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REMOVAL OF VIRUSES AND POLLUTION INDICATORS IN CONSTRUCTED WETLANDS

by

Juan Antonio Vidales Contreras

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Juan Antonio Vidales Contreras

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and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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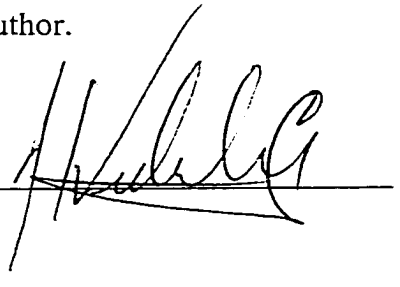
SIGNED 

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1. ABSTRACT

Tracer studies using Br^- and bacteriophage PRD1 in both surface and subsurface flow constructed wetlands were conducted to analyze their hydrodynamic behavior and efficiencies in removing viruses from wastewater. A survival test *in situ* was also conducted to analyze the persistency of PRD1 in wetland environments. Concurrently, a sampling program for microbial and chemical indicators in the surface flow wetland for a period of 16 months was conducted. The tracer studies revealed a reduction of 99 and 84 percent in the subsurface and surface flow wetland, respectively. Bromide recovery at the outlet of both wetland systems was about 75 percent. The Convective-Dispersion Equation was able to predict the observed PRD1 and Br^- breakthrough curves obtained during the tracer study in the surface flow wetland. The monitoring program of pollution indicators showed that biochemical oxygen demand and total suspended solids can be reduced efficiently, reaching the tertiary effluent standard of 10 mg L^{-1} required by The Arizona Department of Environmental Quality. This sampling program suggested that coliphages may be a better indicator of fecal contamination than total and fecal coliforms in surface flow wetlands.

2. INTRODUCTION

2.1 Constructed Wetlands

Constructed wetlands (CW) have been actively used to reduce suspended solids, chemical pollutants and pathogenic microorganisms from wastewater. They are designed to maximize efficiency of natural self-purification processes carried out by plants, microbial populations, and environmental factors. CW can be established almost at any location to treat a wide diversity of wastewater with low cost and minimum trained personnel. Basically, these systems are artificial channels, slightly inclined, and often lined to avoid water seepage. The channels may be filled or not with some type of coarse material like gravel or sand, and vegetated with a single or multiple plant species. Wastewater must pass through the rooted-basin, above the media or through the media resulting in microbial degradation, pathogenic inactivation, and chemical processes. After passing through the wetland, water is typically used for landscape irrigation or is discharged into receiving water bodies or recharge basins for additional soil aquifer treatment.

Classification

Constructed wetlands are classified by Kadlec and Knight (1996) as Surface (SW) and Subsurface (SSW) Flow Wetlands based on whether the flow of water occurs over or below the surface of the wetland substratum. On the other hand, Vymazal *et al.* (1998)

recently proposed a classification based on the type of macrophytes planted in the wetland. In this classification wetlands are labeled: a) free-floating macrophyte systems; b) submerged macrophyte-based systems; or c) rooted emergent macrophyte-based systems.

The terminology proposed by Kadlec and Knight (1996) was adopted for this study. Surface flow wetlands are typically operated at less of 0.4 m of depth and hydraulic loading rates ranging from 4 to 4 mm day $^{-1}$. Generally, subsurface flow wetlands are channels filled with gravel or sand, operated at less than 0.6 m of saturated thickness and hydraulic loading rates ranging from 10 to 60 mm day^{-1} (Geller, 1997).

Wetland Substratum

Wetland substratum used for rooting can be soil, sand or gravel. In SSW, the initial hydraulic conductivity should be adequate to support porosity reduction resulting from growth of plant roots and deposition of suspended solids from wastewater entering the system. Tanner *et al.* (1998) observed a reduction of detention time that was positively related with the level of organic loading received by the wetland. Sanford *et al.* (1995), also, found a considerable reduction of the hydraulic conductivity in a sand-and-gravel bed after a year of landfill leachate addition while beds filled with coarser materials experienced little reduction in hydraulic conductivity.

Wetland Plants

Emergent aquatic macrophytes are plants adapted to grow in water or on a substrate with excessive water content. These macrophytes provide surface area for attachment of microbial populations and the physical matrix for wastewater filtration. Also, they provide oxygen by passive diffusion and convective flow from the atmosphere to their buried parts using their internal lacunal system (Vymazal *et al.* 1998). During internal gas transport, oxygen is supplied to aerobic and facultative anaerobic populations attached to or adjacent to the external root surface. These organisms in turn are responsible for reducing the biochemical oxygen demand (BOD_5) of wastewater (Brix, 1994). The primary aquatic species used in constructed wetlands are common reed (*Phragmites australis*), cattail (*Typha* spp) and bulrush (*Scirpus* spp).

Common reed (*Phragmites australis*)

The common reed can grow under permanent inundation and readily tolerates up to 45 g L^{-1} of salt content, and pH from 2 to 8 (Reed *et al.*, 1988). Its deep root system creates a great volume of active rhizosphere. Geller (1997) found that in German speaking countries SSW planted with common reed were effective in reducing BOD_5, Chemical Oxygen Demand (COD), Phosphorous (P), and Nitrogen (N). Brix (1987) also observed removal efficiencies over 88 percent for BOD_5, P, and N in SSW, vegetated with *Phragmites australis*. In France, this type of ecosystem operated at short residence times

(1 to 4 h) removing 93 percent of the inflow helminth eggs; however, the system was ineffective in reducing COD, N, and P. In the U.S. common reeds have been used successfully in treatment wetlands; however, they are preferentially used in Europe (Reed *et al.*, 1995).

Cattail (*Typha* spp)

Typha angustifolia (narrow-leaf cattail) and *T. latifolia* (broad-leaf cattail) are the most typical plants found in constructed wetlands in the USA. Narrow-leaf plants tolerate salinity levels up to 30 g L^{-1} compared to the much lower tolerance of the broad-leaf plants of less than 1 g L^{-1}. Root penetration of cattail is relatively shallow reaching only about 0.30 m of depth (Reed *et al.*, 1995). Experimental gravel beds based SSW at Richmond, South Wales, Australia, planted with *Typha latifolia* were effective in removing 80, 66, 91 and 90 percent of the influent load of suspended solids, COD, and fecal and total coliforms, respectively. According to Kadlec and Knight (1996) these plants have a superior performance for wastewater treatment compared to bulrushes in SSW. Khatiwada and Polprasert (1998) reported that SW systems planted with *Typha angustifolia* were capable of removing some 99.5 percent of the initial load of fecal coliforms.

Bulrush (*Scirpus* spp)

In Arizona, bulrush and cattail have been used in most of the major demonstration and research wetland projects: Constructed Ecosystems Research Facility (CERF), Tres Rios Demonstration Wetlands, Sweetwater Wetlands and Recharge Project, and Dairy Constructed Wetland Demonstration and Research Project (Karpiscak *et al.*, 1999). Bulrush species used in constructed wetlands include: *Scirpus acutus*, *S. cypernius*, *S. fluviatilis*, *S. robustus*, *S. validus*, *S. lacustris*, and *S. olneyi*. Optimum pH for most of these plants is from 4 to 9 and salinity tolerance ranges from 0 to 5 g L^{-1}. Constructed SSW and SW vegetated with *Scirpus* have shown the ability to remove about 99 percent of viruses from wastewater (Chendorain *et al.*, 1998; Gersberg *et al.*, 1987). Tanner *et al.* (1998) reported that chemical pollutants such as CBOD, total N, and P have been removed in SSW with average efficiencies up to 91, 65, and 38 percent, respectively.

Microbial Populations

Bacteria are the primary microbial population carrying out pollutant removal from wastewater in wetlands. They are found at concentrations ranging from 10^6 to 10^9 cfu g^{-1} of substratum in wetlands vegetated with *Typha* or *Phragmites,* with higher densities in the rhizosphere (Gray and Biddlestone, 1995). In this zone, oxygen is available for aerobic bacteria that degrade organic matter to simpler compounds. Ammonium is transformed to nitrate by nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter*. In anaerobic regions,

fermentative, and methanogenic bacteria transform organic matter to methane using a carbon source such as methanol, acetate, or formate. Methane is emitted to the atmosphere directly or through plant leaves and steams.

Other organisms found in wetlands include fungi, algae, viruses, and protozoa. Fungi are found at high concentrations (1.6×10^6 cfu g^{-1}) in the substrate areas of the rizhosphere (Gray and Biddlestone, 1995). They play an important role in degradation of recalcitrant organic compounds such as cellulose and lignin (Atlas and Bartha, 1993). Algae are photoautotrophic organisms responsible for daily fluctuations of O_2 and CO_2 as well as pH because of their photosynthetic activity. They have been linked to the secretion of substances that are toxic to *E. coli* and *Vibrio cholera* (Maynard *et al.*, 1999). Coliphages are viruses that occur in the intestines of warm-blooded animals infecting coliform bacteria. These viruses are discharged into wetlands by wastewater effluents or wild animals where may be inactivated by bacterial enzymes and algae (Cliver and Herman, 1972; Sobsey and Cooper, 1971). Protozoa are invertebrate organisms and are believed to contribute to the removal of virus and bacteria in treatment facilities (Britton, 1994).

2.2 Pathogens in Municipal Wastewater

Water borne pathogens are frequently transmitted to healthy or immunocompromised hosts by the ingestion of food or contaminated water. The potential risk can be appreciated

by the approximately 76 million people that annually become ill by the ingestion of contaminated food in the USA (CDC, 2000). During 1971 to 1998, groundwater and surface water were responsible for 40 and 50 percent, respectively, of waterborne disease outbreaks related to drinking water (EPA, 1999).

Pathogenic Bacteria

Bacterial diseases caused by *Salmonella*, *Shigella*, *Vibrio cholerae*, *Escherichia coli*, *Yersinia* and *Campylobacter* have been reported as the major causes of waterborne outbreaks related to wastewater (Bitton, 1994). *Salmonella*, alone, is believed responsible for diarrhea in 2 to 4 million people, annually in the USA. These bacteria infect healthy individuals with the elderly, infants, and AIDS infected persons showing more severe symptoms of salmonellosis (Rusin *et al.*, 2000). In 1999, *V. cholerae* caused 254,310 cases of cholera in sixty one countries including the USA (WHO, 1999). *Campylobacter* and *Salmonella* were the primary cause of illness caused by contaminated food from 1996 to 1999 (CDC, 2000). In the USA, drinking water for the period from 1971 to 1996 caused 13 percent of bacterial disease outbreaks (EPA, 1999)

Human Enteric Viruses

The most common enteric viral agents of gastroenteritis are rotavirus, adenovirus, Norwalk virus, astrovirus, and calicivirus (Gerba and Enriquez, 1999). Enteric viruses are

obligate intracellular parasites ranging in size from 20 to 70 nm. Infected individuals may excrete 10^{11} viral particles per gram of feces (Rusin *et al.*, 2000). These viruses are responsible for 5 to 10 million deaths worldwide (Walsh and Warren, 1979). In the USA 210,000 children with a gastrointestinal infection are hospitalized annually at a cost of one billion dollars (Ho *et al.*, 1998). Rotavirus has been reported as the most common cause of infection in the world (Gerba and Enriquez, 1999).

Parasites

In recent years, *Giardia lamblia* and *Cryptosporidium parvum* have been protozoa parasites of major health concern. *C. parvum* was responsible for the largest waterborne outbreak ever documented in the United States (MacKenzie *et al.*, 1994). Whereas, *G. lamblia* is believed to be the agent of 27 percent of waterborne diseases caused by drinking water between 1991 and 1992 (EPA, 1999). These pathogens are more resistant than viruses and bacteria to chlorination; therefore, they may be found in reclaimed wastewater and sediments. Persons infected with *G. lamblia,* may discharge up to 10^8 cysts per day into the environment (Rusin *et al.*, 2000).

2.3 Inactivation in the Environment

Temperature, Sunlight and Metal Ions

Over time pathogens are inactivated in the environment. Commonly, their inactivation follows a first order model with a decay rate that depends on environmental factors such as temperature, sunlight, and metal ions.

Temperature has shown to be a key parameter. Enriquez *et al.* (1995) found a consistent rate of inactivation of poliovirus 1, and adenovirus 40 and 41 by raising tap water temperature from 4 to 23 °C. O'Brien and Newman (1977) observed an inactivation rate for poliovirus of one log-unit reduction per 25 h, when the virus was incubated *in situ* at a temperature between 23 and 27 °C. This same one log reduction was observed after 19 and 7 h for poliovirus 3 and coxsackievirus A-13, respectively. A longer time was observed for an equivalent inactivation of poliovirus 1 and coxsackievirus B-1 at temperatures from 4 to 8 °C. Inactivation of PRD1 and MS2 was similar at 7°C; however, PRD1 was 7 to 10 times more persistent than MS2 between 10 and 23 °C (Yahya *et al.*, 1993). Bacteria are also highly sensitive to temperature which is commonly considered in mathematical models that predict kinetic removal of coliform in the environment (Auer and Niehaus, 1993; Khatiwada and Polprasert, 1998; Marias, 1974).

Sunlight is also lethal for many microorganisms. Inactivation rates were twice as high for CB38Φ and CB7Φ bacteriophages incubated *in situ* in experimental microcosms

than the decay rate observed under dark condition or at 1 m of depth in a water body (Wommack *et al.*, 1996). Noble and Fuhrman (1997) reported that solar radiation always increased the decay rates of marine bacteriophage which ranged from 6.6 to 11.1 percent h^{-1}. In tertiary lagoons, Parhad and Rao (1972) observed a direct correlation of *E coli* inactivation and light intensity; however, algae growth was also positively correlated with light intensity and was the primary mechanism of *E. coli* removal.

