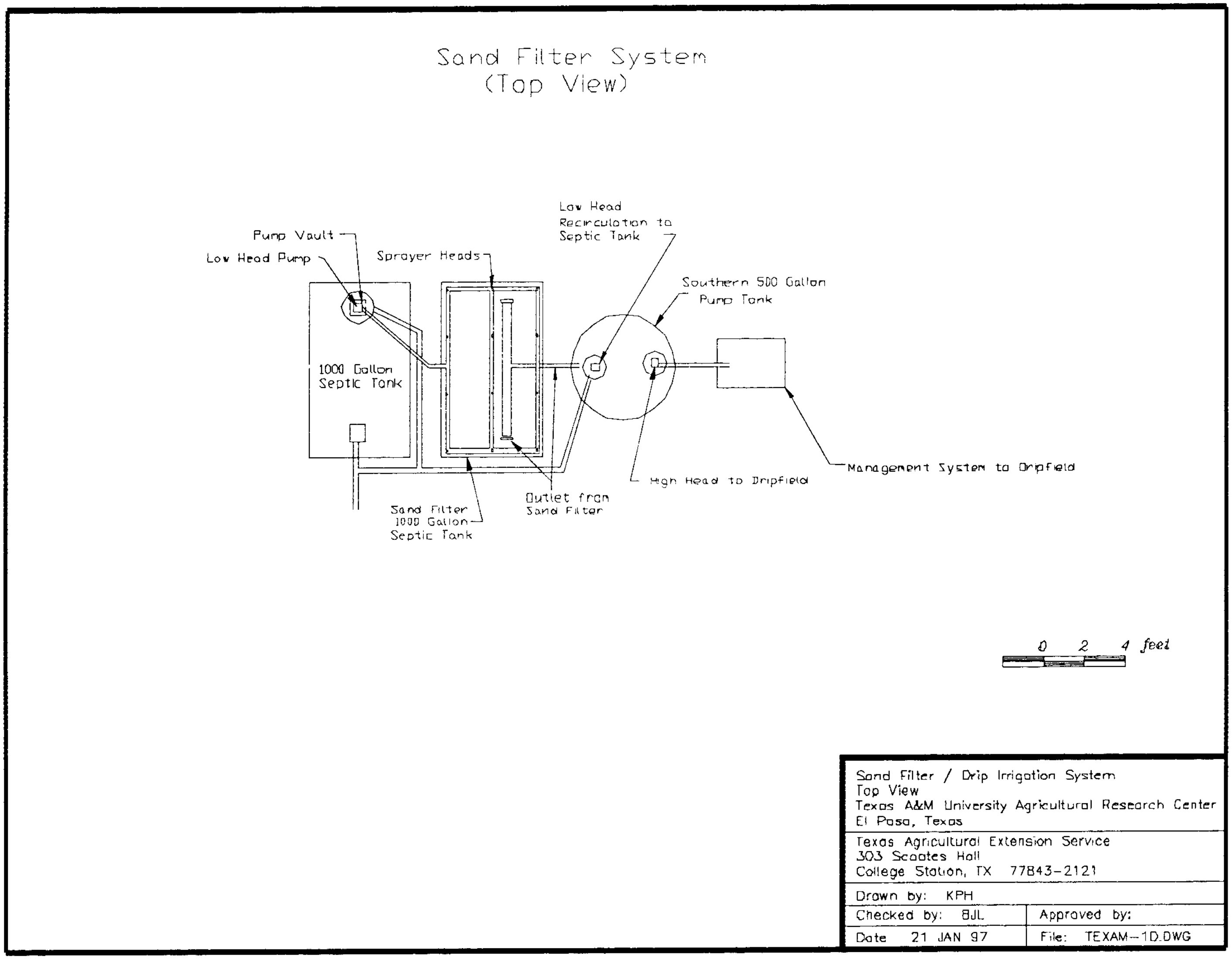

Figure A3: Top view of sand filter treatment system.

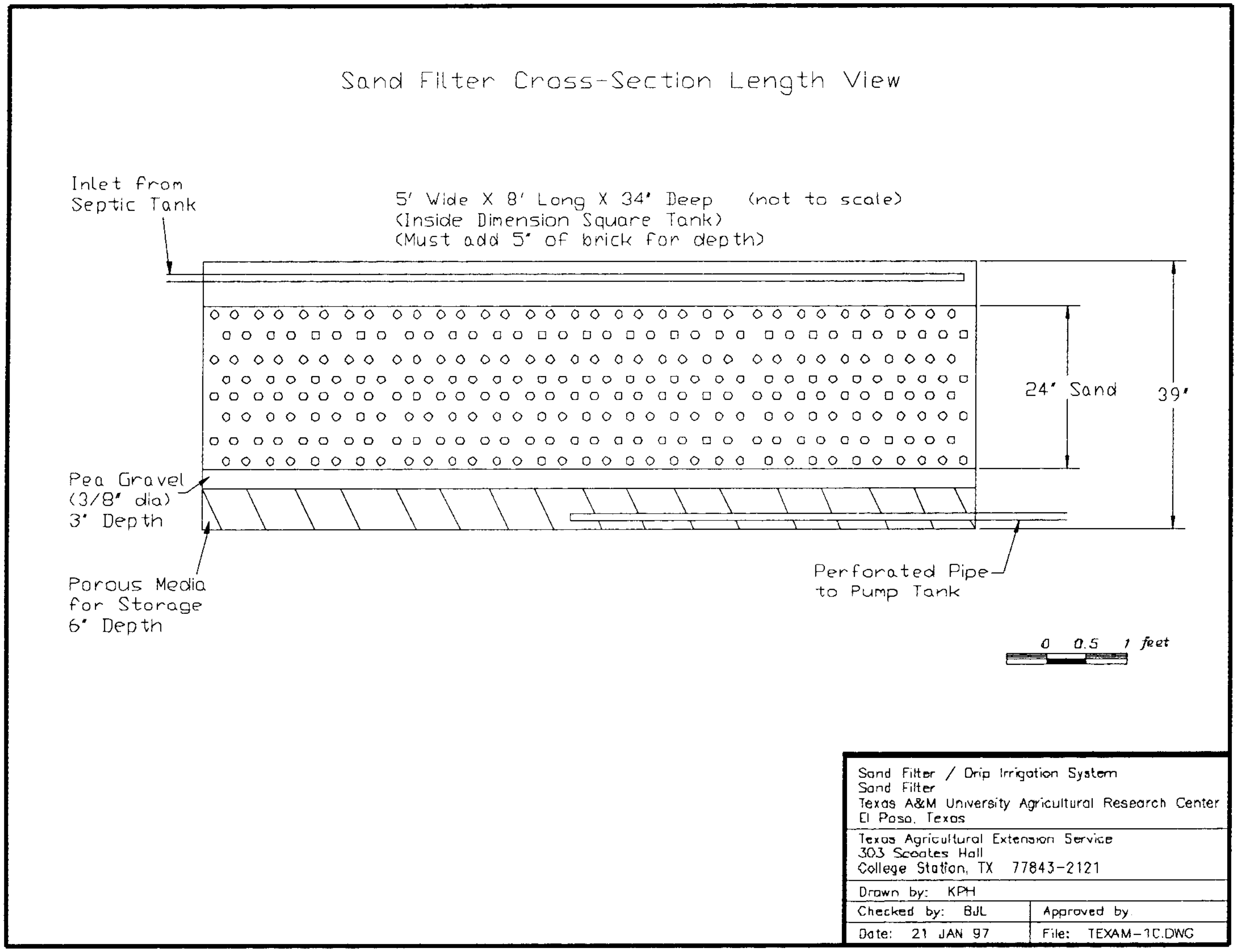

Figure A4: Cross-section of sand filter treatment system.

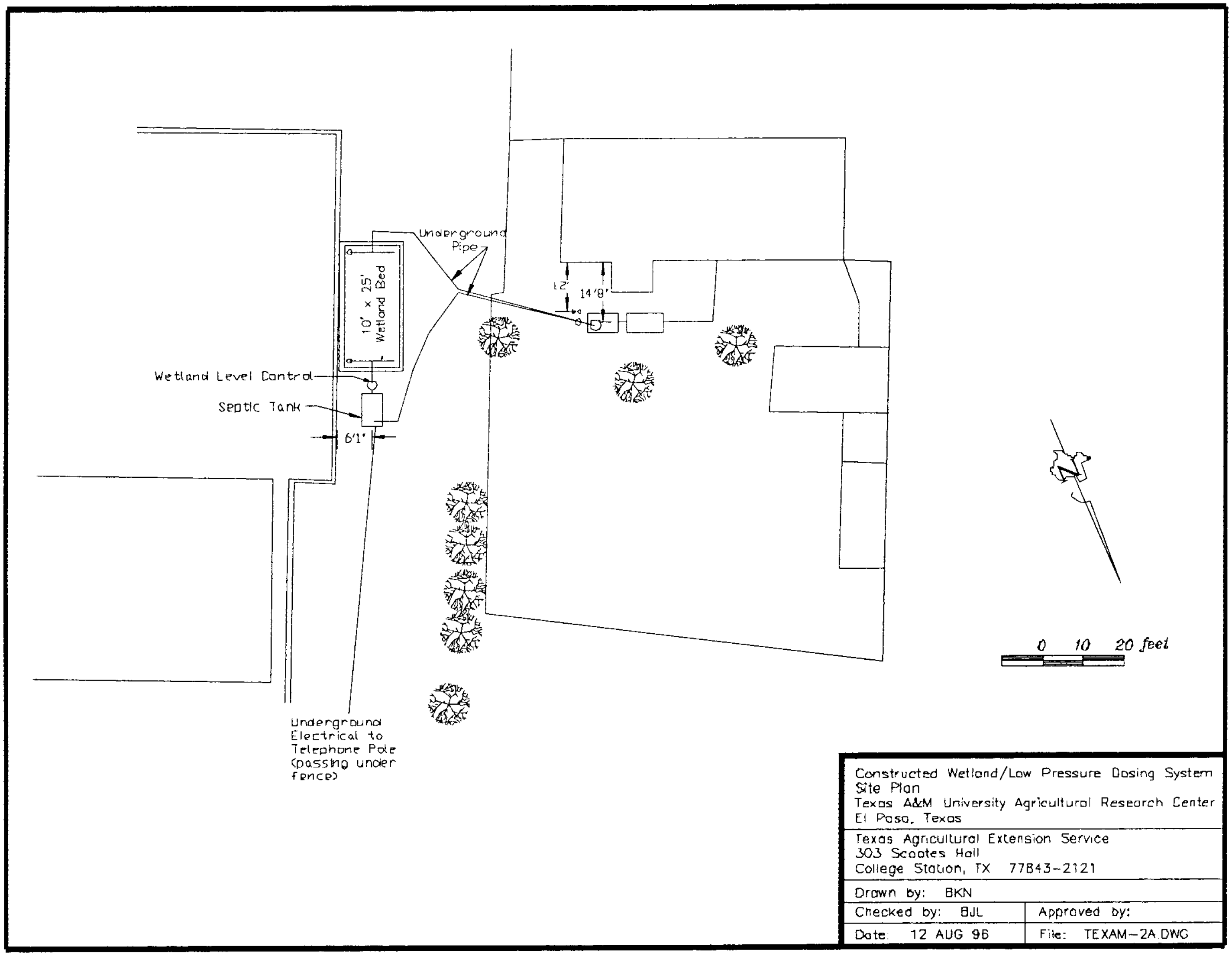

Figure A5: Constructed wetland treatment system.

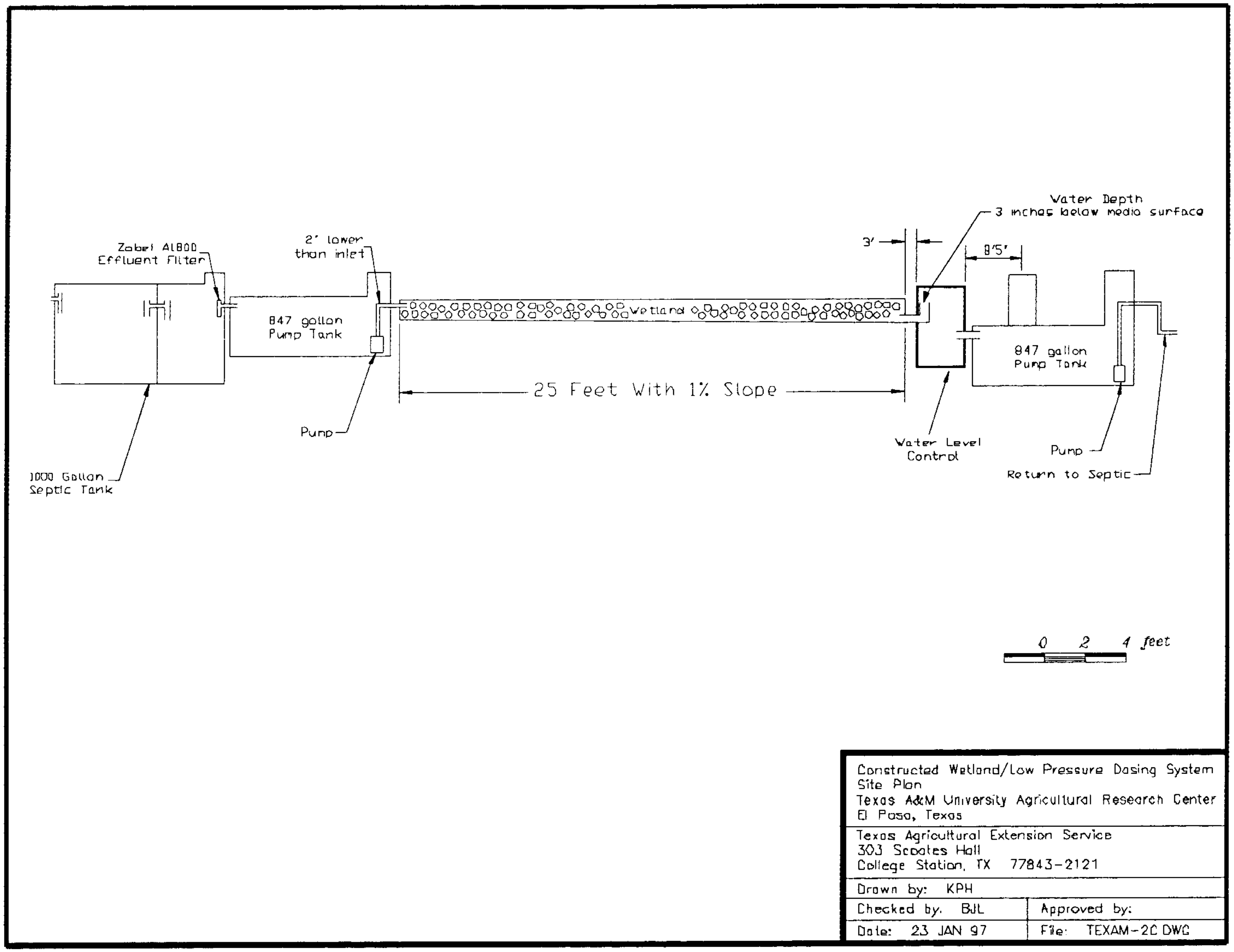

Figure A6: Side view of constructed wetland treatment system.