Metal ions are capable of bacterial and viral inactivation. Sagripanti *et al.* (1993) observed that five type of viruses whether enveloped or noenveloped, single or double stranded, DNA or RNA were inactivated by the addition of cupric or ferric ions. The addition of peroxide increased significantly the antiviral effect of copper. Apparently, the oxidation potential of the metal ion is responsible for viral inactivation. Metal ions bind to functional molecules or groups and subsequent reductions by superoxide radicals and H_2O_2 generate hydroxide radicals causing multiple damages on the binding molecule (Thurman and Gerba, 1988).

Microbial Activity, Organic Matter, and pH

Contradictory results have been reported about the effect of organic matter on virus inactivation (Yates and Yates, 1991). Berry and Noton (1976) found that T2 inactivation was about 7 log in 14 days of incubation in laboratory experiments. However, no antiviral activity was observed in filtered or autoclaved seawater. Ward *et al.* (1986) examined the

inactivation mechanisms of enteric viruses in fresh water. Their results suggested that proteolytic enzymes from bacterial isolates inactivated echovirus particles by exposition of RNA to nuclease enzymes after cleavage of capsid proteins. Survival of enteric adenoviruses was observed to be longer in tap water than in wastewater while polio1 was practically unaffected (Enriquez *et al.*, 1995). On the other hand, La Belle and Gerba (1982) showed that organic matter protect enteroviruses from antiviral factors in seawater.

Pathogen inactivation is also related to pH. In wetlands with open water areas or oxidation ponds, algae proliferation may induce an increase of pH up to 10.4 that might actively increase virus and bacterial inactivation (Kadlec and Knight, 1976; Yates and Yates,1991 ; Maynard *et al.*, 1999).

2.4 Indicator Microorganisms

Detection of pathogenic microorganisms in environmental samples is a difficult, expensive, and time consuming process that requires trained personal. Therefore, indicator microorganisms are commonly used for detection of fecal contamination and indirectly as a presumptive test for the presence of pathogenic microorganisms in food and water. An ideal indicator has the following characteristics (Britton, 1994): 1) it is a member of the intestinal tract of warm-blood animals; 2) it is detectable when enteric pathogens are present and undetectable in uncontaminated samples; 3) it is usually present in higher densities than

pathogens in environmental samples; 4) it is more resistant to environmental stress and disinfection processes than are pathogens; 5) it is incapable of multiplying in the environment; 6) it is inexpensive, easily, and quickly detected; and 7) it is not pathogenic.

The most widely use indicator of fecal contamination in water is the coliform group. Microbiological standards to meet public health protection goals are based on total and fecal coliform concentrations in drinking and wastewater (Gerba, 2000). The coliform group includes: *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter* which are aerobic, facultative anaerobic, gram-negative, non-spore forming, rod-shape bacteria that ferment lactose with gas production at 35 °C within 48 h. Fecal coliforms, a subgroup of coliforms, are characterized by their ability to ferment lactose producing acid and gas within 24 h at 44.5 °C and include *E. coli* and the genera *Klebsilla* (Bitton, 1994).

Coliphages is another indicator of water contamination by fecal material since its size, morphology, and structure are similar to enteric viruses (Gerba, 2000). Stetler (1984) observed that enetroviruses were better correlated with coliphages than total coliforms, fecal coliforms, and fecal streptococci during drinking water treatment.

Fecal streptococci which is also used as an indicator grows in 6.5 percent sodium chloride, at a pH of 9.6, and a temperature of 45 °C. The ratio of fecal coliform concentration versus fecal streptococci is used as an indication of human fecal versus animal contamination. A ratio of 0.7 is an indicator of animal fecal contamination while a ratio

greater than 4 is used as an indicator of human contamination (Geldreich and Kenner, reported in Gerba, 2000).

Removal in Wetlands

Studies conducted in CW have shown a large coefficient of variation of total and fecal coliform populations in the wetland outflowing water. Often, averaged removal efficiencies over 90 percent of the influent load are observed (Bahlaoui *et al.*, 1997; Perkins and Hunter, 2000). This efficiency is inversely related to the hydraulic loading rate (Green *et al.*, 1997; Tanner *et al.*, 1998) and may decrease considerably if vegetation density is low (Gersberg *et al.*, 1989).

In constructed wetland systems, coliforms could be an unreliable indicator of the ability of the system to remove pathogens from wastewater because coliforms can occur naturally in the environment. Van Donsel *et al.* (1967) observed regrow of coliform bacteria associated with an increase of temperature and rainfall in soils amended with nutrients. A similar condition was also found by Elmund *et al.* (1999) in a treatment facility. In this case, the high numbers of *Klebsilla* coincided with an increased of carbohydrates in the incoming wastewater. In subtropical environments, Solo-Gabriele *et al.* (2000) reported several orders of magnitude growth of *E coli* in sediments. Moorhead *et al.*(1998) also found high densities of an "unclear origin" of *E coli* and total coliforms in urban water bodies. The presence of wild animals might explain these high densities of total and fecal

coliforms often found in the environment (Gould and Fletcher, 1978; Have, 1973; Levesque *et al.*, 1993). However, specialized studies should be conducted to identify their origin in natural water systems (Griffin *et al.*, 2000).

2.5 Virus Models

Bacteriophages have been used to simulate transport of enteric viruses in the environment since they are not pathogenic, their analysis is inexpensive, easy, rapid, and highly concentrated stock solutions can be prepared in the laboratory. Many experiments have been conducted using bacteriophage in the laboratory (Bales *et al.*, 1991; Jin *et al.*, 1997; Powelson *et al.*, 1991; Wang *et al.*, 1981; Yahya *et al.*, 1993) and field (Deborde *et al.*, 1999; Schijven *et al.*, 1999). Moreover, their performance in the environment has been compared to animal viruses (Gerba and Goyal, 1981; Goyal and Gerba, 1979; Yates *et al.*, 1985). For example, the bacteriophage MS2 is ssRNA, 23 nm in size, similar to astroviruses, enteroviruses, and Norwalk (Rusin *et al.*, 2000). This bacteriophage has shown little adsorption at pH between 7.5 and 8 in soils under unsaturated (Powelson *et al.*, 1990) and saturated (Jin *et al.*, 1997) conditions. In groundwater MS2 survival is similar to animal viruses (Yates *et al.*, 1985) but shorter than another bacteriophage PRD1 (Yahya *et al.*, 1993), which is also commonly used in tracer studies.

The coliphage PRD1 is dsDNA virion and measures 62 nm with a protein capsid that surrounds an internal lipid-protein membrane (Caldentey *et al.*, 1990) resembling the human rotaviruses. It is positively charged at pH below 4.5 (Bales, *et al.*, 1991) compared with MS2 which is positively charged only below pH 3.9 (Zreda reported in Gerba, 1984). Both MS2 and PRD1 viruses do not adsorb on to silica particles at 7 pH (Bales *et al.*, 1991). At lower pH, PRD1 is more hydrophobic than MS2 and consequently a greater absorption of PRD1 is observed.

Other virus models have also been used in transport experiments. One of these is øX174 which is 27 nm in size and isoelectric between pH 6.6 and 6.8 (Dowd *et al.*, 1998; Fujito and Lytle, 1996). This virus is less hydrophobic than MS2; however, electrostatic interactions might be the primary mechanism of adsorption in soils at a neutral pH (Jin *et al.*, 1997). Another bacteriophage f2, similar to MS2, and øX174, belongs to the group of F-specific RNA bacteriophages. Goyal and Gerba (1979) described it as a virus of very low tendency for attachment to soils particles.

Virus Removal in Wetlands

Virus removal studies in wetlands have been conducted with different approaches. Scheuerman *et al.* (1990) and Karpiscak *et al.* (1995) quantified virus removal by the ratio of the inflow and outflow viral load while Gersberg *et al.* (1987) and Vinluan (1996) conducted *in situ* virus survival studies. Tracer studies using MS2 as a bacteriophage model

in a surface flow constructed wetland were used by Chendoraine *et al.*, (1998) to determine virus removal and model its transport. These studies have reveled that virus removal efficiencies of 99 percent and removal rates of 0.44 day^{-1} can be achieved in wetlands. Apparently, reduction of the inflow virus load is unaffected by climatic conditions; however, it is enhanced by vegetation density and filtration processes through the wetland (Gersberg *et al.*, 1987).

2.6 Problem Definition

Constructed wetlands have been actively used in wastewater treatment making effective use of the processes that occur naturally in the environment for water treatment. They can be applied in a wide diversity of localities, climatic conditions, and wastewater treatment demands. Additionally, their operation costs are lower than traditional wastewater treatment facilities (Kadlec and Knight, 1996). However, it is recognized that their design faces engineering uncertainties because under different circumstances of operation the removal efficiency of the system may vary widely (Unsoeld, 2000).

In the current study, the hydraulic behavior of subsurface and surface flow wetlands have been related to their viral removal efficiency observed during tracer studies and *in situ* survival tests. Removal of bacteriophage PRD1 and transport of a conservative tracer in a surface flow wetland were modeled and information about PRD1 movement in the vadose zone was analyzed. In addition, the spatial behavior of chemical and microbial indicators in

a wetland system operated primarily with backwash water was monitored for a 16 month-period. The usefulness of total and fecal bacteria and indigenous coliphage as indicator microorganisms to evaluate bacteria and virus removal in constructed wetland systems was discussed.

3. PRESENT STUDY

The methods, results, and conclusions of this study are presented in the appendices included in this dissertation. The most important findings and a brief discussion are presented in the following summary.

SUMMARY

The tracer test conducted in the subsurface flow constructed wetland indicated a high degree of plug flow in the hydrodynamic performance of the, 6 year old, gravel bed. During the study, most of the PRD1 was recovered within the following four days after the tracer injection. At the end of the test, the average PRD1 removal was 98.8 percent and a mass recovery of 75 percent for Br^- was observed. The PRD1 reduction was associated with removal and inactivation rates of -1.17 and -0.16 day^{-1}, respectively. Apparently, virus removal in subsurface flow wetlands is mostly due to adsorption processes with a small die-off contribution.

An additional tracer study was conducted in a surface flow constructed wetland that consists of pool-vegetated riffle zone sequences designed to remove suspended solids from backwash water before aquifer recharge. During the study, samples from the surface and subsurface water were obtained. In the vadose zone, PRD1 was observed after the appearance of Br^- in the collected samples while in the surface, the concentration-time distributions of Br^- were simulated by a plug flow model with a high longitudinal dispersion. The convective-dispersion equation and the first order reaction approach were capable of simulating PRD1 displacement and removal through the wetland. At the outlet of the system, recovery of Br^- and PRD1 after 7.3 days was 86 and 16 percent, respectively. PRD1 decay rate was estimated to be 0.3 day^{-1} by regression analysis and mathematical fitness to the experimental data. The findings of these studies suggest that contaminant transport in wetlands can be modeled with the convection-dispersion model (CDE) including the first order reaction approach.

The sampling program was conducted in the east polishing subsystem which consists of a settling basin, a small artificial stream, and the latter surface flow wetland where the tracer study was conducted. Microbial populations of coliphage, total coliforms and fecal coliforms were quantified in samples collected at five sampling sites along the system. In addition to this microbial indicators, chemical and physical parameters such as total

suspended solids (TSS), biochemical oxygen demand (BOD_5), chloride (Cl^-), chlorine (Cl_2), sulphate (SO_4) temperature and pH also were quantified.

Coliphage removal in the system ranged from 46 to 93 percent during the 16 month of sampling. Whereas, the population of TC and FC were consistently higher at the outlet of the polishing wetland than in the water entering the system. In contrast, BOD_5, and TSS, were reduced efficiently below the secondary effluent standard of 30 mg L^{-1} required by The Arizona Department of Environmental Quality. The results of this study suggest that coliphages are a better indicator than coliforms in wetlands since bacterial populations may increase by regrowth or animal fecal contributions. This study also revealed that the stream and wetland sections of the east polishing system are unable to reduce significantly BOD_5, TSS, and turbidity.

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APPENDIX A

RELATIVE TRANSPORT OF Br^- AND PRD1 IN A SURFACE-FLOW CONSTRUCTED WETLAND

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Relative Transport of Br^- and PRD1 in a Surface-Flow Constructed Wetland

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Abstract.- A tracer study using Bromide (Br^-) and bacteriophage PRD1 was conducted in a 3 ha-wetland planted with species of bulrush (*Scirpus* spp) and cattail (*Typha domingensis*) at the Sweetwater Recharge Facility in Tucson, AZ. Transport of the tracers through both the wetland and vadose zone was determined. The constructed wetland consists of pool-vegetated riffle zone sequences designed to remove suspended solids from mixed media filter backwash water before aquifer recharge. During the tracer study, flow rate was maintained at approximately 1.8 m^3 min^{-1} of secondarily treated wastewater effluent. In vadose zone samples, PRD1 was observed after the appearance of Br^- penetrating to a depth of 1.5 m. In the surface, concentration-time distribution of Br^- were simulated by a plug flow model with a high longitudinal dispersion at both the outlet and an internal sample site. The convective-dispersion equation (CDE) and the first order reaction approach were capable of simulating PRD1 displacement and removal through the wetland. At the outlet of the system, recovery of Br^- and PRD1 after 7.3 days was 86 and 16 percent, respectively. PRD1 decay rate was estimated to be 0.3 day^{-1} by regression analysis and model calibration. The findings of this study suggest that CDE, including high dispersion and first order reaction approach, is suitable for modeling virus removal in wetlands.

INTRODUCTION

Water reclamation and reuse preserve and increase water availability in communities where water resources are naturally limited or became limited by population growth. Even, after treatment, however, reclaimed wastewater may contain pathogenic viruses which if used for aquifer recharge, irrigation, or recreational activities increases the risk of waterborne diseases.

Constructed treatment wetlands are a low-cost emergent technology that simulates natural ecosystems to treat diverse types of polluted water. Wastewater treatment occurs through a combination of physical, chemical and microbiological processes with varying degrees of success. Most research in constructed wetlands has been focused on water quality indicators (Bowmer, 1987; Frankenbach and Meyer, 1999; Geller, 1997; Karpiscak *et al.*, 1994; Mandi *et al.*, 1996) such as biological and chemical oxygen demand, total suspended solids, nitrates, and phosphate. Virus removal has been only sporadically studied despite the fact that virus transmission by contaminated water is a health and economical problem recognized worldwide. Annually, one billion dollars is spend in the US in hospitalization of 210,000 children infected by viral agents of gastroenteritis (Ho *et al.*, 1998). Worldwide some 5-10 million die (Walsh and Warren, 1979).

Studies conducted in constructed wetlands have reported virus removal efficiencies as high as 98 percent in subsurface flow wetlands (Gersberg *et al.*, 1987; Karpiscak *et al.*,

1995). In a multi-species surface flow wetland, Vinluan (1996) found 90 percent removal of an indigenous phage, bacteriophage MS2, and PRD1 after 2.05, 3.67, and 13.38 days of incubation *in situ*, respectively. Whereas, only a 38 percent reduction in coliphage was observed at the outlet of a duckweed (*Lemna* spp.) system (Karpiscak *et al.*, 1995). A modeling study conducted in surface flow wetlands found that the first order reaction model, widely used to describe virus inactivation in the environment, was unable to describe MS2 removal because virus removal was considerably higher close to the inlet of the system (Chendorain *et al.*, 1998). Detection of pathogenic viruses is expensive, time consuming and not always possible. Bacteriophage is a suitable option in virus studies because it can be prepared in high concentrations, does not infect humans, and its analysis is relatively easy, inexpensive, and rapid. In this study, bacteriophage PRD1 was used as a human enteric virus model. It is a dsDNA virion, 62 nm in size (Olsen *et al.*, 1974), isoelectric between pH of 3 and 4 (Loveland *et al.*, 1996) and has a protein capsid that surrounds an internal lipid-protein membrane (Caldentey *et al.*, 1990) similar to the human rotaviruses.

This paper reports the findings of a tracer study conducted to analyze PRD1 removal and the hydrodynamic performance of a surface flow constructed wetland designed to remove suspended solids from filter backwash water at a wastewater reclamation plant.

MATERIALS AND METHODS

Site Description

The area of study was the East polishing wetland at the wetland wastewater treatment system, in operation since July of 1997. It is located at the Sweetwater Recharge Facility in Tucson, AZ. The wetland is the terminal component of the East polishing wetland subsystem and is preceded by a settling basin and an artificial stream. It has been supplied for about two years of operation with different proportions of secondary effluent and backwash water. The backwash water comes from the nearby Tucson Water Reclamation Plant during periodic backwashing of pressure mixed media filters used to treat activated sludge effluent prior to its distribution as reclaimed water for use in landscape irrigation. The surface area of the East wetland cell is about 3 ha, the approximate length is 329 m, and the width varies from 72 to 112 m. Pool and vegetated riffle zone sequences is the geometric configuration of the basin. Islands of different size are distributed in the cell mostly in the open water zones where the water depth is approximately 1.2 m. Emergent vegetation consists of bulrush species (*Scirpus* spp.) and cattail (*Typha domingensis*). At the outlet, a calibrated weir is used to measure flow rates and at the inlet a manifold, placed in a small deep zone, distributes the influent water along the east edge of the basin. The volume for water storage was estimated to be 1.74×10^4 m^3.

In the area of the wetland, 4.5 to 6 m of a natural clay overbank deposit is generally found. Therefore, addition of clay to prevent wastewater seepage was unnecessary, only the native clayey soil was compacted to some degree during the east wetland construction. Prior to basin were vegetated with the emergent vegetation in the spring of 1997, four paired suction lysimeters (Soil Measurement Systems, Model SW-071, Tucson AZ) of 0.27 m of length and 0.025 m diameter were installed at the Southeast corner of the polishing cell in the first emergent area. The 8 samplers were splitted in two arrays denominated East and West array and placed into individual wells at 0.3, 0.76, 1.5, and 3 m below the soil surface with a separation of 1 m between adjacent lysimeters. Latter, the installation wells were refilled; only the outlet tubing for pore water samples extraction and vacuum/pressure application were brought to the surface. A constant vacuum source induced by a vacuum pump conduces the pore water sample into the lysimeter through a porous stainless steel membrane of 0.09 m of length located on its upper section.

Production and Enumeration of PRD1

Salmonella typhimurium was used as the PRD1 host bacterium to produce 3.2 L of stock suspension by the agar overlay method (Adams, 1959) with a final concentration of 2.14×10^{10} plaque forming units (pfu) ml^{-1}. To recover PRD1 from the agar overlay, 6 ml of Tris buffered saline, stock solution [Trizma base, Sigma Chemical Co., St. Louis MO

(63.2 g; NaCl, 163.6 g; KCl, 7.46 g; and 1.13 g Na_2HPO_4 anhydrous) dissolved in 1,600 ml of distilled water] diluted 1:10, were placed on the agar surface and incubated for 3 h, at room temperature. The suspension was collected in a 250-ml bottle, and centrifuged at 22,095 x g for 10 min. In addition to further removal bacteria debris the supernatant was filtrated through a 0.45 μm pore size cellulose acetate membrane filters (Costar, Cambridge, MA).

PRD1 was assayed by the double layer agar method described by Adams (1959). A 1-ml aliquot from a *S. typhimurium* culture, previously incubated at 37 °C for 24 h in Trypticase Soy Broth (TSB, Difco, Detroit, MI), was combined with 4 ml of molten agar (TSA, Trypticase Soy Agar; Difco, Detroit, MI) and 1 ml of sample or sample dilutions added. The agar was then poured onto a layer of TSA, and incubated at 37 °C for 18 h in order to enumerate the PRD1.

Tracers

The study was conducted from February 12 to March 18, 1999. Chlorinated secondarily treated wastewater at a flow rate of 1.84 $m^3 min^{-1}$ was entering into the secondary splitter box which is separated from the east wetland cell by the artificial stream and the settling basin. Background concentration of PRD1, Br^-, and Chlorine (Cl_2) were determined in influent and outflow water samples from the basin for one week prior of the start of the tracer study.

Bromide (Br^-) and bacteriophage PRD1 were the conservative and virus tracers used in this study. On March 24, a solution of 340 L of Br^- was prepared in the field adding 47, 34, and 34 preweighted individual 1-kg packages of Sodium Bromide (NaBr) into three containers with 140, 100 and 100 L of wastewater, respectively. The final tracer solution was prepared by adding 180 ml of PRD1 stock suspension, and approximately 2 g of sodium thiosulphate to 20 L of solution. This final tracer solution was then pumped at a rate of 2.5 L min^{-1} at the end of the stream; just prior to discharge into the polishing wetland cell. The input (Co) concentration to the basin was 354 mg L^{-1} of Br^- and 2.56×10^5 pfu ml^{-1} of PRD1.

Sampling Sites and Collection

The sampling sites were situated at the inlet (1), outlet (5), lysimeter area (2), the viewing area (3) located on the North edge of the first open water area and an internal site at the South island from the second open pond (3) (Figure 1). After tracer addition, a surface grab water sample was obtained daily from site 1 for the following three days. At site 2, a surface grab sample and seven pore water samples from the unsaturated soil were collected daily during the study. From the lysimeter at 3 m of depth, in the West lysimeter array, only a few milliliters of water were collected because problems associated with its extraction. The lysimeters were operated at 65 kPa of vacuum by using a pressure/vacuum hand pump (Soil Moisture Equipment, Santa. Barbara, CA.).

Three pneumatic samplers of 0.45 m of length and 0.013 m of diameter were placed vertically below water surface for monitoring tracer concentrations at 0.076, 0.53, and 0.99 m of depth at the sampling site 3. They were constructed to collect water samples applying positive pressure through an outlet tubing for closing an one way steel valve on the bottom of the sampler. The sample was conducted to the surface by tubing connected on the sampler well realizing the internal pressure at the time of collection. Then positive pressure was again applied into the sampler to bring another fraction of water to the surface. After a extraction of 0.5 L of water, a 20 ml-sample was collected.

An automated sampler (American Sigma, Model 702; New York, NY) was installed at the sampling site 4. It was programmed every day to collect 0.6 L of sample per hour into each of 24-polyethylene bottles previously labeled to distinguish their position in the sampler. A second automated sampler (Isco, Model 3700; Lincoln, NE) was set up at the outflow of the wetland where a water level recorder (Leupold & Stevens, Type F1, Beaverton, OR) was also placed for monitoring outflow rates.

After sample collection, 24 clean and disinfected bottles were installed into each of the automated samplers and crushed ice was placed on the base sections. The samples were placed on ice and transported to The University of Arizona for analysis. An ion chromatography system (Dionex 500 Chromatographic System; Sunnyvale, CA) and the agar overlay method were used to analyze Br^- and PRD1, respectively.

Modeling

The experimental concentration-time distributions were simulated by using the one dimensional convective-dispersion equation (CDE) including first order removal:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial X^2} - v\frac{\partial C}{\partial X} - \kappa C$$

an analytical solution of this equation (Harris, 1963) for an instantaneous injection over the cross sectional area satisfying the following initial and boundary conditions (Unice *et al.*, 2000):

$$C(x,0) = 0$$

$$C(0,t) = \frac{m}{Ac}\delta(t)$$

$$C(\infty,t) = 0$$

is given by:

$$C(x,t) = \alpha\frac{m}{2Ac\sqrt{\pi Dt}}\exp\left(-\frac{(x-vt)^2}{4Dt}\right)\exp(-\kappa t)$$

where

δ (t) = Dirac delta function

α = conversion factor (1×10^{-6} and 1×10^{3} for PRD1 and Br-, respectively)

m = mass added into the system (Kg or pfu's)

Ac = flow cross-sectional area (m^2)

v = convective velocity ($m\ day^{-1}$)

D = longitudinal dispersion ($m^2\ day^{-1}$)

X = longitudinal distance (m)

t = time (day)

k = removal rate constant (day^{-1})

C = tracer concentration ($mg\ L^{-1}$ or $pfu\ ml^{-1}$).

The average flow cross-sectional area (Ac) was estimated by drawing an axis, on a copy of original blueprints (horizontal scale 1:40, English units) of the east polishing wetland, from the east edge of the basin to the outlet in order to trace perpendicular cross-sections at different distances from the inlet (Table 1). Each cross-sectional area was calculated considering 0.30 m water depth in the emergent zones and 1.2 m in the open water areas. Using this method, the average cross-sectional area was estimated to be 41.47 m.

Mathematical fitness of CDE to experimental breakthrough curves was conduced using the last square method in Mathcad professional 2000 (Mathsoft, Cambridge MA). Mass recovery was calculated from the outlet breakthrough curve and plugged into CDE solution to determine longitudinal dispersion, and convective velocity. Thereafter, these parameters were used to check the amount of PRD1 added and its decay rate by mathematical fitness. With the outlet Br^- mass recovery, CDE analytical solution was fitted to experimental data collected at the island to calculate the convective velocity and dispersion. These were used together with the previous amount of PRD1, estimated by model calibration, to simulate the experimental breakthrough curve at the island site.

Water Balance

It was assessed by measuring inflow and outflow flow rates, evaporation and precipitation. Evapotranspiration and infiltration were estimated. Wetland evapotranspiration was calculated by:

$$Et = 0.65\ x\ Ev$$

where Et is evapotranspiration (mm day^{-1}), and *Ev* is evaporation (mm day^{-1}) from a class A evaporation pan located at The Constructed Ecosystem Research Facility, on the North side of the wetland. The 0.65- factor was determined by averaging the values recommended

by the Arizona Meteorological Network (AZMET, 2000) to estimate evapotranspiration from open water bodies during Winter (0.7) and Summer (0.6). Seepage losses were calculated by taken the difference between inlet and outlet average flow rate minus Et in m^3 min^{-1} for the period of study.

Mass Balance

Mass recovery was estimated from the zero moment of the outlet breakthrough curve and the average outlet flow rate:

$$M = \phi Q \int_0^{\infty} C(t)\, dt$$

where M is mass recovery (kg), φ is a conversion factor (1.44), Q is the average outlet flow rate (m^3 min^{-1}) and t is time (day^{-1}) .

Mass losses by seepage were estimated by integration of the outlet breakthrough curve using the following expressions:

a) mass in the system (*Ms*) at any time (τ) is given by:

$$Ms(t) = \left(1 - \frac{\int_0^{\tau} C(t)\ dt}{\int_0^{\infty} C(t)\ dt}\right) m$$

where the ratio between integrals is the fraction of mass living the system with a value from 0 to 1. *Ms* is the mass in the system (kg).

b) assuming a homogeneous distribution of *Ms* in the system, losses of mass (*I*) by seepage are given by:

$$I = \frac{q}{V} \int_{t=0}^{\infty} Ms(t)\, dt$$

where I is mass losses by seepage (kg), q is the seepage rate ($m^3\ day^{-1}$), and V is the wetland volume for water storage (m^3).