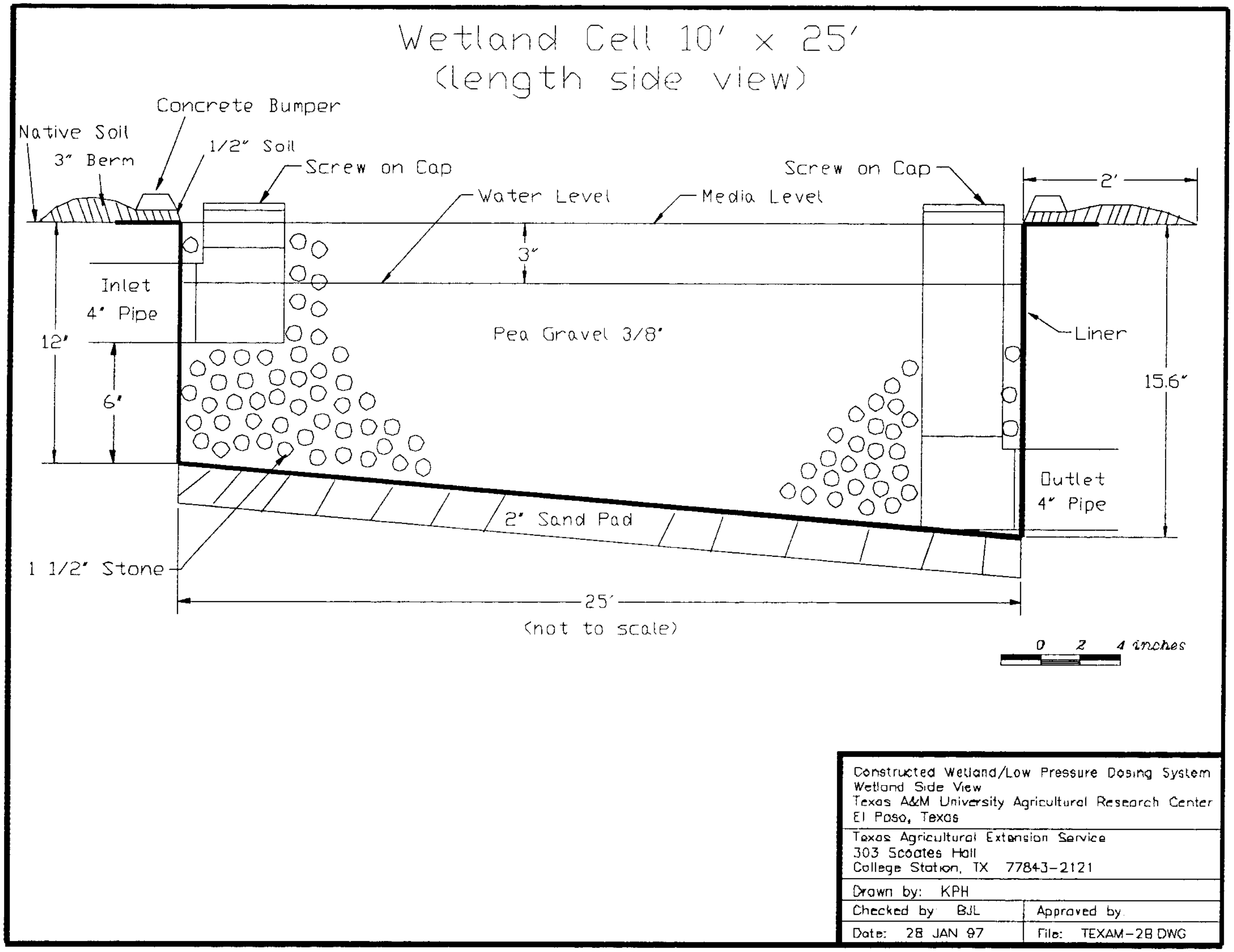

Figure A7: Cross-section of constructed wetland bed.

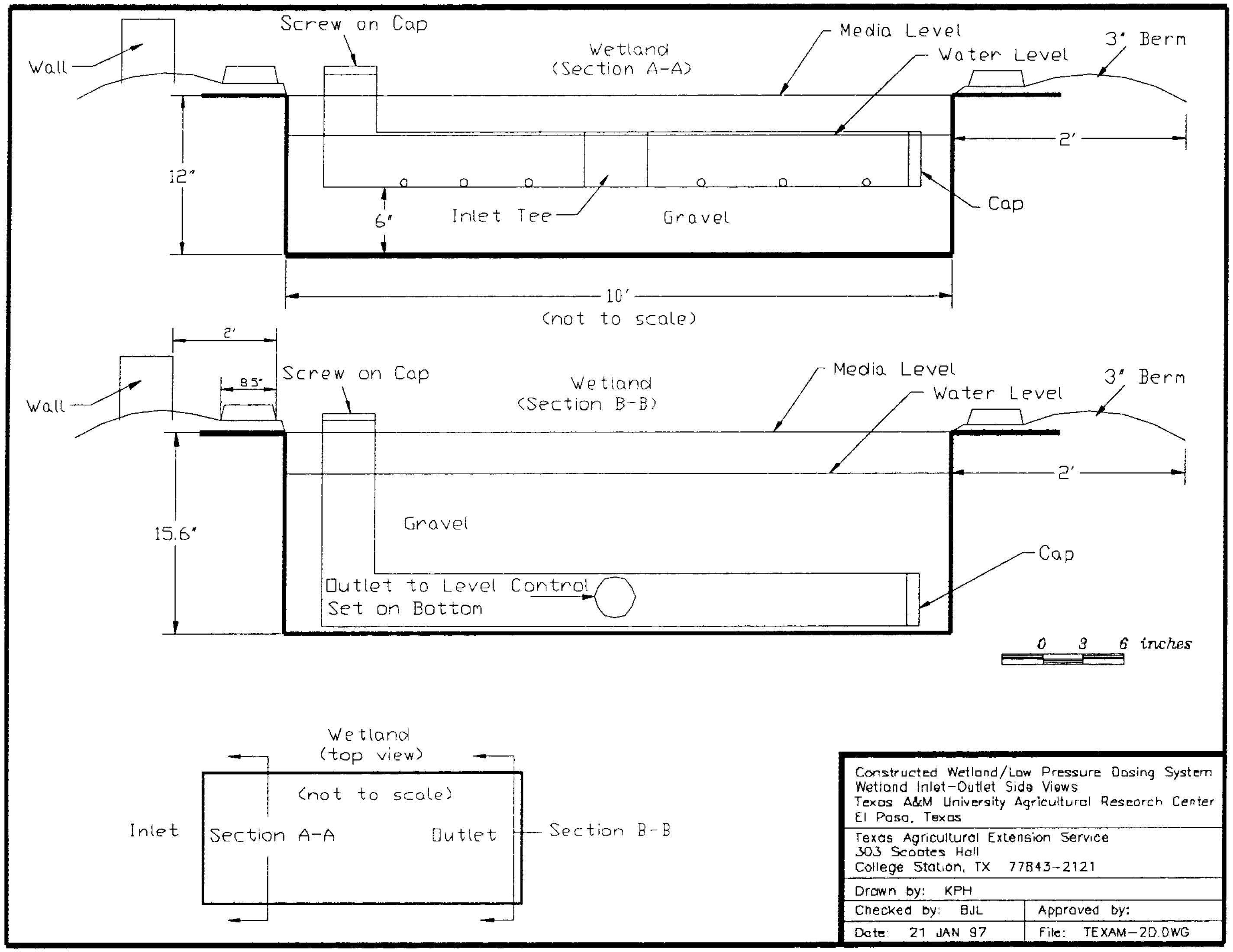

Figure A8: Cross section of piping system supplying and removing water.

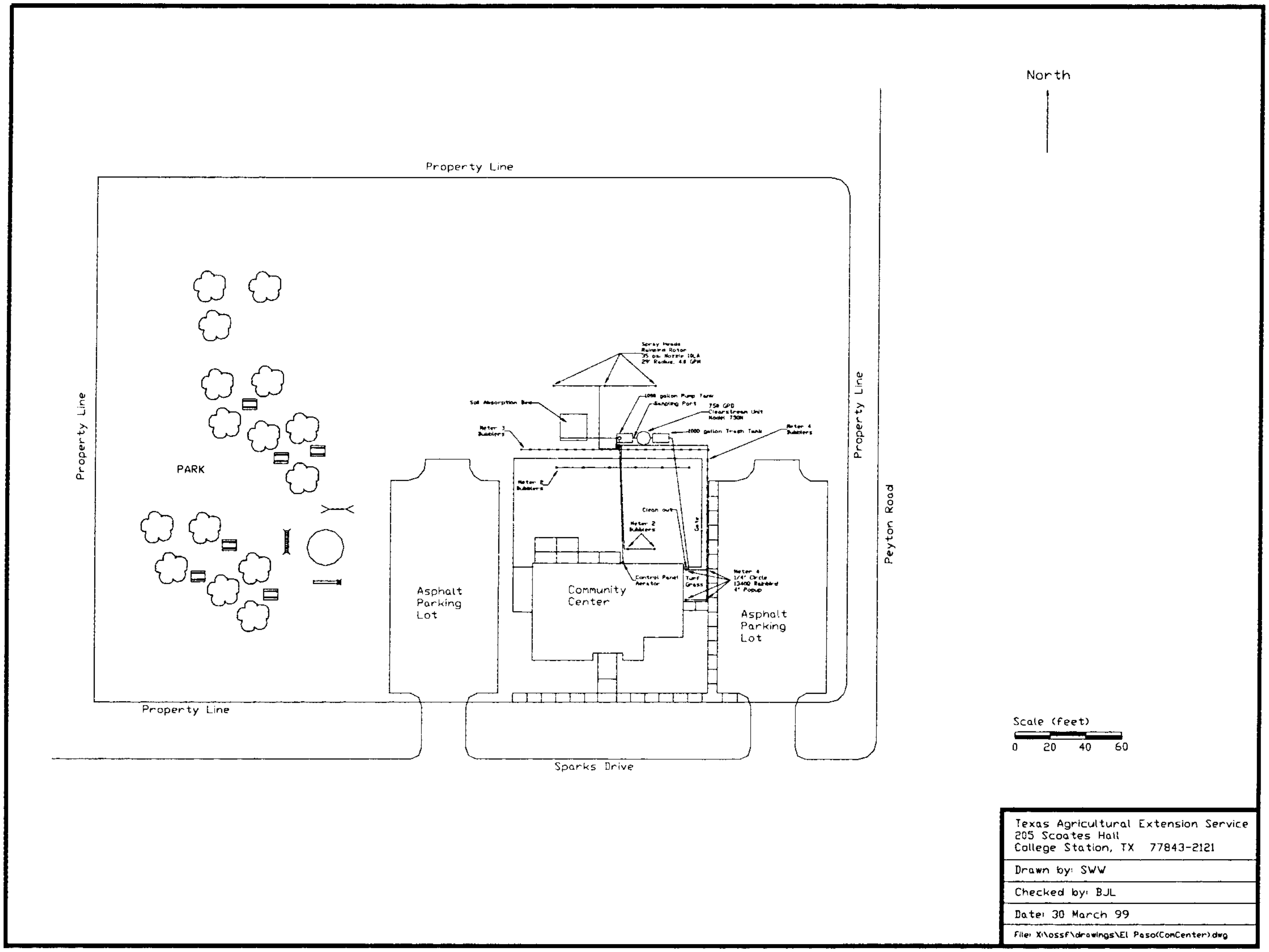

Figure A9: Aerobic treatment unit system

APPENDIX B: WATER QUALITY DATA

Water quality information presented in Tables 1-6 is presented graphically for a visual evaluation. This information will assist in assessing treatment effectiveness.