The normalized first moment or detention time of the system is defined as following:

$$t_d = \frac{\int_0^{\infty} t\, C(t)\, dt}{\int_0^{\infty} C(t)\, dt}$$

where t_d is detention time (day^{-1}). The variance of the detention time distribution with respect to the detention time can be calculated by:

$$\sigma^2 = \frac{\int_0^{\infty} (t - t_d)^2\, C(t)\, dt}{\int_0^{\infty} C(t)\, dt}$$

where σ^2 is the variance of the tracer respond breakthrough curve (days^2).

Dimensionless variance is an indicator of the extension of back-mixing and it is determined by the ratio of the variance and the square of the tracer detention time :

$$\sigma_\theta^{\,2} = \frac{\sigma^2}{t_d^{\,2}}$$

RESULTS AND DISCUSSION

Background Conditions

Background concentration for Br^- was 0.55 mg L^{-1} and for PRD1 less than one pfu ml^{-1}. During the tracer study, chlorinated secondary effluent (containing 1.19 and 0.14 mg L^{-1} of total and free chlorine, respectively, on February 19, 1999) was received into the secondary splitter box (Figure 1). This water then flowed through the small stream into the East Polishing Basin. Total and free chlorine levels were below 0.1 mg L^{-1} in samples from the tracer injection point at the end of the stream. Relative concentration of Br^- was 13 percent lower than PRD1 a few minutes before the end of the tracer injection at the influent end of the wetland. PRD1 and Br^- concentrations, for the first three days of the study, are reported in Figure 3. Apparently, PRD1 performance at the sampling site 1 was similar to

Br^- showing that the bacteriophage inactivation was unimportant at this initial stage of the study.

Hydrologic Conditions

Flow conditions were close to a steady state in the basin during the study (Table 2). Hydraulic loading rate at the outlet of the system was 84 percent of influent water. Water losses by seepage and evapotranspiration were estimated to be 8.2 and 3.5 mm day^{-1}, respectively. Kadlec and Knight (1996) suggest that wetlands can be consider similar to an open water body for estimating their evapotranspiration losses. These losses can be represented by about 70 to 80 percent of class A pan evaporation from an adjacent open site. Arizona Meteorological Network recommends a factor of 0.6 for Summer and 0.7 for Winter to estimate reference evapotranspiration of a large water body like a pond or lake; however, factors such as temperature, turbidity, depth, size of the pond, and surface area may drastically modify this relationship (AZMET, 2000).

Lysimeter Area

The highest PRD1 concentration observed in surface grab samples from 2 was 3185 pfu ml^{-1} on February 25, the day after injection of the tracers. In Figures 4 and 5 Br^- and PRD1 concentrations were plotted versus lysimeter depth. Each curve corresponds to the date in which Br^- or PRD1 concentration was the highest one observed during the 21-day study

in one of the four lysimeters at the East and West site. Because the absolute concentration of PRD1 is much higher than Br^-, only the curve for the conservative tracer maximum concentration noticed in surface samples was plotted.

Conservative Tracer

The higher Br^-concentration in the vadose zone (Figures 4 and 5A) than in the surface grab sample, taken on February 25, revels a short residence time of the main cloud of Br^- that passed over the lysimeter field. In the vadose zone, the uneven concentration profile of Br^-, mostly in the West array of samplers, and its rapid displacement, in contrast with the calculated seepage rate, appears to indicate preferential flow and a different degree of water content surrounding the lysimeters at identical depths. A major disadvantage of using lysimeters for water sampling from the vadose zone is their installation. Soil conditions are altered because a hole has to be dug and refilled after sampler installation promoting the possibility of preferential flow.

Bacteriophage

PRD1 was observed to arrive later than Br^- (Figures 4 and 5B) in the soil water samples reaching, apparently, a maximum depth of 1.5 m. A similar virus performance was reported in a tracer study conducted in an artificial recharge basin where hydrophobic

interactions between virus and organic colloids from secondary effluent, used in the study, were apparently responsible of such behavior (Powelson *et al.*, 1993). Several previous studies (Bales, 1991; Jin, *et al.*, 1997; Powelson *et al.*, 1991; Powelson and Gerba., 1994) have found that hydrophobic and electrostatic interactions as well as unsaturated conditions are mostly responsible for virus removal in soils. Since July of 1997, the East Polishing Basin had been flooded with different proportions of secondary effluent and backwash water. Vadose zone accumulation of organic matter from wastewater may have been preferentially deposited in the shortcut flow paths resulting in virus hydrophobic interactions (virus adsorption and retardation) in spite of preferential flow.

PRD1 was detected at very low concentrations (1 pfu ml^{-1}) after 10 days of study at a depth of 0.76 m in the West array. Unsaturated conditions were higher at this location than at the other samplers. The importance of unsaturated conditions for virus removal was shown by Powelson and Gerba (1994) who found that removal rates were greatly increased by decreasing soil water content. In the East array, PRD1 was not observed at depths of 1.5 and 3 m, probably do to a longer contact time and a greater degree of unsaturated conditions, in contrast with the lysimeters affected by preferential flow, limited movement of PRD1.

Deep Water

During the first day of sampling both Br^- and PRD1 were observed in samples collected from 3 (Figure 6). After an apparent initial distribution Br^- concentration decreased significantly, the following day. The highest concentration observed was at a depth of 1 m. Bacteriophage was better mixed than Br^- one day after the tracer addition ; later, its concentration decreased drastically.

Modeling

Recovery of Br^- at 5 was about 76 percent of the mass originally added into the system; therefore, the CDE was fitted to the experimental data assuming an initial injected mass of 67 kg, steady state conditions, and a 100-percent of the tracer recovery. Calibration of the model was conducted as described above, the input and calculated parameters are presented in Table 3.

Conservative tracer Br^-

The breakthrough curves at the sampling sites 4 and 5 were adequately simulated by CDE. (Figure 7). A steeper profile and higher velocity were shown by Br^- for its arrival at the island than at the outlet which is consistent with the convective velocity and longitudinal

dispersion calculated by model calibration. At the outlet, Br^- depicted a longer tailing probably because of contraction of the cross-sectional area that induced dead zones formation at the end of the basin.

Concentration of Br^- evidenced a nearly uniform evolution over time; although, the dispersion of experimental Br^- concentrations in contrast to CDE curve is higher at site 4 than 5 (Figure 7), probably because of its closer position to the inlet. Near the site of solute addition, spatial movement of a tracer is three dimensional. When CDE is reduced to one dimensional form the non-uniform displacement is averaged in a longitudinal dispersion coefficient. Gradually, the equation will show a better fitness to experimental data depending on how well the tracer is mixed across flow sectional area. This is a function of transport time or distance and is negatively related to the solute diffusion coefficient. Transport time or distance from the inlet to the island was not enough to reach full mixed conditions in the flow cross-sectional area.

Similarly to the findings of other studies (Chendorain *et al.*, 1998; Kadlec, 1994), a high longitudinal dispersion was observed through the basin (Levenspiel and Smith, 1957). In the present study, it may be attributed to non-uniform advection induced by islands and pool-riffle sequences as reported by Seo (1990) in laboratory studies, vegetation (Coutanceau and Bouard, 1977; Nepf *et al.*, 1997), and flow cross-section variation. This intensifies the lateral and vertical concentration gradients.

Bacteriophage PRD1

The one dimensional convection-dispersion equation was capable of simulating PRD1 concentration-time distribution at both the island and outlet (Figure 8 and 10). Similar to other tracer studies conducted in surface flow wetlands (Chendorain *et al.*, 1998), both figures show a wide variation of PRD1 concentrations suggesting a high analytical variability or that concentration gradients of PRD1 can change greatly in short distances within wetlands because of insufficient mixing.

PRD1 decay rate was estimated to be -0.30 day^{-1} ($r^2 = 0.98$) by regression analysis between the normalized zero moment of the breakthrough curve at the inlet (pulse added into the system), island, and outlet, and the time at their center of mass (Figure 9). This value was practically equal to the decay rate calculated by mathematical fitness of CDE to the outlet experimental curve. Likewise, *m* obtained by CDE calibration was only 3.6 percent higher than the PRD1 in the 3.2 L of stock suspension added initially into the influent water. The first order model included into CDE as well as convective velocity and longitudinal dispersion obtained by mathematical fitness were on average consistent with PRD1 transport conditions through the wetland. Apparently, the calculated decay rate described adequately PRD1 removal over time at the outlet and the internal site. This differs somewhat from MS2 removal studies conducted in surface flow wetlands by Chendorain *et al.* (1998). In these studies, MS2 decay rate was a site dependent coefficient ranging from 0.076 to 5.81 day^{-1}.

Detention Time and Mass Recovery

The wetland detention time for Br^- was 7.3 and 6.8 days calculated from the experimental and CDE breakthrough curve, respectively (Table 4). Also for PRD1, both first moments were practically equal. However, these were shorter than those for Br^- because virus removal reduces the PRD1 breakthrough curve and consequently its first moment becomes shorter than the first moment of Br^- breakthrough curve. Mass recovery and losses by seepage for Br^- at the outlet, based on outflow rates, were estimated to be 76.6 and 10 percent, respectively. Mass recovery based on model simulation was 74 percent.

Tracer studies in treatment wetlands are characterized by a highly variable mass recovery (Table 5). Factors such as microbial degradation, sorption, inadequacies of analysis and sampling, flow conditions, and inaccurate measurement of outflow rates have been responsible of this variability. Probably, small contributions from sampling, analysis, and flow rates may account for the mass balance underestimation.

Bacteriophage PRD1 was removed 84 percent during the 7.3 days-detention time in the East Polishing Wetland. Dimensionless variance (σ_θ^2) and arrival time (t_{10}/t) of Br- at the outlet of the East polishing wetland were estimated to be 0.23 and 0.52, respectively. These parameters were 0.18 and 0.58 in a tracer study conducted in a multi-species subsurface flow wetland where 98.24 percent removal of PRD1 was observed. In this multi-species wetland, the removal and inactivation rate (in dialysis bags) were estimated to be -1.17 and -0.16 day^-

[1], respectively. Apparently, adsorption promoted by filtration was the primary mechanism of PRD1 fixation on the wetland substratum followed by a longer inactivation process. Compared with MS2 removal reported by others, this efficiency is low. For example, Gersberg *et al.* (1989) found 91.5 percent removal of MS2 in a surface flow wetland and Chendorain *et al.* (1998) reported 93 to 98 percent also in surface flow wetlands. Studies conducted by Yahya *et al.* (1993) and Vinluan (1996) reveled that PRD1 is more persistent in the environment than MS2; however, the latter study conducted in multi-species subsurface wetland and other conducted by Gersberg *et al.* (1987) suggest that survival of MS2 and PRD1 in wetland environment receiving municipal wastewater is comparable.

Only few research studies have been conducted to determine the ability of constructed wetlands to remove animal enteroviruses. Karpiscak (1995) found human enterovirus removal of 98 percent and Thurston (1997) over 83 percent in the same multi-species subsurface wetlands. Other studies conducted in forested and subsurface flow wetlands have observed removal rates for indigenous coliphage to be smaller than for enteroviruses (Scheuerman, *et al.*,1997). For example, Gersberg *et al.* (1987) reported that indigenous FRNA coliphage was removed more slowly than polioviruses and MS2 in vegetated gravel beds receiving municipal wastewater. Comparable findings were reported comparing indigenous coliphage to human enteroviruses in forested wetlands (Scheuerman *et al.*, 1997).

CONCLUSIONS

Preferential flow of Br- was observed along the soil profile below the wetland. Movement of PRD1 was delayed in contrast to Br^- and largely removed, although, it was detected at 1.5 m of depth. Apparently, hydrophobic interactions and unsaturated conditions limited soil penetration of PRD1 at the lysimeter sampling site. One dimension CDE was capable of simulate acceptably Br^- and PRD1 transport performance in the basin. Although, a higher longitudinal dispersion has to be included into CDE to simulate Br^- and a first order model to predict PRD1 removal. In contrast with Br^-, PRD1 traveled without retardation through the basin suggesting that virus removal was mostly irreversible. A 7.3 day detention time for Br- resulted in a 84 percent reduction of PRD1. The removal rate of PRD1 in the current study was several times smaller than removal rates reported for MS2 and PRD1 in subsurface flow wetlands (Gersberg *et al.*,1987; Vidales *et al.*, 2000). Given the high persistency of PRD1 in the environment, survival does not appear to be the primarily removal mechanism. Apparently, its removal in wetlands is related to a filtration process and probably sedimentation of virus colloidal carriers (LaBelle and Gerba, 1979 and 1980).

According to this study and results reported by Gersberg *et al.* (1989), Yaya *et al.* (1993), Vinluan (1996), and Chendorain *et al.* (1998) PRD1 may be a better virus model for human virus than MS2 in wetland systems.

The East Polishing Basin at the Sweetwater Wetland showed a lower effectiveness in removing PRD1; however, a higher removal efficiency might be achieved by reducing inflow in order to promote a more ideal plug flow conditions and a greater contact time.

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DISCLAIMER

The American Water Works Association Research Foundation and the other agencies listed above not had opportunity to review and comment on this paper, therefore, none of these agencies necessarily endorse the findings presented here.

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Table 1. Flow cross-sectional area at different distances from the inlet.

	Distance from the inlet (m)												
	20	38	64	85	123	150	168	187	206	240	270	293	315
Ac *	27.93	25.86	36	72.86	29.74	25.86	66.85	47.70	17.45	21.33	80.37	65.73	21.8
Avg*													41.47

*Ac, cross sectional area (m^2); Avg, average cross sectional area (m^2)

Table 2. Daily hydraulic loading rate applied on the east polishing basin during the study.

Hydraulic Load Rate	Inlet	Outlet
Average (mm day^{-1})	88.1	76.4
Standard deviation (mm day^{-1})	5.6	5.2
Variation coefficient (%)	6.4	6.8

Table 3. Parameters for mathematical fitting of the convection-dispersion equation (CDE) to experimental breakthrough curves obtained at the sampling sites 4 and 5.

	Input						Output				
Br^-	**Ac**	**M**	**D**	**V**	***k***	**x**	**Ac**	**M**	**D**	**V**	***k***
Outlet	41.5	67				328.88			1600	57.5	
Island		67				182	40.4		1879	62.6	
PRD1											
Outlet	41.5		1600	57.5		328.88		7.1 x 10^{13}			0.298
Island	40.4	7.1 x 10^{13}	1879	62.6	0.298	182					

Ac (m^2); **M** (kg for Br^- and pfu's for PRD1); **D** (m^2 day^{-1}); **V** (m day^{-1}), ***k*** (day^{-1}); **x** (m).

Table 4. First moments calculated from experimental and the predicted concentration-time distribution curves at the sampling sites 4 and 5.

Site	First moment (Days)			
	Experimental distribution		Predicted distribution	
	Br^-	**PRD1**	**Br^-**	**PRD1**
Outlet	7.3	5.3	6.8	5.26
Island	3.92	3	3.84	3

Table 5.- Mass recovery in tracer studies conducted in treatment wetlands.

Wetland	Surface area m^2	Tracer	Recovery (%)	Infiltration	Source
[a]SSF	500	Lithium	15-100	d	Netter (1997)
		Uranin	48-50		
		Eosin	6-7		
		Bromide	47-99		
SSF	100	[c]EAR	86	d	Bowmer (1987)
SSF	19	Bromide	87-94	d	Tanner *et al.* (1998)
SF and-SSF	300	Lithium	96	d	King *et al.* (1997)
[b]SF	1050	Bromide	75 ±11	negligible	Chendorain *et al.* (1998)
SF	$2x\ 10^4$	Lithium	106 ± 32	negligible	Kadlec (1994)

[a]SSF, subsurface flow; [b]SF, surface flow; [c]EAR, Eriochrome Acid Red A

[d]lined with some type of impermeable material.

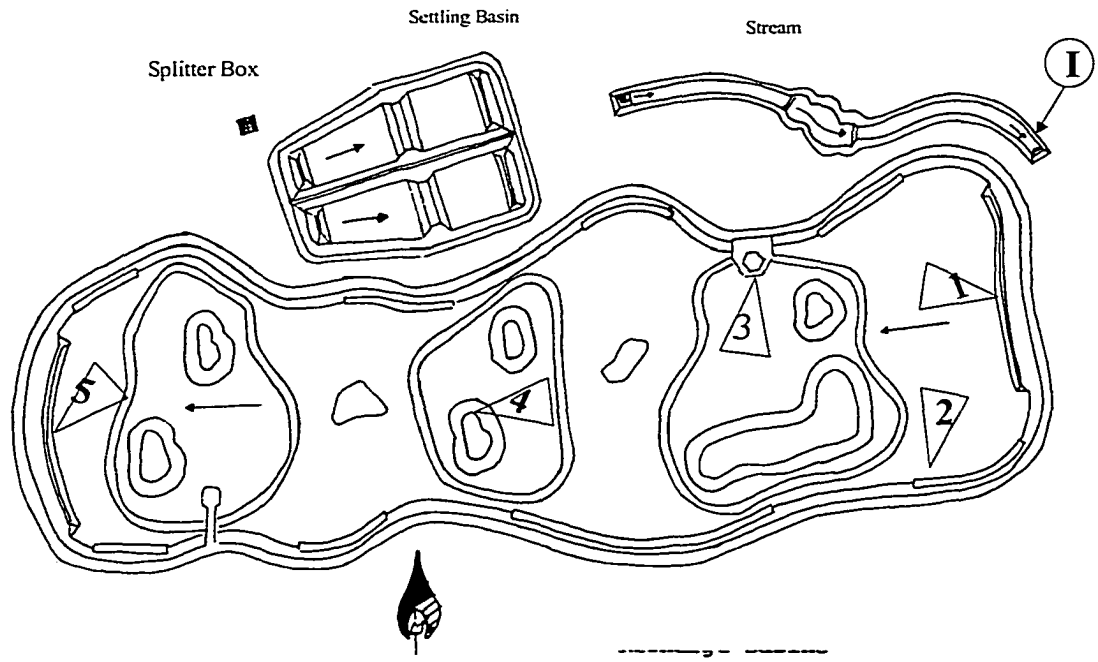

Figure 1. Schematic representation of the east polishing basin showing the injection (I) and sampling sites at the inlet (1), lysimeter area (2), first (3) and second (4) open water zones, and outlet (5) of the system.

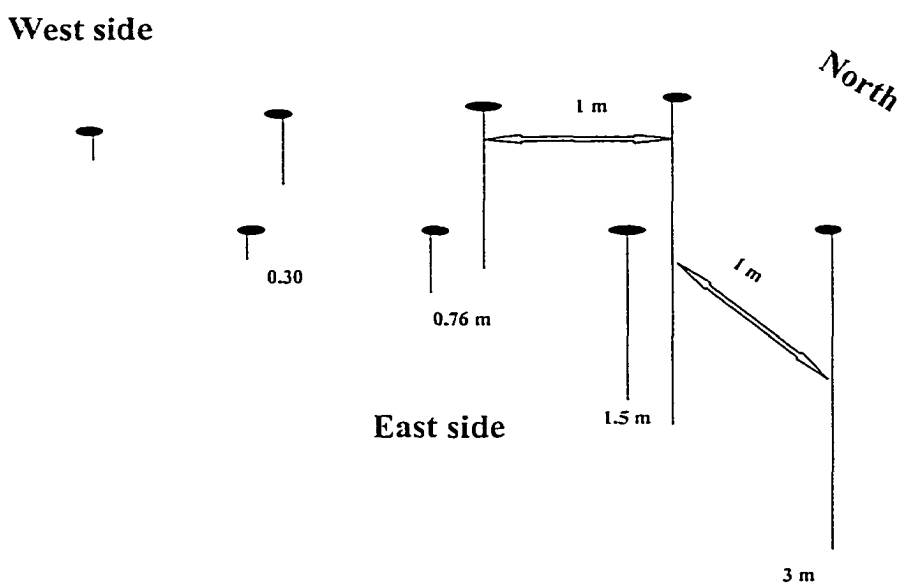

Figure 2. Schematic representation of the East and West lysimeter arrays depicting installation depths (0.3, 0.76, 1.5, and 3 m) and separation between adjacent lysimeters (1 m).

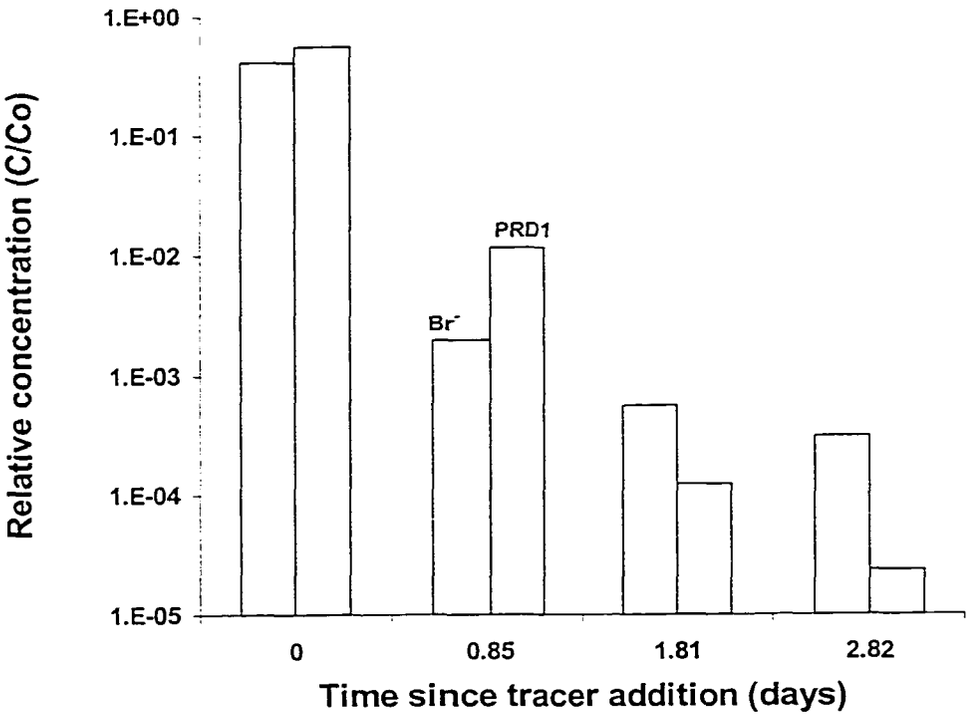

Figure 3. Relative concentration of PRD1 and Br^- at the sampling site 1 given by the ratio between actual (C) and input (Co) concentration.

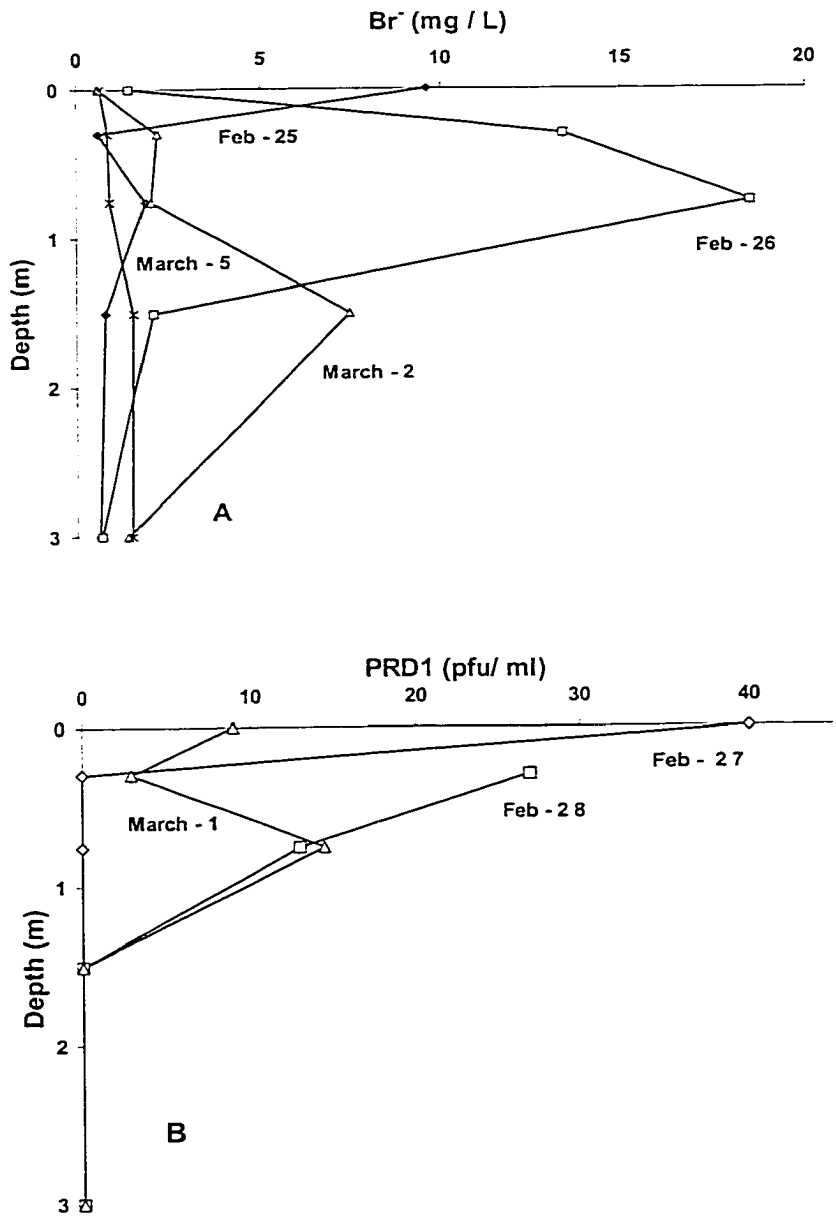

Figure 4. Concentration profiles of Br- (A) and PRD1 (B) at the East lysimeter array, sampling site 2.