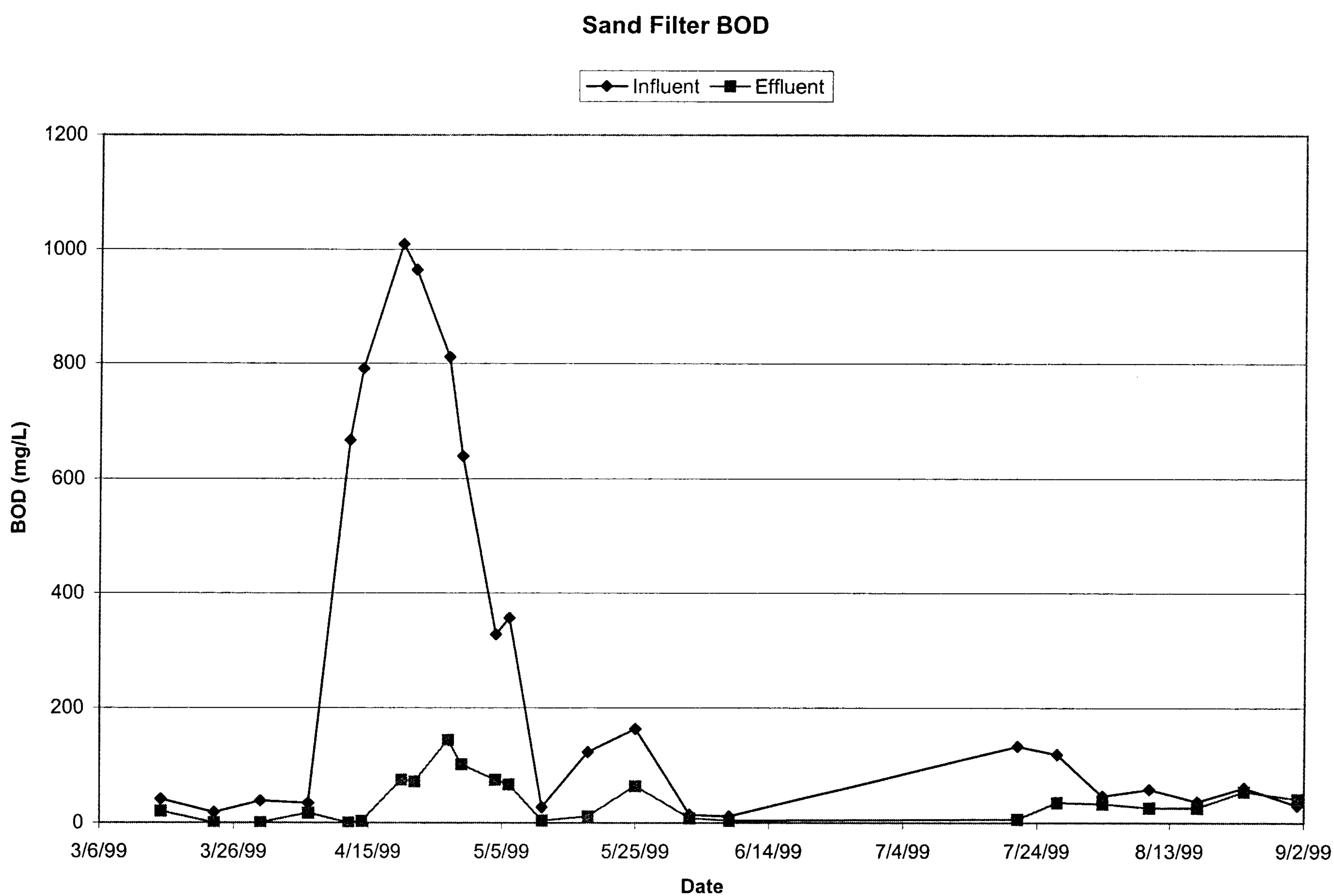

Figure B1: BOD_5 (mg/l) monitoring data for sand filter.

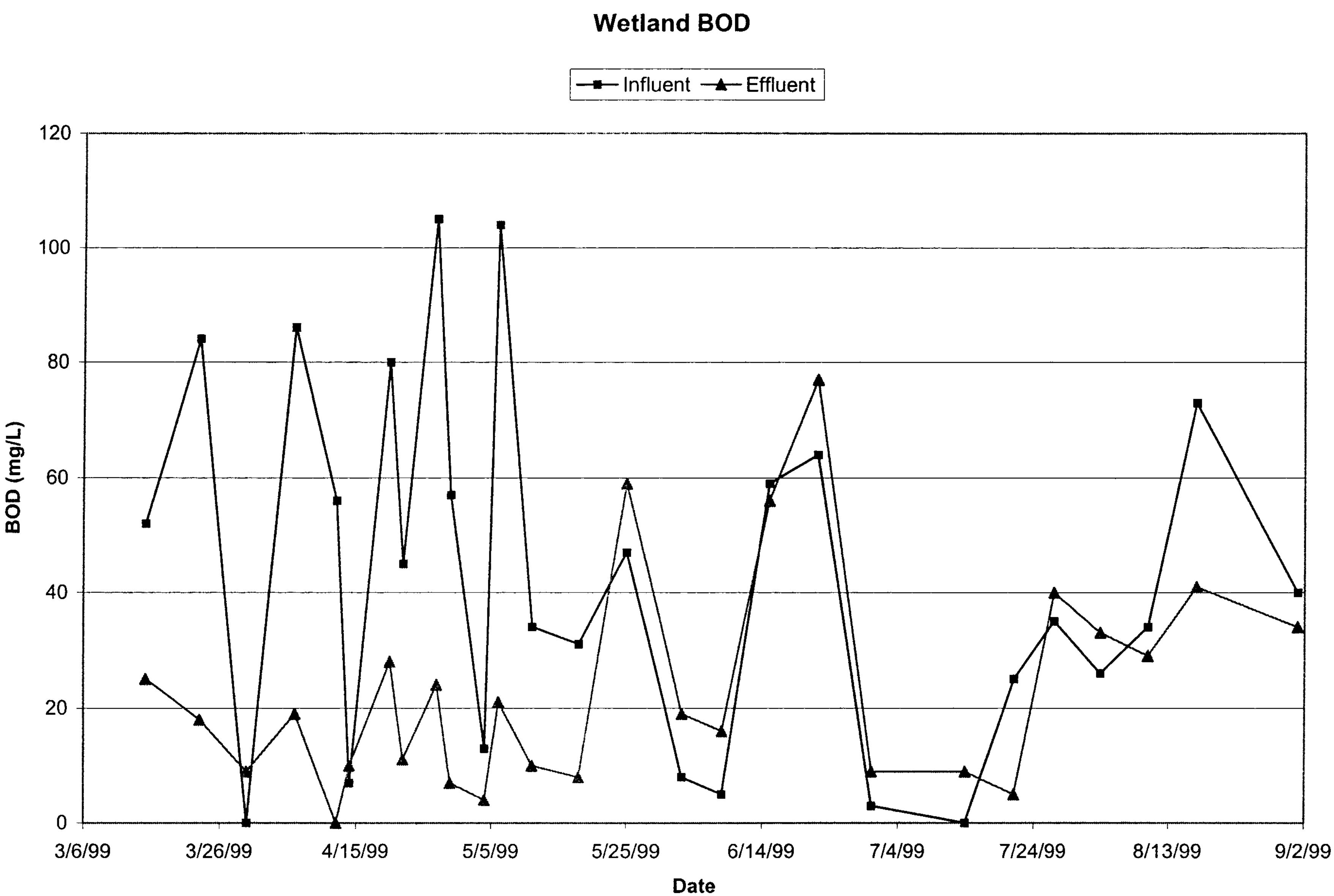

Figure B2: BOD_5 (mg/l) monitoring data for wetland.

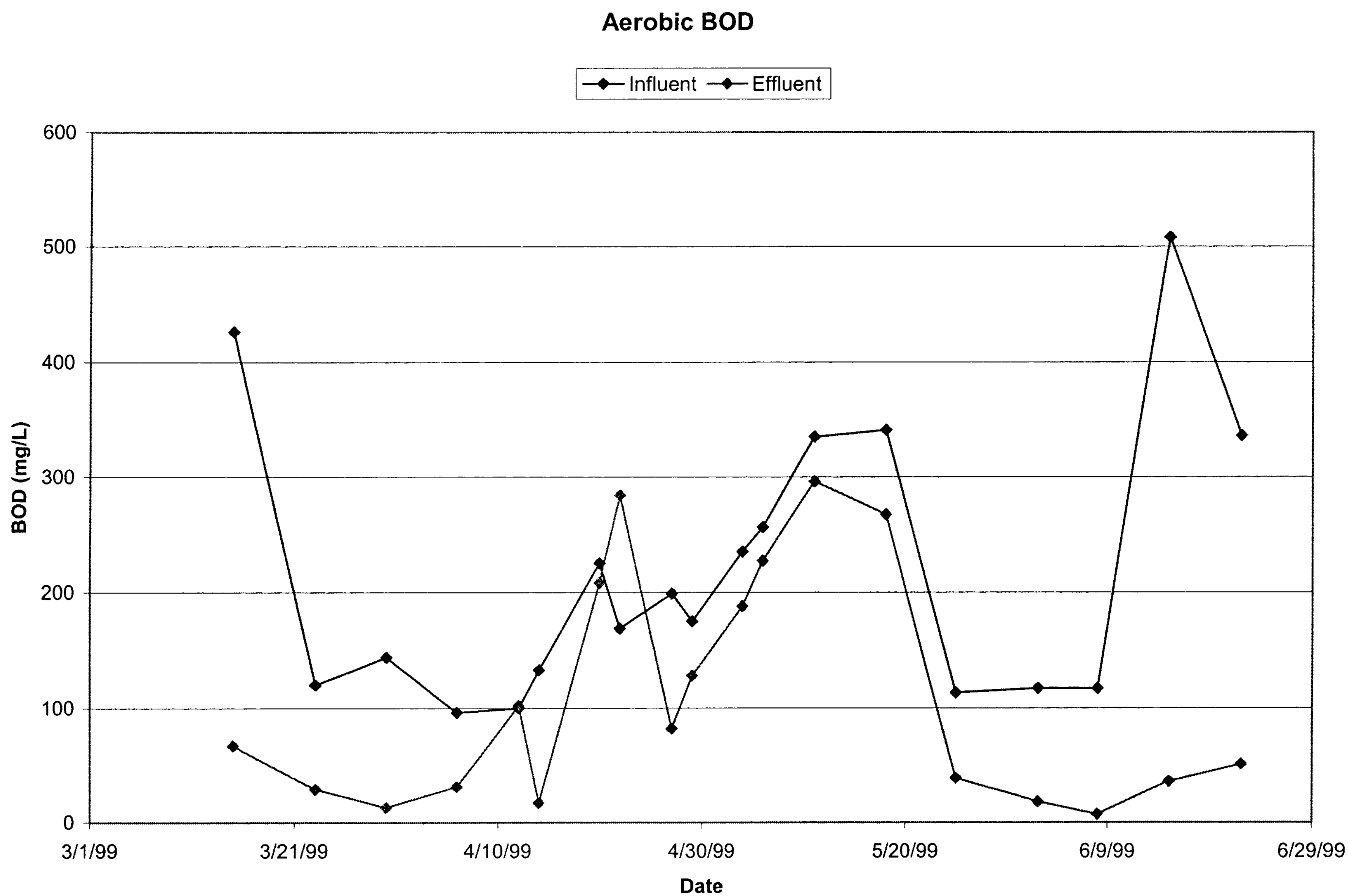

Figure B3: BOD_5 (mg/l) monitoring data for aerobic system.