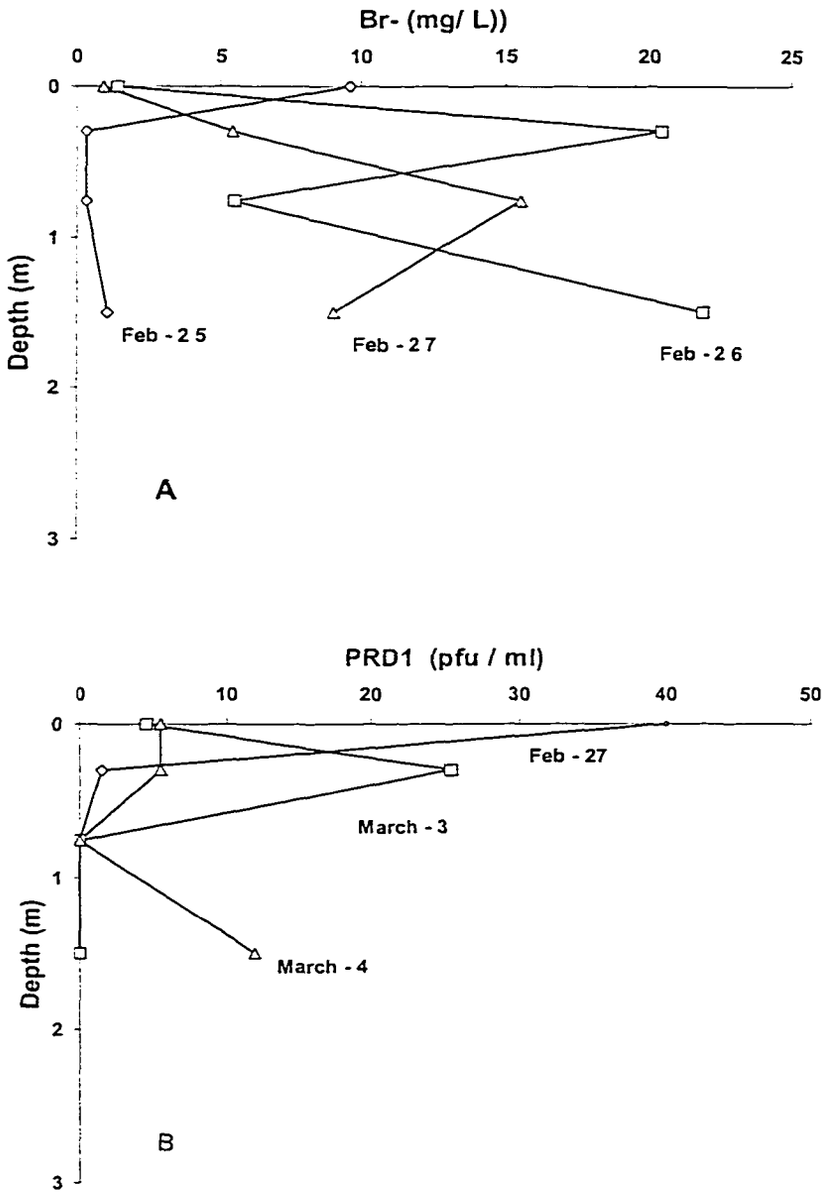

Figure 5. Concentration profiles of Br- (A) and PRD1 (B) at the West lisymeter array, sampling site 2.

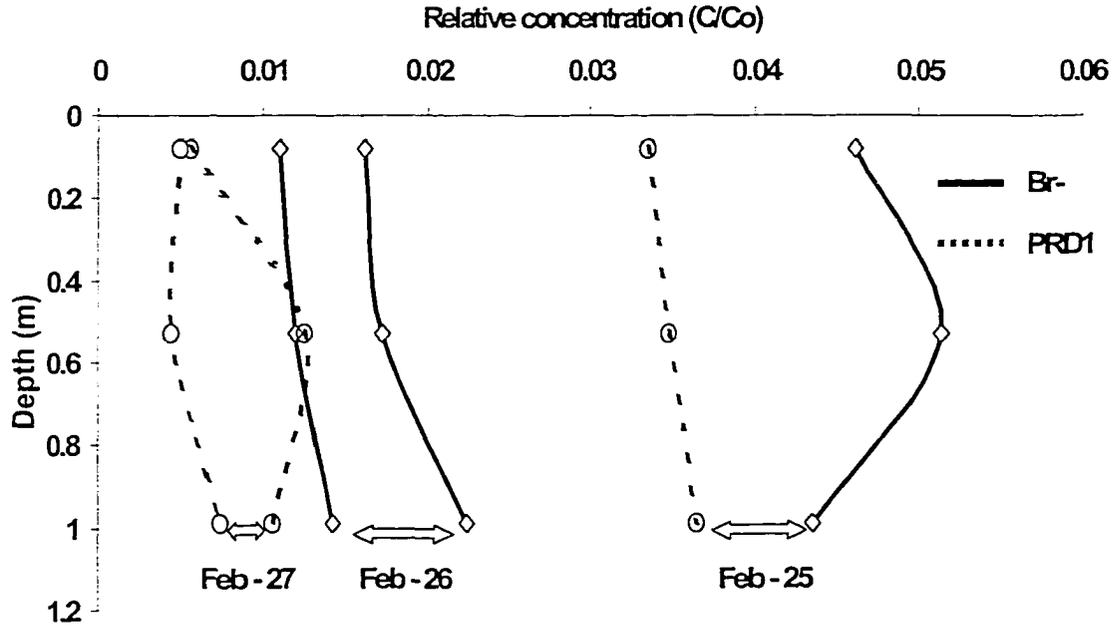

Figure 6. Relative concentration of Br- and PRD1 expressed by the ratio between actual (C) and input (Co) concentration observed at three different depths within the first open water zone, site 3.

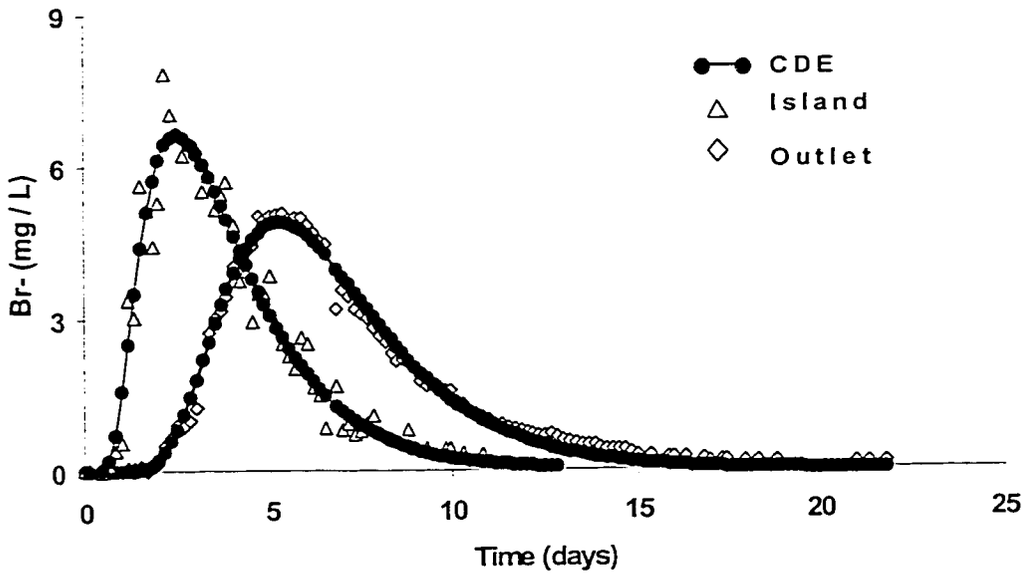

Figure 7. Concentration-time distribution of Br- at sites 4 and 5.

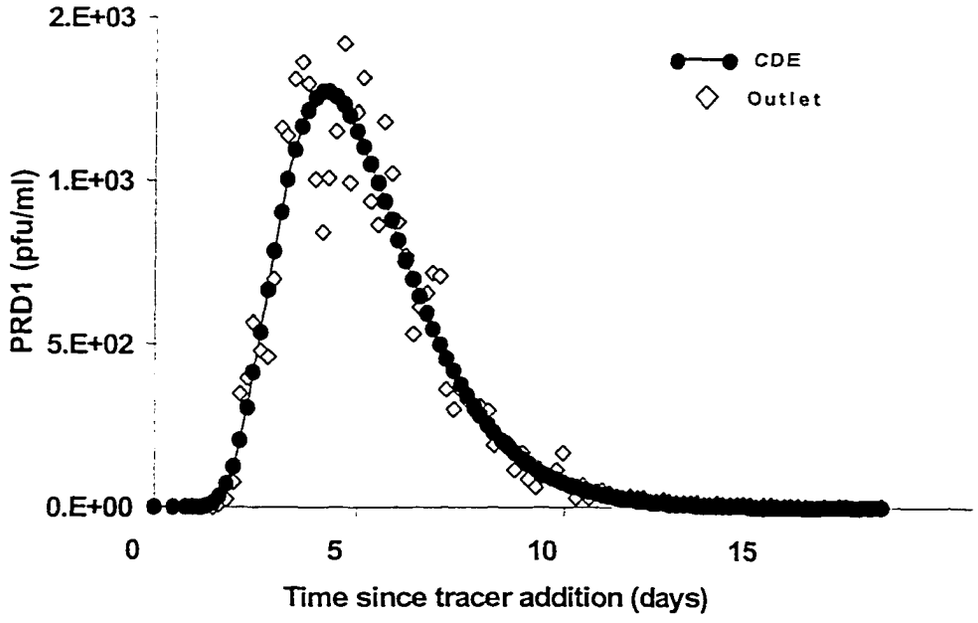

Figure 8. Concentration-time distribution of PRD1 at the sampling site 5.

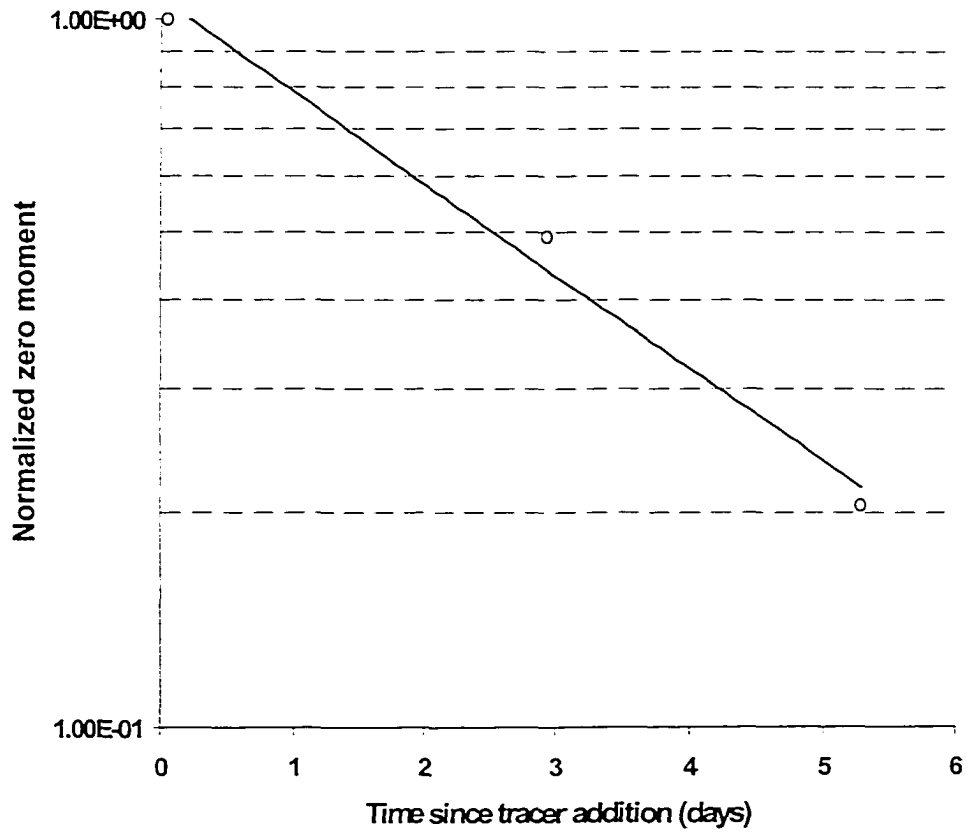

Figure 9. Lineal regression of the normalized zero moment and time at the center of mass of the pulse added into the system and breakthrough curves at sites 4 and 5.

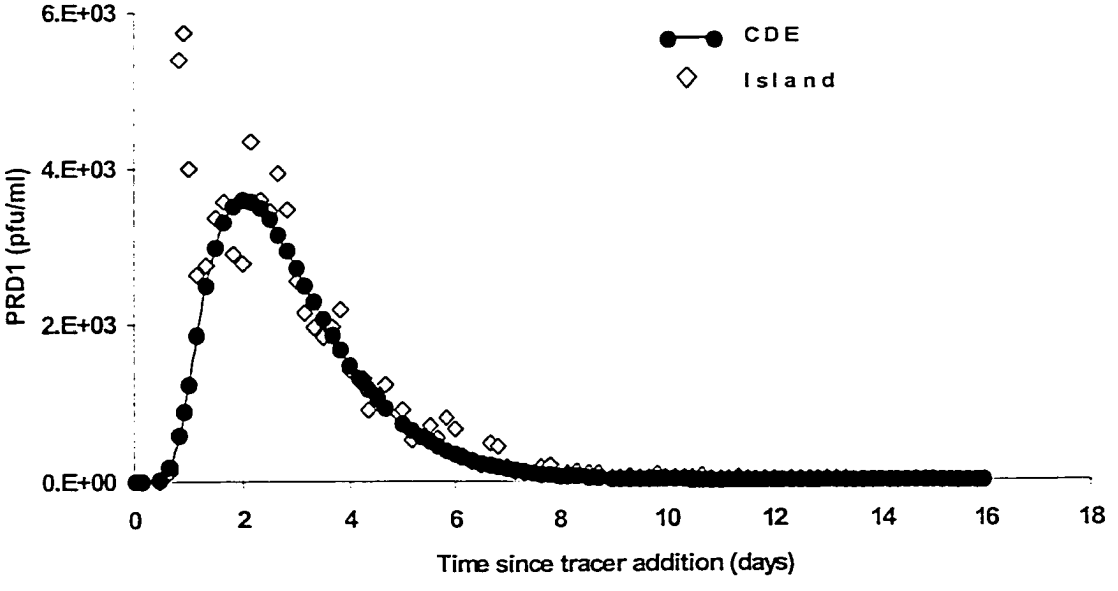

Figure 10. Observed and predicted concentration-time distribution of PRD1 at the sampling site 4.