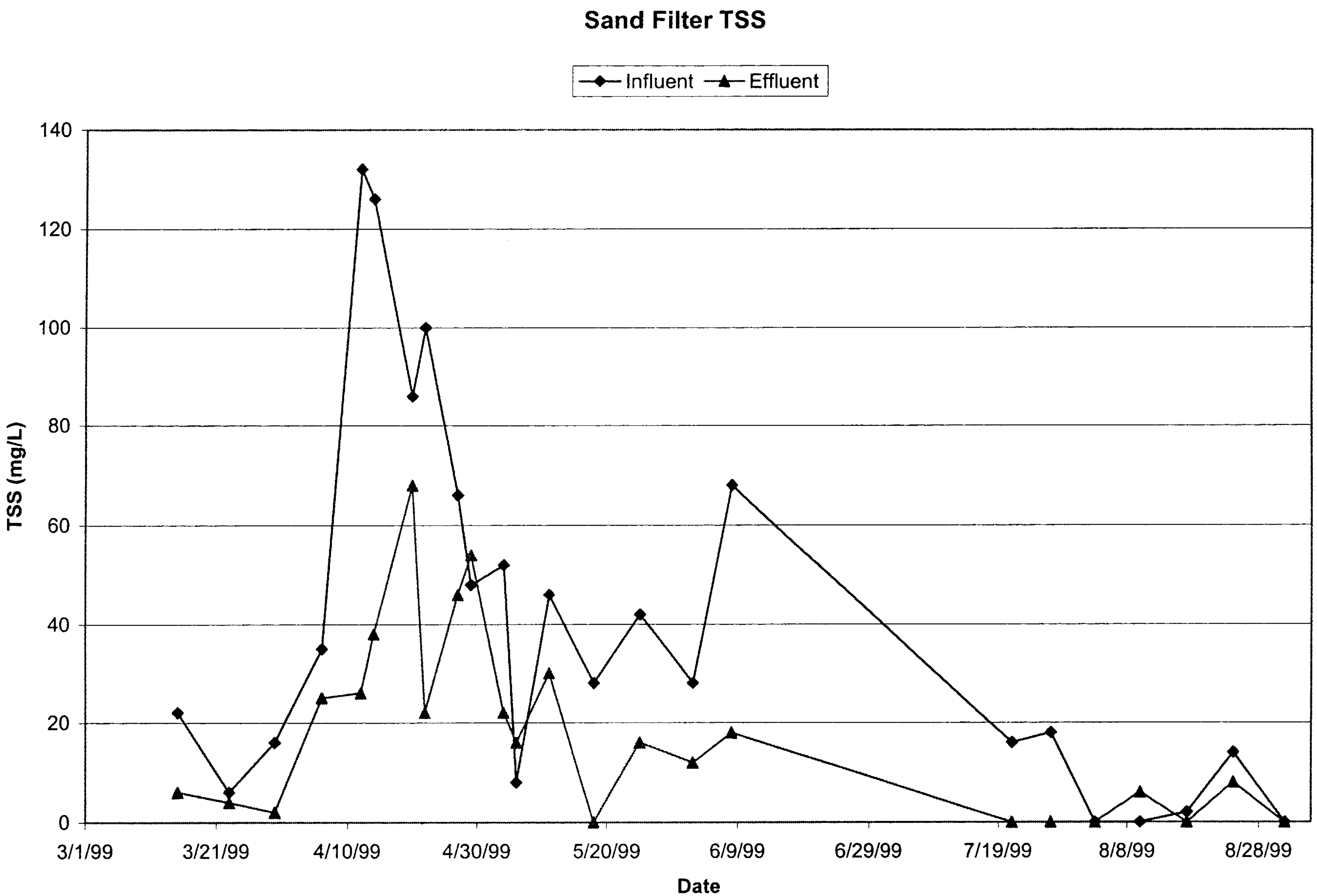

Figure B4: TSS (mg/l) monitoring data for sand filter.

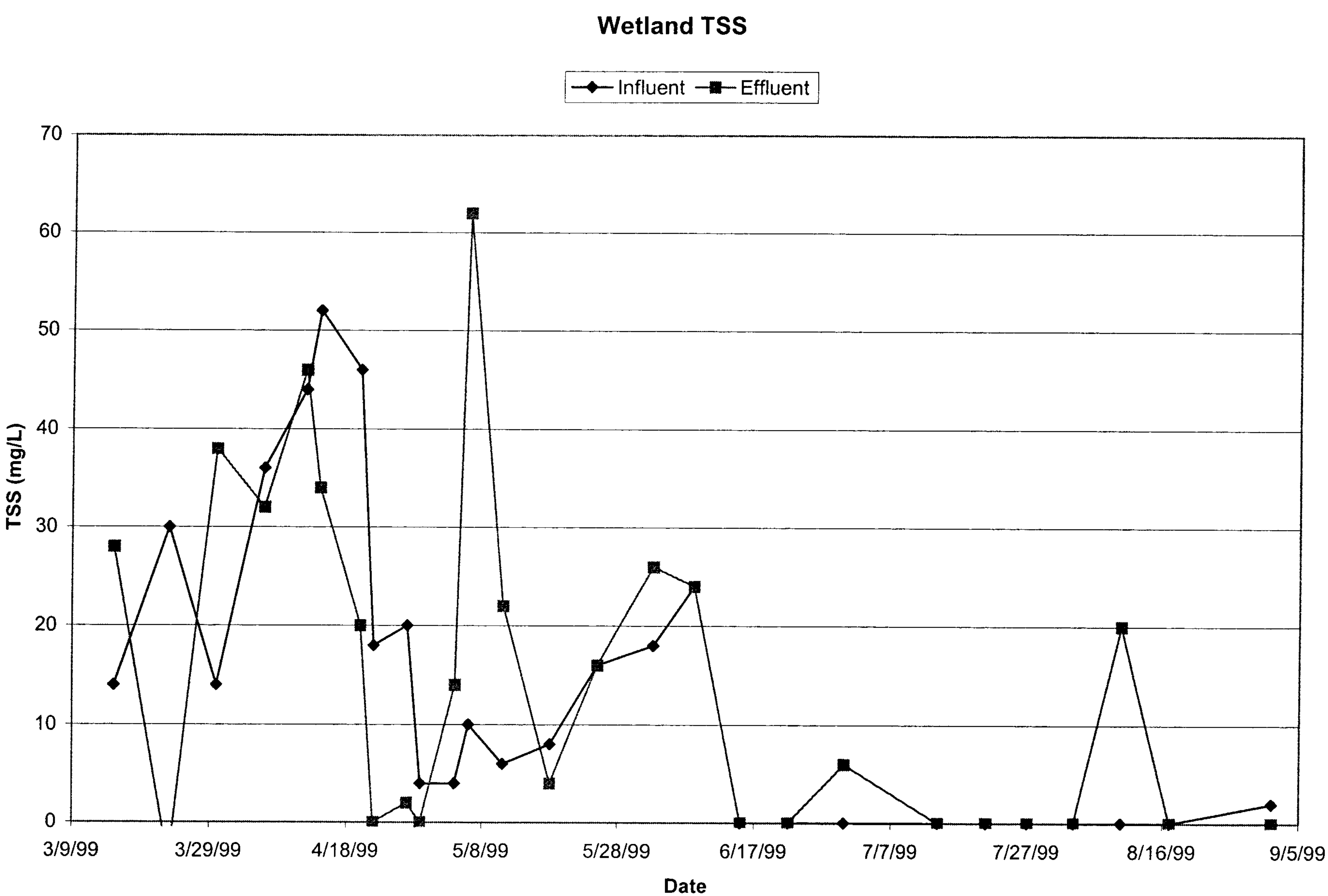

Figure B5: TSS (mg/l) monitoring data for wetland.

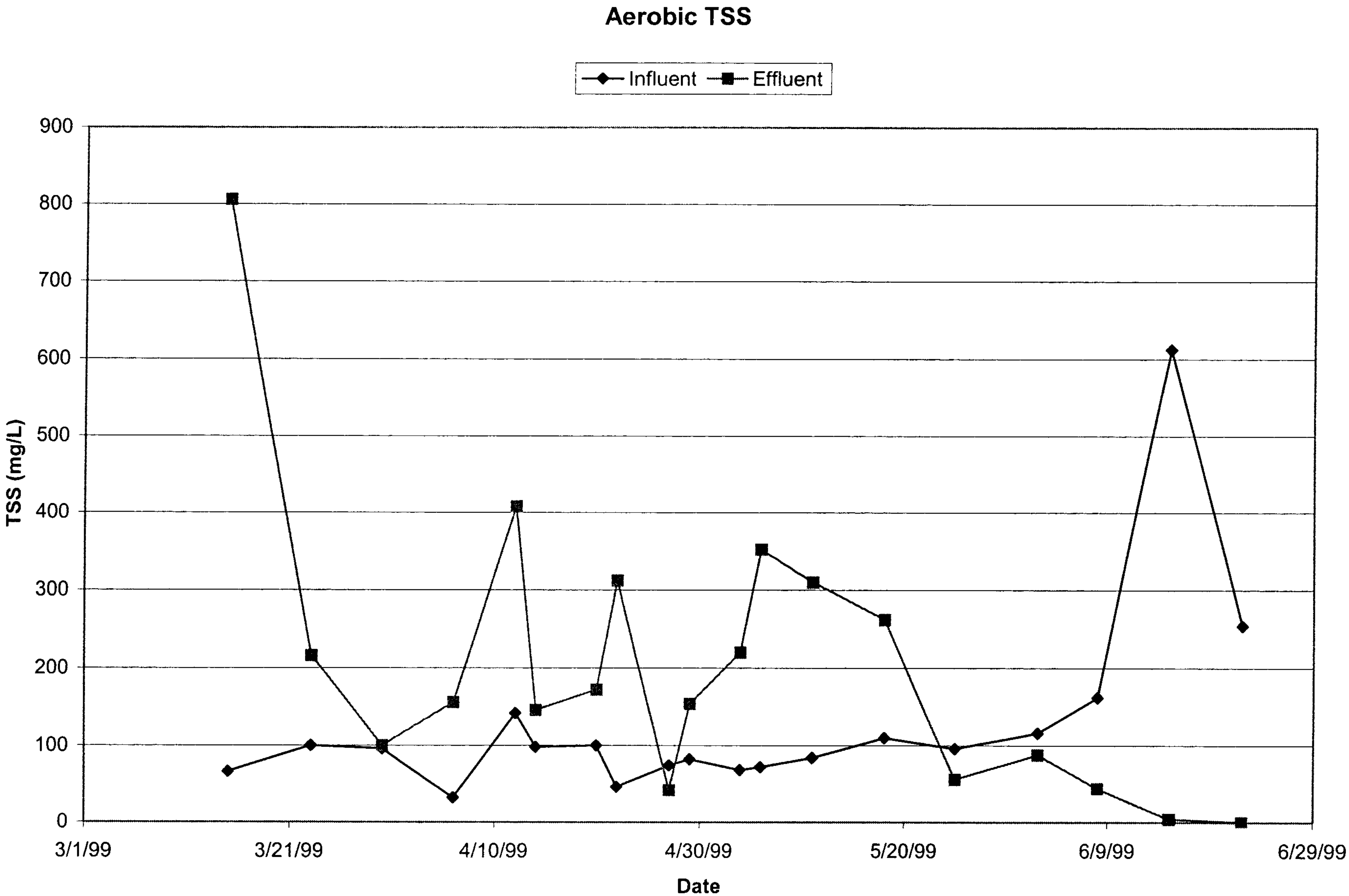

Figure B6: TSS (mg/l) monitoring data for aerobic system.

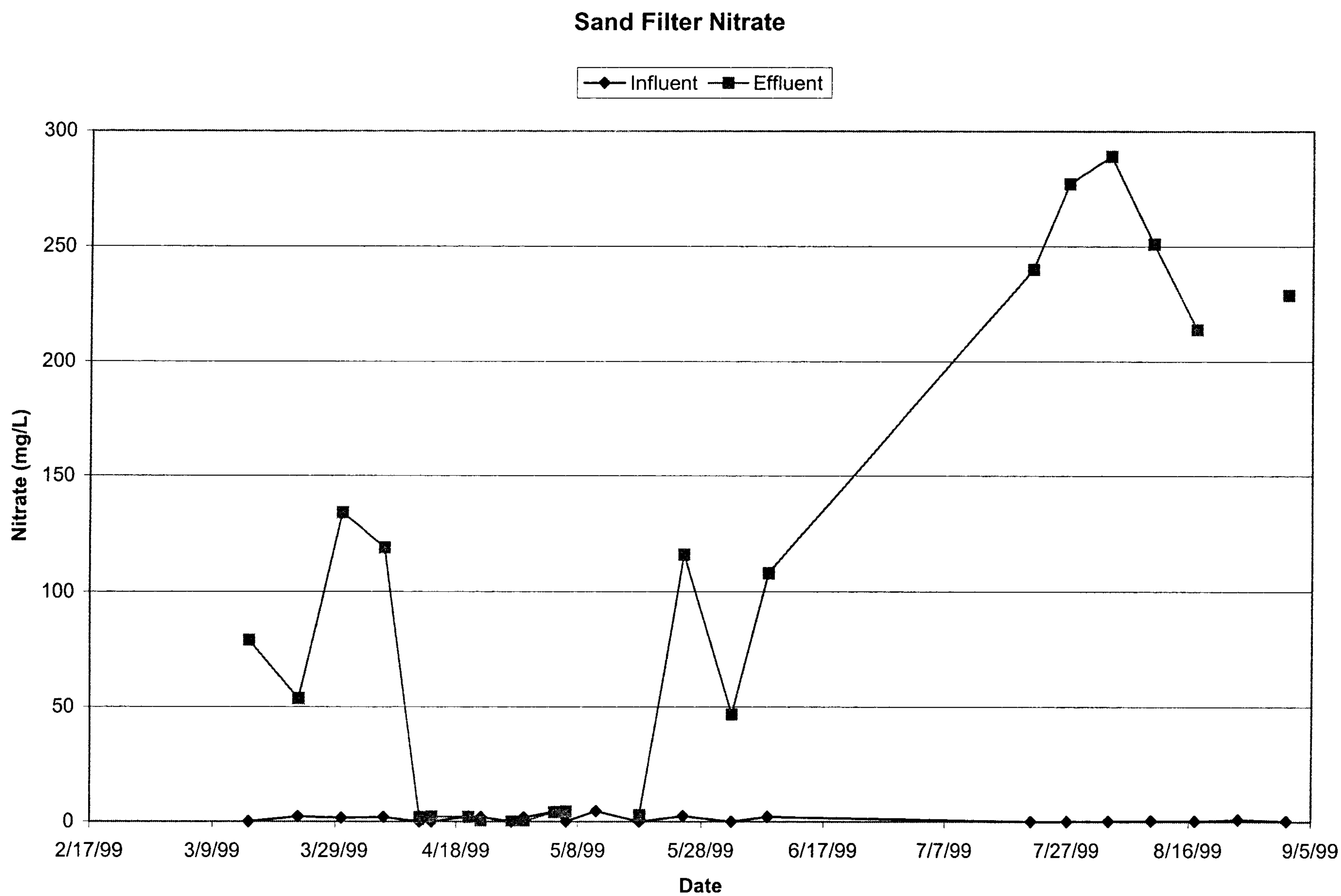

Figure B7: Nitrate (mg/l) monitoring data for sand filter.

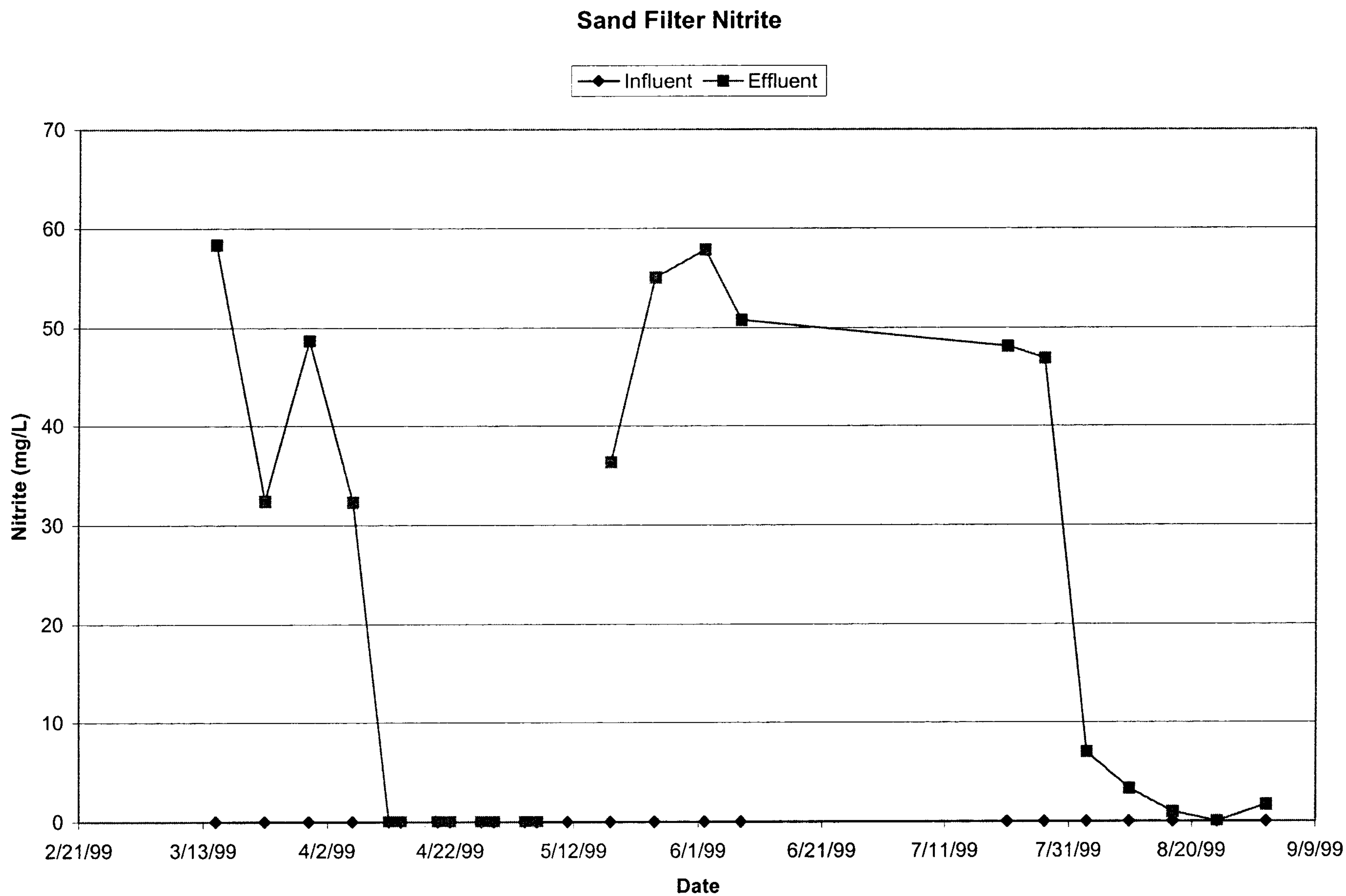

Figure B8: Nitrite (mg/l) monitoring data for sand filter.

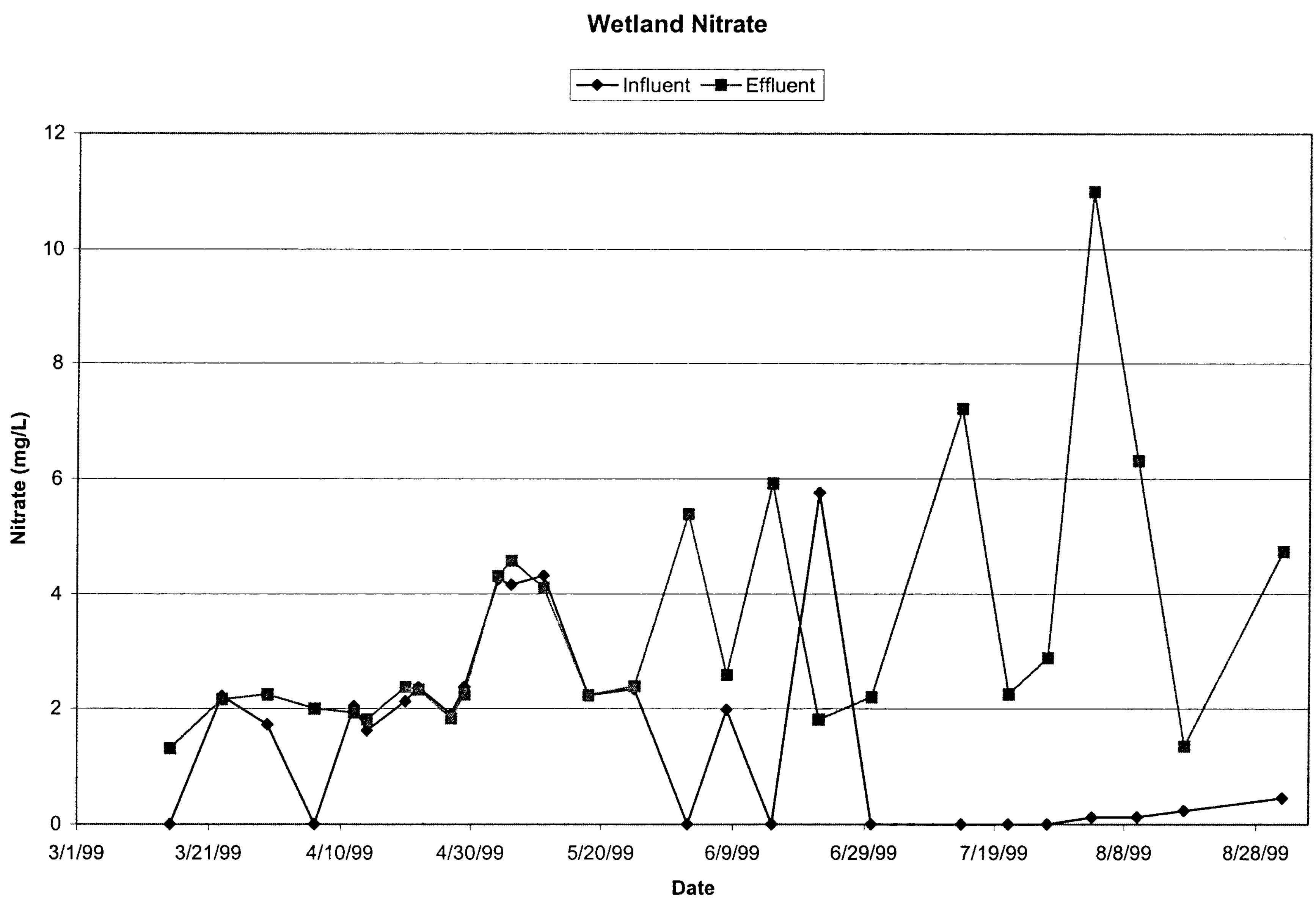

Figure B9: Nitrate (mg/l) monitoring data for wetland.

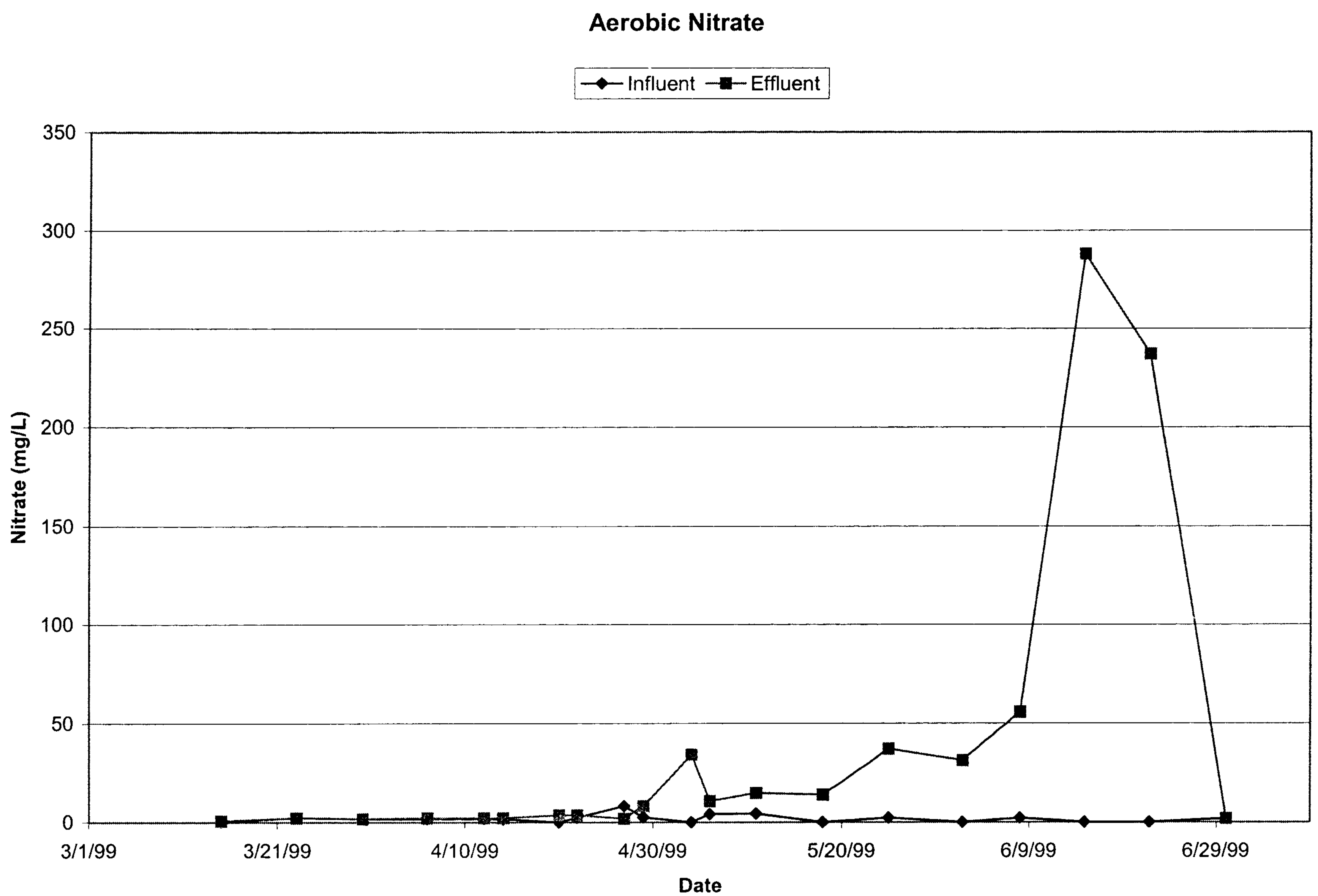

Figure B30: Nitrate (mg/l) monitoring data for aerobic system.