APPENDIX B

VIRUS REMOVAL FROM WASTEWATER IN A MULTI-SPECIES SUBSURFACE-FLOW CONSTRUCTED WETLAND

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Virus Removal from Wastewater in a Multi-Species Subsurface-Flow Constructed Wetland

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Abstract.- Virus removal was studied in a multi-species subsurface flow constructed wetland. Tracer studies and a virus survival test were conducted using Bromide (Br^-) and bacteriophage PRD1. These were added simultaneously into the wetland which received unchlorinated secondary effluent, pre-treated by a duckweed pond. A high degree of plug flow existed in the hydrodynamic performance of the, 6 year old, gravel bed during the study. Most of the PRD1 was recovered during the first four days of sampling; however, PRD1 concentration did not reached the background concentration at the end of the study. The average bacteriophage removal was 98.8 percent whereas Br^- mass recovery was 75 percent in the spring tracer study. The removal rate of PRD1 was estimated to be -1.17 day^{-1}; in contrast, its inactivation rate *in situ* for a 12.4 day-period was -0.16 day^{-1}. Apparently, virus removal is governed by an initial irreversible attachment followed by a comparatively long inactivation period. The findings of this study suggest that subsurface flow wetland can remove about 99 percent of the virus load.

INTRODUCTION

Water is the principal route for transmission of disease-causing viruses that leads to significant morbidity and mortality worldwide. Once released into the environment by infected individuals, viruses can contaminate surface waters and infect, directly or indirectly, susceptible persons, causing diseases such as diarrhea, fever, poliomyelitis, gastroenteritis, hepatitis, meningitis, and paralysis (Bitton, 1994; Rusin *et al.*, 2000).

Subsurface flow wetlands represent a low-cost emerging technology that mimics natural occurring processes to treat wastewater. These constructed ecosystems can potentially reduce the wastewater virus load by filtration of influent water through vegetated gravel-filled beds. Gersberg *et al.* (1987) found that 99 percent of indigenous F-specific bacteriophage was removed by a 18.5 m x 3.5 m x 0.76 m subsurface flow wetland operated at a hydraulic loading rate of 5 cm day^{-1}. The authors suggested that attachment promoted by filtration was the primary removal mechanism. Vinluan (1996) conducted a removal and survival experiment of indicator microorganisms in a multi-species surface flow wetland. She found that removal of 90 percent of bacteriophages MS-2, PRD1, and indigenous phage required 3.67, 13.38 and 2.05 days, respectively. Virus decay showed a slow reduction along the wetland with an increasing removal rate in the last ten meters of the system. Similarly, a 6 month-study reported by Karpiscak *et*

al. (1995) indicated that pathogenic viruses can be removed significantly from secondary wastewater effluent in vegetated gravel beds.

Studies of virus removal in subsurface flow wetlands are scarce. Most studies have analyzed virus removal as the ratio of inlet and outlet concentration associated to calculated detention time. A tracer test is the best approach to determine the actual detention time and the amount of mixing within the system. Additionally, it may reveal the effect of mixing conditions on virus removal. A tracer study conducted simultaneously with a survival experiment could establish the contribution of virus inactivation over the removal performance of the system. Gersberg *et al.* (1987) have suggested that virus removal is mostly related to a filtration process in a subsurface flow wetland. This paper reports removal of bacteriophage PRD1 and the hydrodynamic performance of a multi-species subsurface flow wetland, analyzed by tracer studies; as well as, the results of a survival test in situ conducted to determine the inactivation behavior of PRD1.

MATERIALS AND METHODS

Bacteriophage PRD1

Bacteriophage PRD1 is a *ds*DNA virion, 62 nm in size (Olsen *et al.*,1974), and isoelectric between pH of 3 and 4 (Loveland *et al.*, 1996). It has a protein capsid that

surrounds a lipid-protein membrane similar to the human rotaviruses (Caldentey *et al.*,1990). Several researchers have used it as virus model in soil and aquifer studies (Powelson and Gerba, 1994; Schijven *et al.*, 1999; Ryan *et al.*,1999) since its size is similar to rotaviruses and adenoviruses (Rusin *et al.*, 2000).

Production and Enumeration of PRD1

PRD1 stocks were grown by the agar overlay method (Adams, 1959) and harvested from the agar surface by addition of 6 ml of Tris buffered saline, stock solution [Trizma base, Sigma Chemical Co., St. Louis MO (63.2 g; NaCl, 163.6 g; KCl, 7.46 g; and 1.13 g Na_2HPO_4 anhydrous) dissolved in 1,600 ml of distilled water] diluted 1:10, per petri dish. After 3 h supernatant was collected in a 250-ml bottle, centrifuged at 22,095 **x g** for 10 min, and filtrated through a 0.45 μm pore size cellulose acetate membrane filters (Costar, Cambridge, MA).

PRD1 was assayed by the double layer agar method described by Adams (1959) using Trypticase Soy Agar (TSA, Difco, Detroit, MI). A 1-ml aliquot from a *Salmonella typhimurium* culture, previously incubated at 37 °C for 24 h in Trypticase Soy Broth (TSB; Difco, Detroit, MI) was combined with 4 ml of molten agar (TSA, Trypticase Soy Agar; Difco, Detroit, MI) and 1 ml of sample or sample dilutions added. The agar was then poured onto a layer of TSA and incubated at 37 °C for 18 h in order to enumerate the PRD1.

Study Site

The study site (Figure 1) was a subsurface-flow wetland at the Constructed Ecosystem Research Facility (CERF) in Tucson, AZ. The individual plastic lined cells are trapezoidal in cross-section and measure 8.2 m wide (W) on top, 61 m long (L), and 0.9 m in depth (Karpiscak *et al.*, 1994). The volume available for water storage before planting was calculated to be 153 m^3 estimating an initial 40 percent porosity (θ). On the bottom of the trench, a layer of 6 to 9 cm of gravel is overlay by 15 to 30 cm of cobbles. Subsequent gravel additions filled the basin up to 0.9 m of depth (Karpiscak *et al.*, 1993). At the influent end, a 4 m-length section, approximately, was filled with 0.9 m of cobbles over a thin layer of gravel. A pipe with several manually-controlled openings introduced unchlorinated secondary effluent from the Roger Road Municipal Wastewater Treatment Facility, previously treated in a duckweed (*Lemma gibba*) pond at CERF. At the outlet, water overflowed into a vertical stand pipe (0.19 m in diameter) to leave the system. The emergent vegetation in the gravel-filter consists of cottonwood (*Populus fremontii*), black willow (*Salix nigra*), coyote willow (*Salix exigua*), sycamore (*Platanus wrightii*), desert willow (*Chilopsis linearis*), seep willow (*Baccharis glutinosa*), bulrush (*Scirpus olneyi*), cattail (*Typha domingensis*), yerba mansa (*Anemopsis californica*), and giant reed (*Arundo donax*).

Tracer Addition and Sampling

Summer 1997

On July 1997, 50 ml of PRD1 stock solution at a concentration of 1.8×10^{11} pfu ml^{-1} and 150 g of NaBr were mixed in 20 L of influent wastewater. This mixture was then added to the wastewater flowing into the wetland for a 2 h-period. Both tracers were monitored for 10 days by collecting samples every 4 hours at the outlet. Additional samples were taken from PVC pipes that had holes drilled in their side walls. These pipes were placed vertically to a depth of 0.45 m at a distance of 20 and 40 m from the inlet. At the end of the study, during the last three days, 3, 2 and finally one sample per day were collected at each sampling site. An specific-electrode (ATI Orion Model 9435, Boston, MA.) was used to determine Br^- concentration in the collected samples. At the inlet, flow rates were monitored continuously by a totalizer flow meter. At the outlet, they were measured every 4 h. A volume of 2.86×10^{-3} m^3 was labeled on the internal well of the exit pipe and then its bottom neck were blocked with an adjustable sphere-shape rubber. The filling time of this volume was obtained and the flow rate calculated by the ratio between the labeled volume and filling time.

Spring 2000

On March 26, a second tracer study was performed. Two automatic samplers were placed at 36 m (Isco, Model 3700; Lincoln, NE) from the inlet and at the outlet of the wetland (American Sigma, Model 702; New York, NY); later, both samplers were programed to take a sample every 3 h for 12 days. At 36 m from the inlet, the sampler collected samples from a previously installed steel pipe with a 0.025 m-diameter and a length of 0.95 m. Holes were drilled into the pipe wall to permit sampling at a depth of 0.6 m. The tracer suspension consisted of a volume of 20 L of influent water as well as 838 g of NaBr, and 100 ml of bacteriophage PRD1 stock suspension at a concentration of 1.2×10^{11} pfu ml^{-1}. It was added at the inlet for a 1.5 h-period. The inflow rate was 0.9 m^3 h^{-1} therefore the input concentration (Co) for each tracer was 468 mg L^{-1} of Br^- and 9.3×10^6 pfu ml^{-1} of PRD1.

Flow rates were measured as in the summer tracer test of 1997. At the outlet, the monitoring frequency was changed to three times per day (early morning, afternoon, and evening). Each day, cleaned and disinfected 1 L-bottles were placed in the sampler and crushed ice was added to keep the collected samples cooled. The samples were kept in ice, transported to the laboratory at the University of Arizona, and assayed the same day of collection. Bromide and bacteriophage were analyzed by using Ion-Chromatography (Dionex 2020i; Sunnyvale, CA) and the double agar layer assay, respectively.

Spring 2000: Survival test

Pieces of approximately 10 cm of dialysis tube (Spectra/pro 4, Houston, TX) were used for incubating PRD1 *in situ* at 36 and 54 m from the inlet. The membrane of this tube has a molecular weight cut off between 12000-14000 which is defined as the solute molecular weight that is 90 percent prevented from permeating through the dialysis tube membrane. The cut off is around 3 orders of magnitude smaller than the molecular weight of the DNA (24×10^6; Olsen *et al.*, 1974) of PRD1; thus this virus is unable to pass through the membrane. However, it is large enough to allow exchange of different types of hydrocarbons, alcohols, ketones, esters, oxides, acids, alkalis, and solvents as well as metal salts.

On same day that the tracers were added, 24 dialysis bags were prepared by placing 10 cm-pieces of dialysis tube in distilled water to soften them and tying off one end of each bag. At the same time, a solution of PRD1 at a concentration of 2.47×10^7 pfu ml^{-1} was prepared by diluting one ml of the PRD1 stock suspension in influent wastewater. From this solution, 2 ml-aliquots were poured into the dialysis bags which were closed by tying their upper end.

A set of 12 bags was placed at both 36 and 54 m from the inlet into a small 0.2 m-deep well. From each site, a dialysis bag was collected daily and the water temperature measured in the wells, except on March 5. The collected bags were put into 50 cm-

centrifuge tubes, placed in ice and taken to the laboratory. The samples were analyzed the same day.

Parameters Estimation

Mass recovery was calculated by the following expression:

$$M_o = \alpha Q \int_0^{\infty} C(t)\, dt$$

where Mo is the tracer mass recovery (Kg), α is a conversion factor (1.44), Q is the average flow rate at the outlet during the experiment (m^3 min^{-1}), t is time (days) and C is the outlet tracer concentration (mg L^{-1}). Mass recovery can be compared with the added mass as a criterion of evaluation. Discrepancies between both amounts may be an indicator of tracer degradation or adsorption, inaccurate measurements of flow rates or tracer concentrations, or low frequency of sampling. Detention time was determined by integration of the detention time distribution by the following expression:

$$t_d = \frac{\int_0^{\infty} t\, C(t)\, dt}{\int_0^{\infty} C(t)\, dt}$$

where t_d is detention time of the system (days) and t is time (days). The arriving time (t_{10}) was evaluated by the time at 10 percent of Br^- mass recovery at the outlet.

The variance of the detention time distribution with respect to the detention time can be calculated by:

$$\sigma^2 = \frac{\int_0^\infty (t - t_d)^2 C(t)\,dt}{\int_0^\infty C(t)\,dt}$$

where σ^2 is the variance of the tracer respond breakthrough curve (days^2).

Dimensionless variance (σ_θ^2) is an indicator of the extension of back-mixing and it is determined by the ratio of the variance and the square of the tracer detention time:

$$\sigma_\theta^{\,2} = \frac{\sigma^2}{t_d^{\,2}}$$

Relative breakthrough curve is the ratio of the normalized concentration distributions of two tracers (Harvey and Garabedian, 1991) that can be used to evaluate relatively removal or transformation of a non-conservative tracer. It is estimated by the following relationship:

$$RB = \left(\frac{\int_{t_o}^{t} \frac{[C_{PRD-1}]_t}{[C_{PRD-1}]_{t_o}} dt}{\int_{t_o}^{t} \frac{[C_{Br^-}]_t}{[C_{Br^-}]_{t_o}} dt} \right) x100$$

where $[C_{PRD\text{-}1}]_{to}$ and $[C_{Br^-}]_{to}$ is the PRD-1 and Br^- pulse concentration during the tracer injection. Relative attenuation results from subtracting the relative breakthrough curve from one hundred (RA= 100-RB).