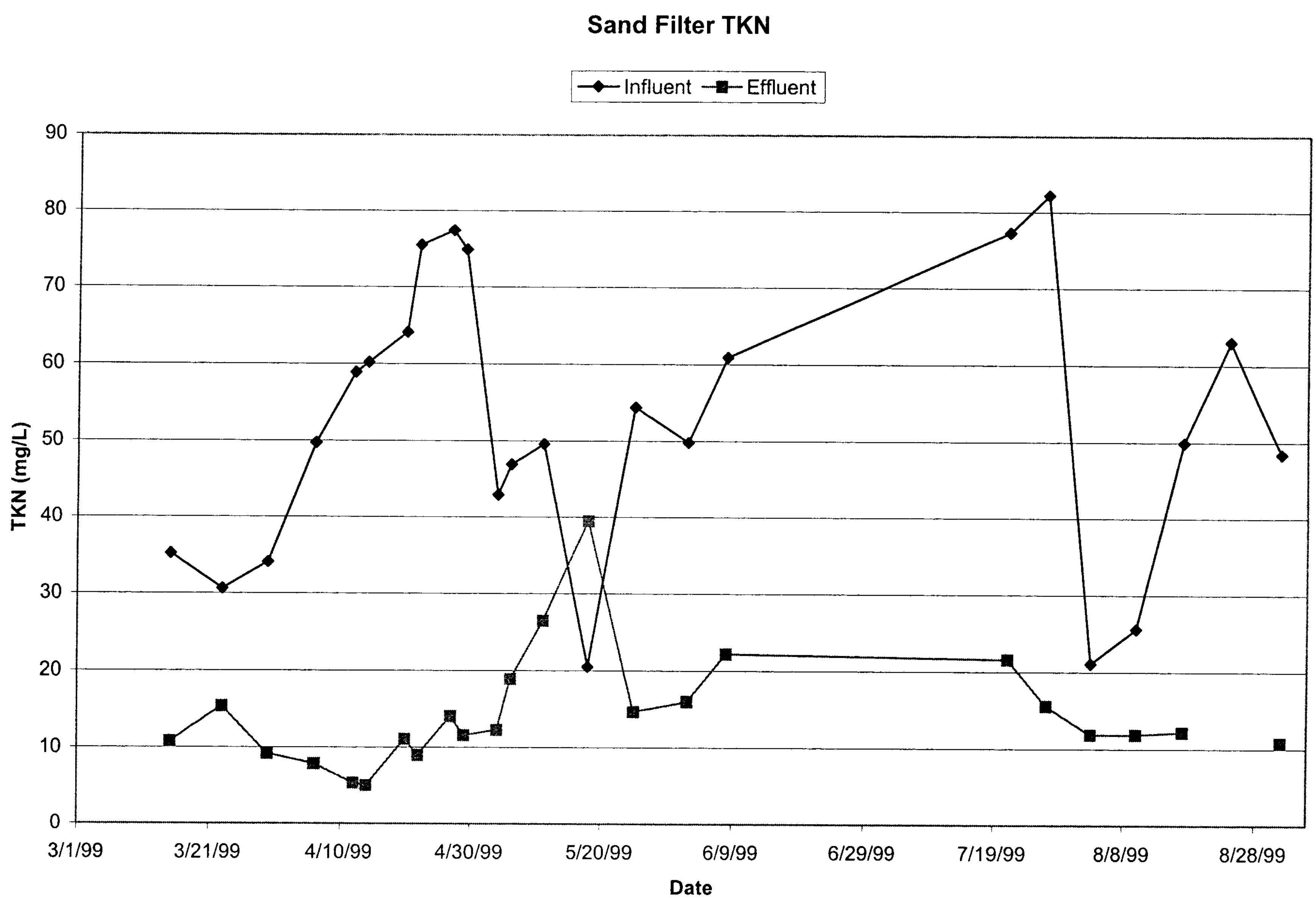

Figure B11: TKN (mg/l) monitoring data for sand filter.

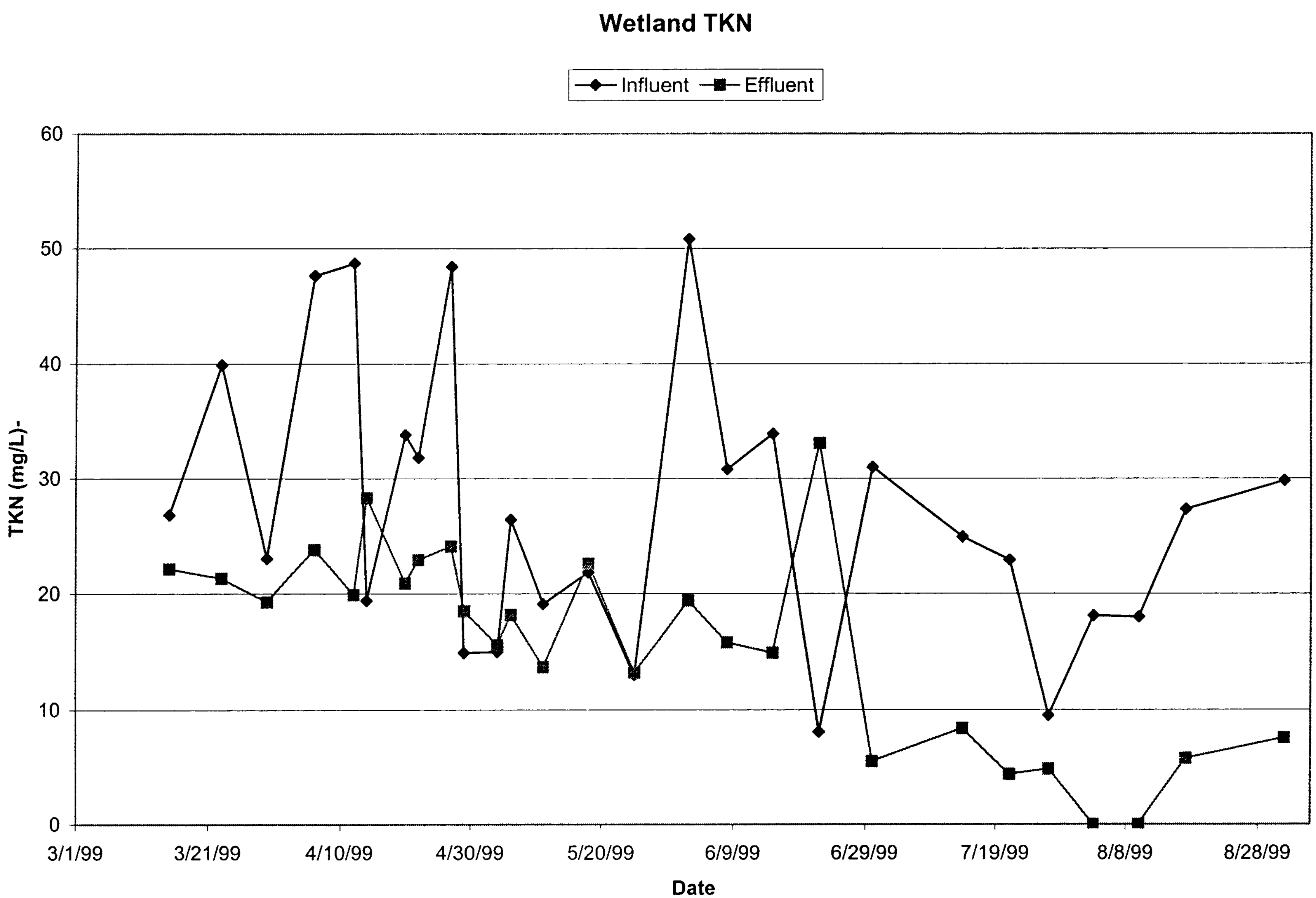

Figure B12: TKN (mg/l) monitoring data for wetland.

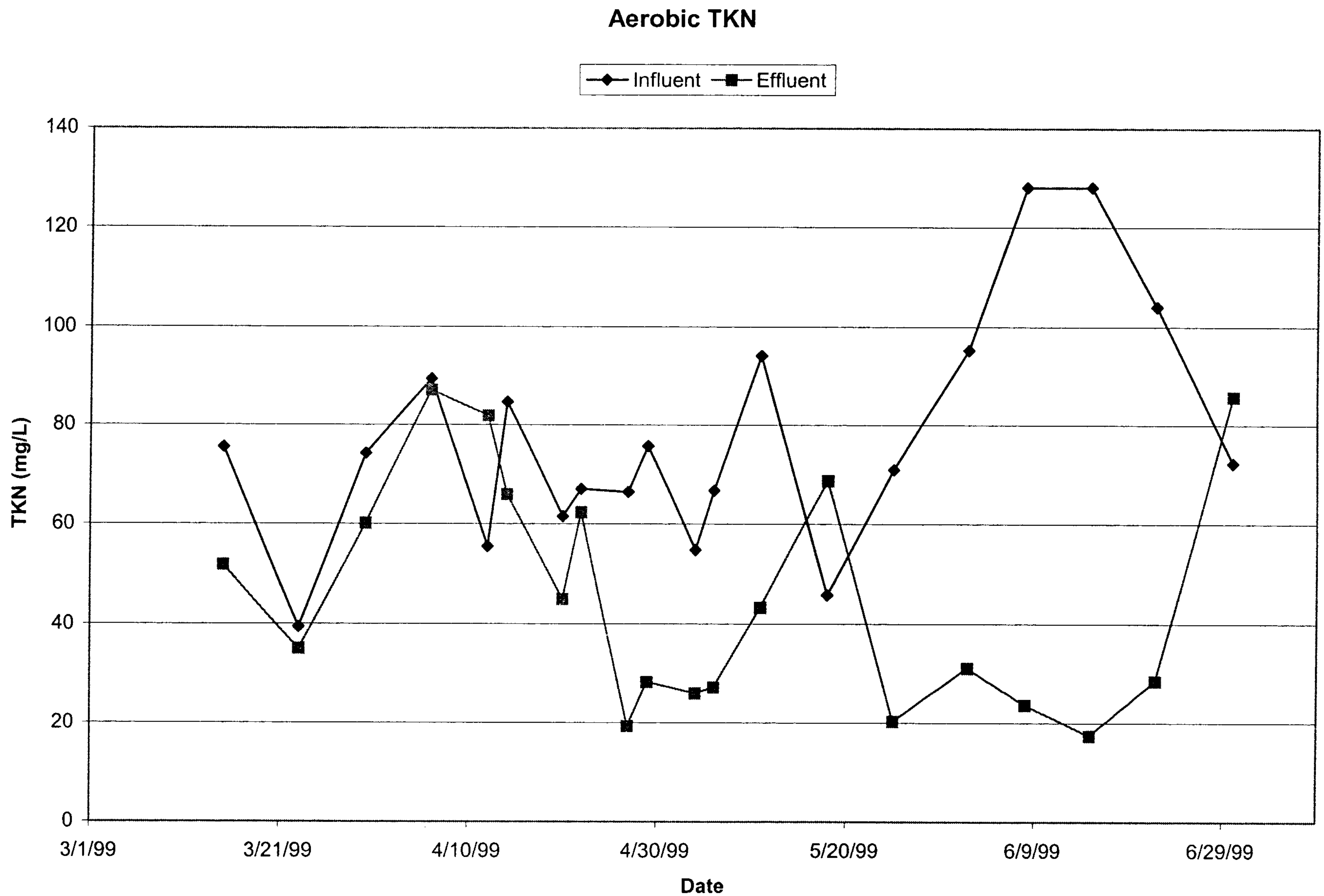

Figure B13: TKN (mg/l) monitoring data for aerobic system.

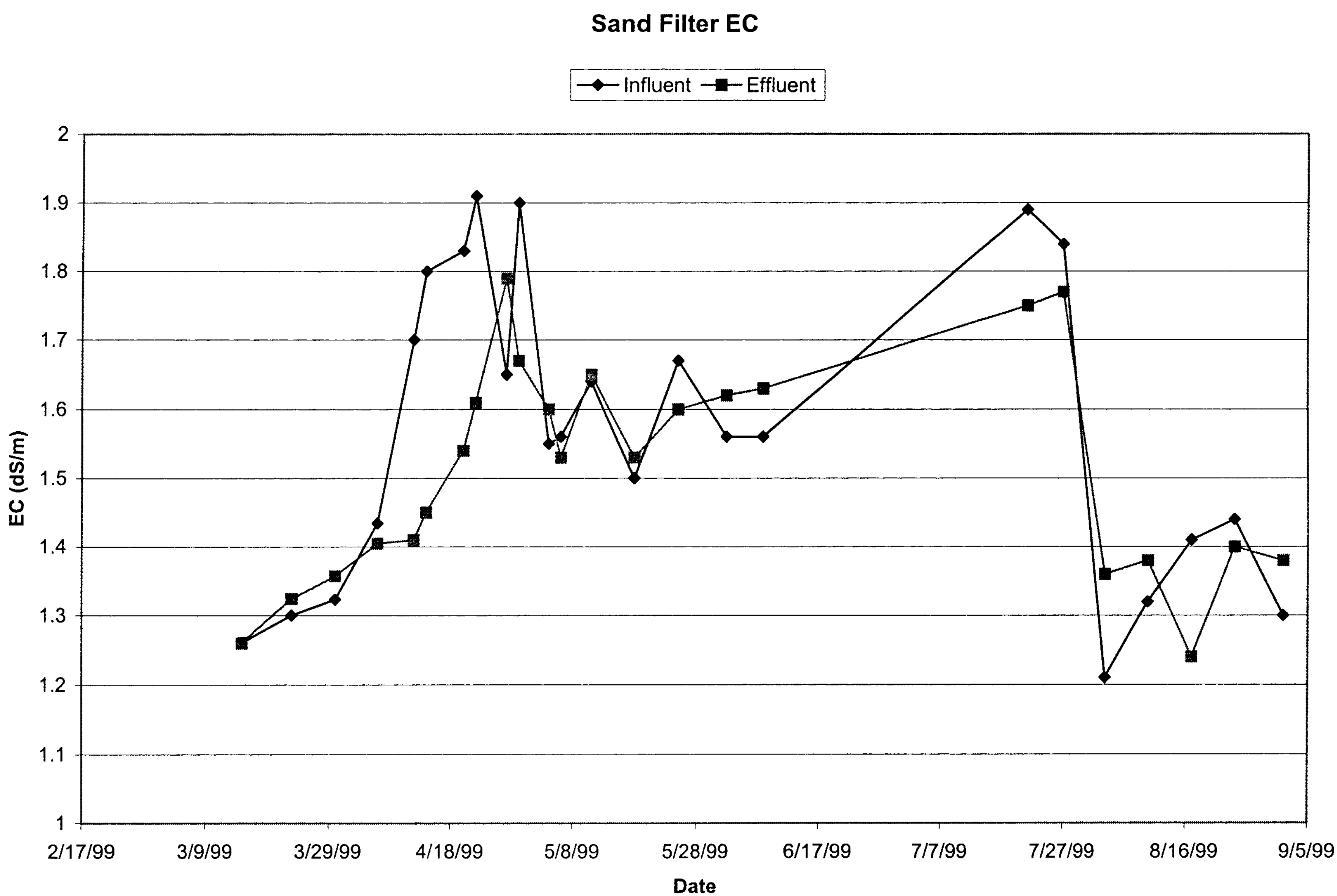

Figure B14: EC (dS/m) monitoring data for sand filter.

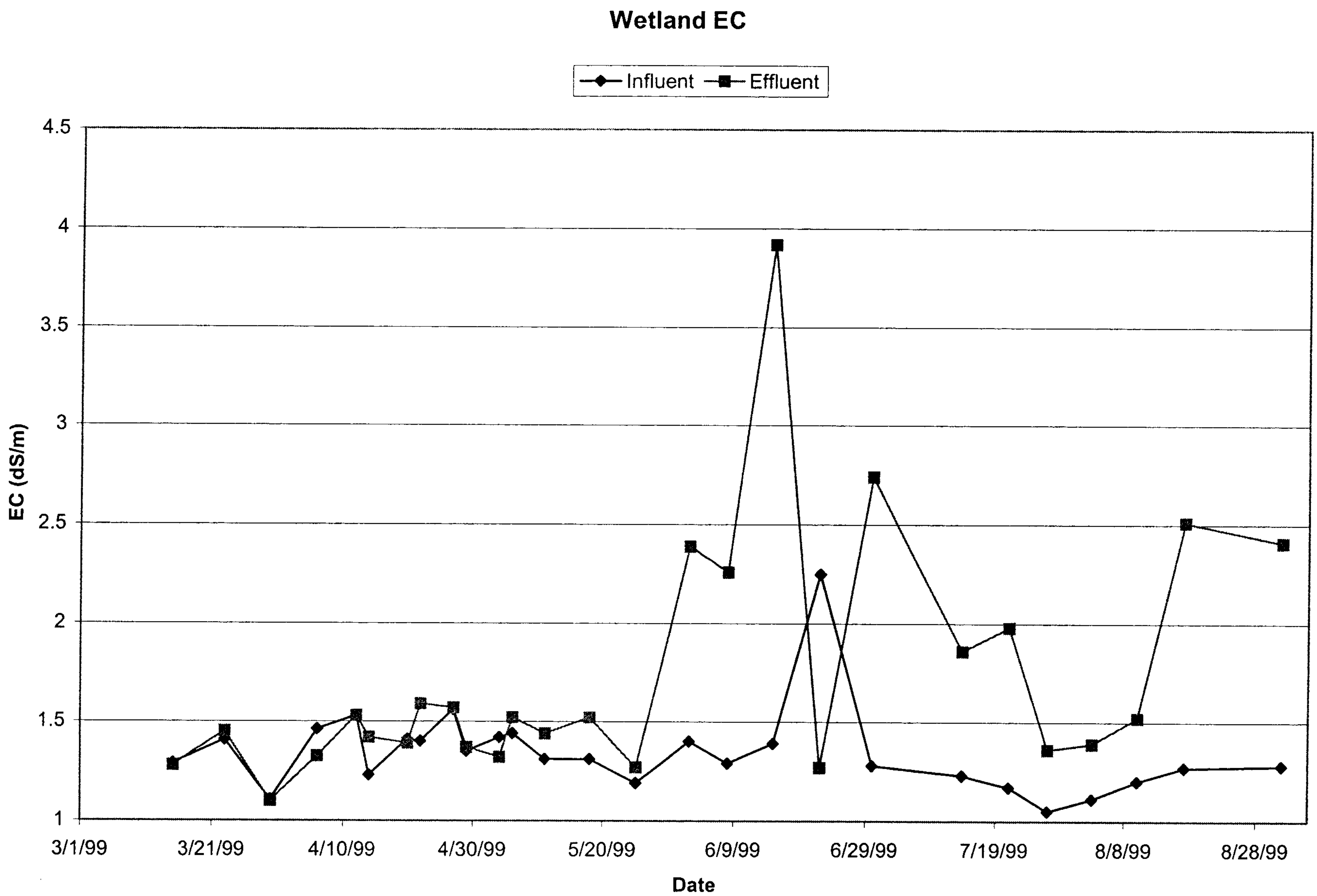

Figure B15: EC (dS/m) monitoring data for wetland.

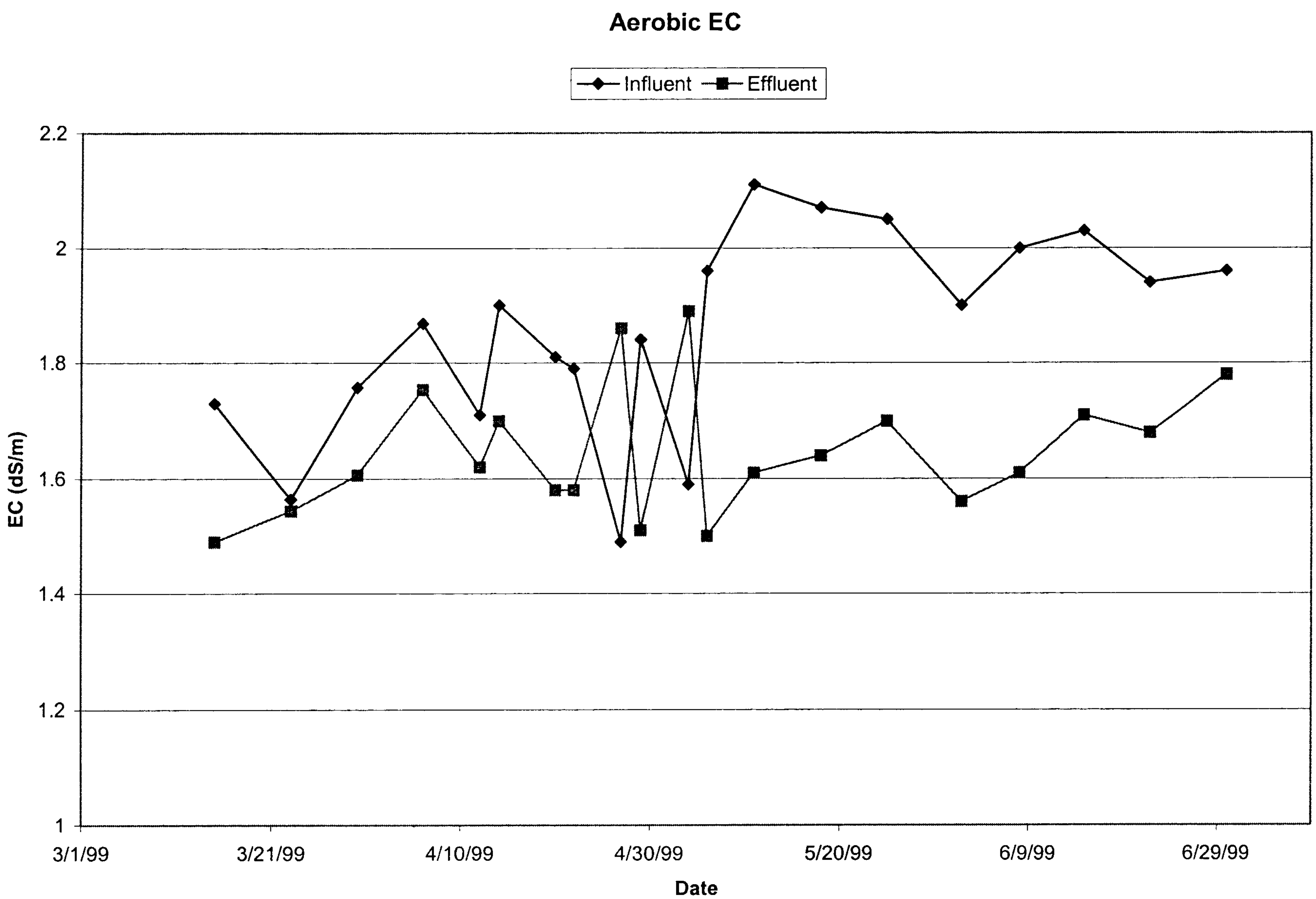

Figure B16: EC (dS/m) monitoring data for aerobic system.

APPENDIX C: Flow Rate Data

Summary flow rate data is presented in Tables 5, 7, and 9. The raw meter readings are presented here. This information will assist in assessing treatment effectiveness and mass removal rates in the technologies.

Table C 1:Flow rate data for sand filter system (gallons).

Date	Days	Meter Reading Zone 1	Zone 1 Increase	Meter Reading Zone 2	Zone 2 Increase	Total Increase	Avg. Daily Flow (gal)
4/1/99	1	4215		2627			
4/2/99	1	4250	35	2662	35	70	70
4/3/99	1	4269	19	2681	19	38	38
4/4/99	1	4271	2	2683	2	4	4
4/5/99	1	4276.5	5.5	2688	5	10.5	10.5
4/6/99	1	4276.5	0	2688	0	0	0
4/7/99	1	4356	79.5	2768	80	159.5	159.5
4/8/99	1	4401	45	2813	45	90	90
4/9/99	1	4460	59	2872	59	118	118
4/10/99	1	4475	15	2887	15	30	30
4/11/99	1	4475	0	2887	0	0	0
4/12/99	1	4483	8	2895	8	16	16
4/13/99	1	4490	7	2902	7	14	14
4/14/99	1	4499	9	2911	9	18	18
4/15/99	1	4515	16	2927	16	32	32
4/16/99	1	4560	45	2972	45	90	90
4/17/99	1	4610	50	3022	50	100	100
4/18/99	1	4610	0	3022	0	0	0
4/19/99	1	4677	67	3089	67	134	134
4/20/99	1	4715	38	3127	38	76	76
4/21/99	1	4715	0	3127	0	0	0
4/22/99	1	4776	61	3188	61	122	122
4/23/99	1	4791	15	3203	15	30	30
4/24/99	1	4791	0	3203	0	0	0
4/25/99	1	4816	25	3228	25	50	50
4/26/99	1	4822	6	3234	6	12	12
4/27/99	1	4822	0	3234	0	0	0
4/28/99	1	4822	0	3234	0	0	0
4/29/99	1	4836	14	3248	14	28	28
4/30/99	1	4877	41	3288	40	81	81
5/1/99	1	4982	105	3405	117	222	222
5/2/99	1	5136	154	3565	160	314	314
5/3/99	1	5193	57	3668	103	160	160
5/4/99	1	5193	0	3668	0	0	0
5/5/99	1	5265	72	3716	48	120	120
5/6/99	1	5284	19	3735	19	38	38
5/7/99	1	5306	22	3754	19	41	41

Date	Days	Meter Reading Zone 1	Zone 1 Increase	Meter Reading Zone 2	Zone 2 Increase	Total Increase	Avg. Daily Flow (gal)
5/8/99	1	5320	14	3770	16	30	30
5/9/99	1	5363	43	3805	35	78	78
5/10/99	1	5363	0	3805	0	0	0
5/11/99	1	5399	36	3839	34	70	70
5/12/99	1	5415	16	3860	21	37	37
5/13/99	1	5476	61	3881	21	82	82
5/14/99	1	5476	0	3881	0	0	0
5/15/99	1	5577	101	3977	96	197	197
5/16/99	1	5626	49	4015	38	87	87
5/17/99	1	5690	64	4079	64	128	128
5/19/99	2	5886	196	4221	142	338	169
5/20/99	1	5935	49	4278	57	106	106
5/23/99	3	6000	65	4322	44	109	36.3
5/24/99	1	6000	0	4322	0	0	0
5/25/99	1	6063	63	4373	51	114	114
5/26/99	1	6137	74	4437	64	138	138
5/27/99	1	6166	29	4453	16	45	45
5/28/99	1	6207	41	4486	33	74	74
8/1/99	64	7539.7	1332.7	5704.5	1218.5	2551.2	39.9
8/2/99	1	7539.7	0	5704.5	0	0	0
8/2/99	1	7539.7	0	5704.5	0	0	0
8/5/99	2	7596	56.3	5754.9	50.4	106.7	53.35
8/6/99	1	7596	0	5754.9	0	0	0
8/7/99	1	7641.2	45.2	5804.2	49.3	94.5	94.5
8/8/99	1	7641.2	0	5804.2	0	0	0
8/9/99	1	7641.2	0	5804.2	0	0	0
8/10/99	1	7641.2	0	5804.2	0	0	0
8/11/99	1	7707.3	66.1	5853.7	49.5	115.6	115.6
8/12/99	1	7707.3	0	5853.7	0	0	0
8/13/99	1	7707.3	0	5853.7	0	0	0
8/14/99	1	7753	45.7	5904.2	50.5	96.2	96.2
8/15/99	1	7753	0	5904.2	0	0	0
8/16/99	1	7753	0	5904.2	0	0	0
8/17/99	1	7753	0	5904.2	0	0	0
8/18/99	1	7753	0	5904.2	0	0	0
8/19/99	1	7819.4	66.4	5954.6	50.4	116.8	116.8
8/20/99	1	7819.4	0	5954.6	0	0	0
8/21/99	1	7819.4	0	5954.6	0	0	0
8/22/99	1	7878.2	58.8	6017.7	63.1	121.9	121.9
8/23/99	1	7878.2	0	6017.7	0	0	0

Date	Days	Meter Reading Zone 1	Zone 1 Increase	Meter Reading Zone 2	Zone 2 Increase	Total Increase	Avg. Daily Flow (gal)
8/24/99	1	7923.9	45.7	6068.1	50.4	96.1	96.1
9/24/99	31	9105.3	1181.4	7193.6	1125.5	2306.9	74.4

Table C 2: Raw flow rate data for wetland system (gallons).

Date	Days	Inflow Meter	Outflow Meter	Inflow Gallons	Outflow Gallons	Daily Avg Inflow Gallons	Daily Avg Outflow Gallons
11/11/98		80753	122317				
11/12/98	1	81085	122620	332	303	332.0	303.0
11/13/98	1	81280	122837	195	217	195.0	217.0
11/15/98	2	81423	122837	143	0	71.5	0.0
11/16/98	1	81580	123064	157	227	157.0	227.0
11/17/98	1	82361	123846	781	782	781.0	782.0
11/21/98	4	83982	125365	1621	1519	405.3	379.8
11/22/98	1	84126	125365	144	0	144.0	0.0
11/23/98	1	84126	125365	0	0	0.0	0.0
12/1/98	7	85830	127130	1704	1765	243.4	252.1
12/2/98	1	86679	128007	849	877	849.0	877.0
12/6/98	4	87520	128883	841	876	210.3	219.0
12/7/98	1	87520	128893	0	10	0.0	10.0
12/8/98	1	87666	128893	146	0	146.0	0.0
12/12/98	4	89445	130764	1779	1871	444.8	467.8
12/21/98	8	90876	132225	1431	1461	178.9	182.6
12/22/98	1	90877	133055	1	830	1.0	830.0
12/28/98	6	90883	137255	6	4200	1.0	700.0
12/29/98	1	90884	137255	1	0	1.0	0.0
1/4/99	6	90996	137921	112	666	18.7	111.0
1/5/99	1	90999	137921	3	0	3.0	0.0
1/7/99	2	92643	137930	1644	9	822.0	4.5
1/11/99	4	94260	137989	1617	59	404.3	14.8
1/12/99	1	94268	137989	8	0	8.0	0.0
1/14/99	2	95016	138183	748	194	374.0	97.0
1/15/99	1	95132	138247	116	64	116.0	64.0
1/19/99	4	96976	138609	1844	362	461.0	90.5
1/27/99	8	97264	138902	288	293	36.0	36.6
3/5/99	37	105130	147053	7866	8151	212.6	220.3
3/8/99	3	106009	147996	879	943	293.0	314.3
3/10/99	2	106130	148170	121	174	60.5	87.0

Date	Days	Inflow Meter	Outflow Meter	Inflow Gallons	Outflow Gallons	Daily Avg Inflow Gallons	Daily Avg Outflow Gallons
3/14/99	4	107735	149857	1605	1687	401.3	421.8
3/22/99	8	109089	151214	1354	1357	169.3	169.6
3/24/99	2	109089	151214	0	0	0.0	0.0
3/25/99	1	109089	151214	0	0	0.0	0.0
3/30/99	5	111005	153181	1916	1967	383.2	393.4
3/31/99	1	111005	153181	0	0	0.0	0.0
4/1/99	1	111005	153181	0	0.2	0.0	0.2
4/4/99	3	111342	153428	336.6	246.7	112.2	82.2
4/5/99	1	111489	153428	147.2	0	147.2	0.0
4/7/99	2	111649	153724	160.3	296.3	80.2	148.2
4/8/99	1	111805	153724	156.3	0	156.3	0.0
4/9/99	1	111980	154000	174.8	276.1	174.8	276.1
4/10/99	1	112178	154190	197.7	189.8	197.7	189.8
4/11/99	1	112320	154190	142.1	0	142.1	0.0
4/12/99	1	112320	154190	0	0	0.0	0.0
4/14/99	2	112464	154429	144.4	238.7	72.2	119.3
4/15/99	1	113206	155171	741.1	741.7	741.1	741.7
4/16/99	1	113206	155171	0	0	0.0	0.0
4/18/99	2	113559	155484	353.6	313.7	176.8	156.9
4/19/99	1	113702	155529	142.4	45.1	142.4	45.1
4/20/99	1	113956	155810	254	280.6	254.0	280.6
4/21/99	1	114316	156203	360.6	393	360.6	393.0
4/22/99	1	114452	156203	136	0	136.0	0.0
4/23/99	1	114629	156519	177.2	316.5	177.2	316.5
4/24/99	1	114902	156683	272.4	163.4	272.4	163.4
4/25/99	1	115014	156683	112.2	0	112.2	0.0
4/26/99	1	115730	157999	715.7	1316.2	715.7	1316.2
4/27/99	1	116214	158266	484.7	267.3	484.7	267.3
4/28/99	1	117231	159042	1016.6	775.9	1016.6	775.9
5/1/99	3	117534	159258	302.7	215.3	100.9	71.8
5/2/99	1	117534	159258	0	0	0.0	0.0
5/3/99	1	117873	159674	339.8	416.2	339.8	416.2
5/5/99	2	118200	159901	326.6	227.5	163.3	113.8
5/6/99	1	118919	160391	718.6	489.9	718.6	489.9

Date	Days	Inflow Meter	Outflow Meter	Inflow Gallons	Outflow Gallons	Daily Avg Inflow Gallons	Daily Avg Outflow Gallons
5/9/99	3	119943	160721	1024.5	329.9	341.5	110.0
5/10/99	1	120681	161292	738.2	571.2	738.2	571.2
5/11/99	1	121019	162679	338.1	1386.7	338.1	1386.7
5/12/99	1	121324	162679	304.7	0	304.7	0.0
5/13/99	1	121492	162919	167.8	239.8	167.8	239.8
5/17/99	4	122550	163812	1058.5	893.4	264.6	223.3
5/18/99	1	122706	163812	155.7	0	155.7	0.0
5/19/99	1	122720	163812	13.5	0	13.5	0.0
5/20/99	1	123094	164501	374.8	688.7	374.8	688.7
5/21/99	1	123728	164892	633.3	391.5	633.3	391.5
5/22/99	1	124453	165212	725.1	319.7	725.1	319.7
5/23/99	1	124817	165733	363.9	521.2	363.9	521.2
5/24/99	1	125502	166194	685.2	460.8	685.2	460.8
5/25/99	1	125591	166194	89.4	0	89.4	0.0
6/4/99	10	127588	166882	1996.8	688	199.7	68.8
6/5/99	1	127642	168919	54.2	2037.4	54.2	2037.4
6/6/99	1	127708	169001	65.5	81.2	65.5	81.2
6/7/99	1	127750	169082	41.9	81.6	41.9	81.6
6/8/99	1	127807	169191	57.5	109	57.5	109.0
6/9/99	1	127863	169267	56	75.4	56.0	75.4
6/10/99	1	127916	169352	52.9	85.7	52.9	85.7
6/11/99	1	128039	169547	123.3	194.9	123.3	194.9
6/12/99	1	128099	169579	59.5	31.9	59.5	31.9
6/13/99	1	128143	169734	43.7	154.6	43.7	154.6
6/16/99	3	128327	169917	184.8	182.8	61.6	60.9
6/17/99	1	128351	170010	23.6	93	23.6	93.0
6/19/99	2	128483	170102	131.9	92.2	65.9	46.1
6/20/99	1	128483	170186	0	84.6	0.0	84.6
6/21/99	1	128527	170254	44.4	67.3	44.4	67.3
6/22/99	1	128608	170368	80.6	114.1	80.6	114.1
6/23/99	1	128692	170368	84.5	0	84.5	0.0
6/24/99	1	128789	170435	96.7	67.2	96.7	67.2
6/25/99	1	128836	170549	47.3	114.3	47.3	114.3
6/26/99	1	128937	170652	100.9	102.6	100.9	102.6

Date	Days	Inflow Meter	Outflow Meter	Inflow Gallons	Outflow Gallons	Daily Avg Inflow Gallons	Daily Avg Outflow Gallons
6/27/99	1	129083	170813	145.6	161.2	145.6	161.2
6/29/99	2	129266	170843	183.5	29.6	91.8	14.8
6/30/99	1	129345	170926	78.7	83.7	78.7	83.7
7/2/99	2	129346	171000	1.1	73.4	0.5	36.7
7/6/99	4	129348	171000	1.7	0	0.4	0.0
7/7/99	1	129432	171186	84.5	186.5	84.5	186.5
7/8/99	1	129597	171186	164.3	0	164.3	0.0
7/9/99	1	130249	171356	651.8	169.9	651.8	169.9
7/12/99	3	130410	171356	161.7	0	53.9	0.0
7/14/99	2	130731	171580	320.7	223.9	160.3	111.9
7/15/99	1	131121	172056	390.1	476.3	390.1	476.3
7/18/99	3	131884	172772	763.2	715.6	254.4	238.5
7/20/99	2	131998	172772	114.2	0	57.1	0.0
7/21/99	1	132045	172772	46.5	0	46.5	0.0
7/22/99	1	132119	172772	74.3	0	74.3	0.0
7/23/99	1	132362	172933	243.2	161.3	243.2	161.3
7/24/99	1	132621	173189	258.6	255.4	258.6	255.4
7/26/99	2	132891	173297	270.4	108.8	135.2	54.4
7/27/99	1	133406	173382	514.1	84.2	514.1	84.2
7/28/99	1	133441	173467	35.4	85.3	35.4	85.3
7/30/99	2	133810	173981	368.8	514.3	184.4	257.2
7/31/99	1	133887	174024	77.4	42.4	77.4	42.4
8/1/99	1	134080	174163	192.6	139.4	192.6	139.4
8/2/99	1	134300	174443	220.2	279.8	220.2	279.8
9/24/99	53	139130	180032	4830.1	5589.2	91.1	105.5

Table C 3: Raw Flow rate data for the aerobic system.

		Meter Readings (Gallons)				Increase in Flow (Gallons)					Daily
Date	Days	Zone 1	Zone 2	Zone 3	Zone 4	Zone 1	Zone 2	Zone 3	Zone 4	Total	Average
1/27/99		4335.8	91213.4	99831.8	43275.5						
2/3/99	7	4335.8	91213.4	99831.8	43275.5	0.0	0.0	0.0	0.0	0.0	0.0
2/8/99	5	4335.8	91213.4	99831.8	43275.5	0.0	0.0	0.0	0.0	0.0	0.0
2/12/99	4	4412.3	91328.7	99892.2	43345.1	76.5	115.3	60.4	69.6	321.8	80.4
2/25/99	13	4412.3	91328.7	99892.2	43345.1	0.0	0.0	0.0	0.0	0.0	0.0
2/26/99	1	4453.2	91328.7	99901.6	43345.1	40.9	0.0	9.4	0.0	50.3	50.3
3/1/99	3	4579.1	91402.0	100087.1	43476.4	125.9	73.3	185.5	131.3	516.0	172.0
3/3/33	2	4660.8	91444.7	100124.7	43557.1	81.7	42.7	37.6	80.7	242.7	121.3
3/8/99	5	5252.1	92147.5	100904.4	44075.9	591.3	702.8	779.7	518.8	2592.6	518.5
3/9/99	1	5448.5	92261.8	101054.4	44223.5	196.4	114.3	150.0	147.6	608.3	608.3
3/13/99	4	5675.3	92466.3	101248.4	44460.1	226.8	204.5	194.0	236.6	861.9	215.5
3/17/99	4	5807.4	92559.4	101359.1	44617.9	132.1	93.1	110.7	157.8	493.7	123.4
3/22/99	5	6034.1	92733.1	101539.4	44910.0	226.7	173.7	180.3	292.1	872.8	174.6
3/24/99	2	6076.0	92747.9	101569.3	44948.2	41.9	14.8	29.9	38.2	124.8	62.4
3/30/99	6	6332.4	92948.7	101737.4	45118.0	256.4	200.8	168.1	169.8	795.1	132.5
4/1/99	2	6450.0	93039.7	101809.4	45169.2	117.6	91.0	72.0	51.2	331.8	165.9
4/5/99	4	6504.6	93077.0	101815.4	45194.4	54.6	37.3	6.0	25.2	123.1	30.8
4/6/99	1	6548.5	93101.9	101851.2	45235.5	43.9	24.9	35.8	41.1	145.7	145.7
4/7/99	1	6625.5	93133.1	101881.2	45276.2	77.0	31.2	30.0	40.7	178.9	178.9
4/8/99	1	6672.0	93195.2	101924.5	45317.5	46.5	62.1	43.3	41.3	193.2	193.2
4/12/99	7	7112.2	93499.2	102189.2	45585.4	440.2	304.0	264.7	267.9	1276.8	182.4
4/28/99	16	7919.9	93779.9	102744.6	46116.9	807.7	280.7	555.4	531.5	2175.3	136.0
4/29/99	1	7963.2	93802.8	102783.1	46147.6	43.3	22.9	38.5	30.7	135.4	135.4
5/6/99	7	8275.9	93972.3	103006.9	46349.4	312.7	169.5	223.8	201.8	907.8	129.7
5/14/99	8	8523.8	94108.9	103400.1	46485.0	247.9	136.6	393.2	135.6	913.3	114.2
5/26/99	12	8867.8	94259.4	103910.0	46924.9	344.0	150.5	509.9	439.9	1444.3	120.4
9/14/99	112	12767.6	96949.0	108382.2	50183.0	3899.8	2689.6	4472.2	3258.1	14319.7	127.9