RESULTS AND DISCUSSION

Flow rates and evapotranspiration

Differences in flow rates at both inlet and outlet for the summer and spring tracer experiments are shown in Table 1. During the summer experiment, evapotranspiration was estimated to be 42 percent based on influent and outflow average flow rates. Some periods of non-flow at the outlet were commonly observed in the afternoons during Summer. Forty- two percent is probably an underestimation of the actual value because the influent water was insufficient to satisfy losses the high evapotranspiration losses during the non-flow periods. Thus during this part of the day, water losses were more than 100 percent. However, given the impossibility of measuring the actual losses an estimation of 100 percent was used.

The spring tracer test showed a small variation of flow rates at both ends of the wetland, the flow rate was considerably more constant than during the summer experiment. However, flooded conditions at the outlet, mostly in the mornings, altered

the schedule of flow rate monitoring. It was only possible to take the morning, afternoon, and evening flow rate measurements on five of 12 days. Loses by evapotranspiration were determined to be 26 percent of average influent flow.

Conservative Tracer (Br^-)

Summer 1997

A non-typical breakthrough curve at 40 and 56 m from the inlet were observed during the summer of 1997 (Figure 2). This behavior may be explained by the small Br^- pulse, introduced into the system, that did not increased Br^- concentrations enough in the wetland water to diminish the effects of interfering ions, probably, reconcentrated because of evapotranspirational losses.

At 20 m form the inlet, a sharp peak in concentration was seen after 1.3 days following introduction of the tracers. Thereafter, a long tailing effect was observed. Apparently, the first moment of the breakthrough curve up to this point was 2.2 days. At the outlet, Br^- mass recovery was 140 percent and a detention time of 5.6 days was calculated after integration of the breakthrough curve.

Spring 2000

After extrapolation of the outlet breakthrough curve of Br^- to the background concentration, mass recovery was estimated to be about 75 percent of the initial injected

mass. This underestimated mass recovery of Br^- may be the result of limited frequency of monitoring during the flooded periods. Bromide started to arrive at the outlet 1.9 days, after its addition into the wetland, reaching the background concentration 17.6 days, later, on the extrapolated breakthrough curve. The actual and nominal detention time (t_n) were estimated to be 5.5 and 8.1 days, respectively. The nominal detention time was calculated with the average of inlet and outlet flow rate and the effective volume of the system.

According to the ratio between the actual and nominal detention time, the gravel-bed porosity appeared to have decreased about 33 percent from the original effective volume after 6 years of operation. This is in agreement with findings by Blazejewski and Murat-Blazejewska (1997) and Tanner *et al.* (1998) who recently noted that gravel-bed porosity decreases by accumulation of organic and inorganic matter from wastewater and vegetation, reducing the hydraulic conductivity and detention time in the wetland.

Sanford *et al.* (1995) observed a consistent reduction over time of the hydraulic permeability in four reed-vegetated subsurface flow beds. The high initial hydraulic conductivity of coarse-gravel and pea-gravel matrix seemed to prevent surface flow. However, in a sand-and-gravel bed the hydraulic conductivity decreased 87 percent after one year of leachate addition causing surface flow and a prominent reduction of treatment efficiency. Tanner *et al.* (1998) reported that the original void space of a 9.5 m

x 2 m x 0.4 m vegetated gravel bed operating at an organic loading rate of 5.8 g m^{-2} day^{-1} was reduced 50 percent after 5 years of operation. Accumulation of organic and inorganic material primarily take places near the inlet of the wetland system. Kadlec and Watson (1993) noticed high buildup of solids in the first 100 m of a gravel-bed wetland in Benton, KY, and Tanner and Sukias (1995) reported a significantly higher accumulation of organic material in planted wetlands than in unplanted ones. Preferentially, the accumulation occurred near the inlet and the superficial 10 cm of the gravel bed.

Mixing of Br^-

An ideal reactor would function under either plug flow or completely mixed conditions with a σ^2_θ value of zero or one, respectively (Kadlec and Knight, 1996). Ideally, back mixing becomes infinite in completely-mixed conditions and fractions of entering water reach the outlet of the system with zero detention time (Teefy, 1996). Ideal plug flow is opposite in behavior; the solute travels along the filter without back-mixing residing in the basin for a period equal to the nominal detention time.

Asymmetry and large dispersion have been common characteristics of the breakthrough curves from tracer studies conducted in subsurface-flow wetlands. (Bowmer, 1987; Netter, 1994.; King *et al.*, 1997; Tanner *et al.*, 1998). This hydrodynamic performance is expected for water flow through a medium with stagnant zones that promote solute retention as the main cloud moves through the system.

Eventually, the solute is released slowly back into the convective zones promoting back-mixing and making its breakthrough curve asymmetric.

A negative relationship of σ^2_θ with dimensionless arriving time (t_{10}/t_d) and effective porosity from different experimental sites is illustrated in Table 2. Even though, the sites are different in dimensions, vegetation, and hydraulic load mixing parameter, t_{10}/t_d and σ^2_θ, values are comparable. Values of σ^2_θ indicate that subsurface flow wetlands are more closely related to plug flow than to full-mixed conditions. However, values such as 0.3 and 0.46 of σ^2_θ indicate a higher degree of full-mixing conditions, an early arrive of the breakthrough curve (Figure 3), and probably a reduction of the treatment efficiency. The tendency of hydrodynamic performance of a vegetated gravel-filled bed to plug flow behavior is a result of characteristics such as: a) relatively large L/W ratio that promote enough time for a uniform distribution of the entering water across the sectional cross area of the system (Polprasert and Bhattarai, 1985); b) satisfactory effective volume, the larger dead volume the higher dispersion; consequently, a closer condition to full mixed performance; c) even distribution of entering water at the inlet to reduce the time for uniform distribution across the water front (Perrson *et al.*, 1998), and d) appropriate hydraulic load of operation in contrast with the hydraulic conductivity of the solid matrix to avoid surface flow.

PRD1 Removal and Inactivation

The average background concentration of phage infecting PRD1 host bacteria in the influent water was 119 pfu ml^{-1} fluctuating in a range of 104 to 150 pfu ml^{-1} for both summer and spring experiments. Detention time distribution for Br^- and PRD1 is noticeably different (Figure 4). Because bacteriophage removal reduces its original load through the rooted-gravel filter, the relative virus attenuation was estimated to be 97.6 percent.

Bacteriophage PRD1 was mostly recovered during the early stage of the Br^- breakthrough curve. The dashed line in Figure 4 delimits 10 percent of Br^- mass recovery at 3.2 days after the tracer injection; at this time, 65 percent of total recovered PRD1 already had been recovered. Presumably, PRD1 traveled through the shortest paths of the vegetated gravel bed to reach the outlet of the system. The rooted filter design suggest that the cobble layer is the wetland fraction of shortest detention time where PRD1 was removed inefficiently. Apparently, PRD1 is removed by adsorption on the wetland substratum where the virus had a longer contact period.

Dialysis bag suspension incubated in the gravel bed can be considered to have a similar thermal, chemical, and biological environment to the wetland aqueous phase to simulate and evaluate virus survival in the wetland during the tracer experiment (O'Brien and Newman 1977; Gersberg et al., 1987).

Wetland water temperature averaged 16.3 °C. This temperature is in the range (10-25 °C) where PRD1 inactivation rates from -0.01 to -0.18 day^{-1} in secondary effluent have been reported (Schijven and Hassanizadeh, 2000). Figure 5 shows the inactivation of PRD1 at 36 and 54 m from the inlet. At both sites, PRD1 behavior was similar with an estimated inactivation rate of -0.16 day^{-1} (0.70 R^2). This result was comparable to the inactivation rate reported by Vinluan (1996) in a survival test conducted in a multi-species surface flow wetland under shadow conditions at CERF if the inactivation rate reported by the author in Log_{10} day^{-1} is transformed to units per day^{-1} (2.3 x Log_{10} day^{-1}). On the other hand, the removal rate during the spring experiment was -1.17 day^{-1} (0.99 R^2) calculated from the normalized zero moment of injected pulse of PRD1, the breakthrough curves at 36 and 54 m, and the time at its center of mass. Gersberg *et al.* (1987) found a significant difference of MS2 removal in a vegetated gravel bed and its inactivation *in situ* by using dialysis bags. Removal rates were estimated to be -0.48 ± 0.19 and -1.14 ± 0.09 day^{-1} under stagnant and flowing conditions, respectively; whereas, -0.12 day^{-1} was the inactivation rate in the dialysis bags. Somewhat, these results suggest a similar or longer persistency in subsurface flow wetlands of MS2 than PRD1. Which contradicts results reported by Yahya *et al.* (1993) about a longer persistency of PRD1 in groundwater. Apparently, in the current study, virus inactivation and rhizosphere effects were not the primary removal mechanisms. Studies conducted by Gesberg *et al.* (1987)

suggest that a mechanism responsible of irreversible colloidal collection by solid surfaces during the filtration enhanced virus adsorption. Additionally, virus attachment on suspended solids, eventually removed from wastewater by sedimentation or adsorption, might have played an important role on virus removal from wastewater during the first 4.2 days of the tracer study conducted in CERF.

During the summer experiment of 1999, attenuation of PRD1 was characterized apparently by two processes with very different removal rates (Figure 5). Near the influent, PRD1 removal was 96.3 percent but after 20 m, a more gradual reduction was observed, reaching 99.38 percent at the end of the wetland. This apparently non-first order PRD1 removal may result from the sampling pipe location, the heterogeneous distribution of the tracer between the rooted-gravel bed layers and the high variation of the flow rates during the study. In contrast, in the spring experiment of 2000, PRD1 reduction showed an apparent first order process; probably, as a consequence of more even flow rate conditions and a better location of the sampling pipe, intersecting the cobble layer. The concentration of PRD1 in the summer experiment was 36 percent of the spring test, probably, because of the greater amount added in the former test. However, the differences in the removal efficiency between both experiments was negligible, PRD1 removal in the second experiment was only 1.12 percent less than in the summer test.

Only few studies have been conducted to determine the ability of constructed wetlands to remove animal enteroviruses. Results reported by Thurston (1997) showed that they were reduced by over 83 percent while Karpiscak *et al.* (1995) found a 98 percent-reduction. Both experiments were conducted in subsurface flow wetlands whit a five day detention time. Scheuerman, *et al.* (1997) compared removal rates for indigenous coliphage and enteroviruses in forested wetlands. The decay rates of indigenous coliphage were slower than the observed rates for enteroviruses. Similar results were found by Gersberg *et al.* (1987) comparing decay rates of human polioviruses to an indigenous FRNA bacteriophage in vegetated gravel beds receiving municipal wastewater. The indigenous phage was reduced at slower rates than the human virus and MS2.

PRD1 Flushing

Schijven and Hassanizadeh (2000) indicated that laboratory and field evidence suggests that virus removal in soils is mainly governed by kinetic processes. Figure 6 shows the long tailing of PRD1 in both the summer of 1997 and the spring of 2000. In these studies, the outflow PRD1 concentration at the end of study was higher than the background concentration observed in the sampling sites before tracer injection. In soil column experiments, Dowd *et al.* (1998) found that low virus concentrations can be

observed for extended periods of time after the virus input had ceased. Schijven *et al.* (1999) found a long tailing of the virus concentration distribution for long periods of sampling in soil tracer studies conducted under field conditions. Apparently, this behavior is produced by virus detachment from the solid phase which is relatively much slower than attachment. Once retained on the surface of collectors, virus inactivation may predominantly occur with a small inactivation rate.

CONCLUSIONS

The detention time of the vegetated-gravel bed estimated from the outlet Br^- breakthrough curve observed in the spring 2000 was 5.5 days. The PRD1 removal and inactivation rate was -1.17 and -0.16 day^{-1}, respectively. Even though, PRD1 recovery during the summer experiment was 36 percent of the spring one, the total removal was similar in both experiments. Climatic conditions appear somewhat irrelevant to PRD1 removal, at least for the summer and spring period in a subsurface flow wetland. Bacteriophage PRD1 was mostly recovered during the early stages of the Br^- breakthrough curve suggesting that the recovered fraction of PRD1 has a low tendency for attachment or that its removal is strongly affected by preferential flow.

In agreement with the inactivation rate obtained in the survival experiment, PRD1 can survive in the wetland for a significant period of time. Approximately, 13.9

days would be required for 90 percent inactivation of the initial amount of bacteriophage. This is similar to results reported by Vinluan (1996) for 90 percent PRD1 reduction in a survival test conducted in surface flow wetland at CERF under shadded conditions. The inactivation rate *in situ* was similar to reported values from others for PRD1 (Vinluan, 1996; Schijven and Hassanizadeh, 2000) and comparatively, this decay rate is almost one order of magnitude less than the removal rate estimated in the spring 2000 tracer test. Presumably, adsorption was the primary mechanism of virus reduction during the first 4.2 days of PRD1 displacement through the rooted-filter. For longer periods, inactivation may be of greater importance. The long tailing of PRD1 breakthrough curve at the outlet of the system suggests that virus detachment from wetland substratum can be a long term source of viruses for water passing through a subsurface flow wetland which appears capable of reducing PRD1 by 99 percent of the initial virus load, if the hydrodynamic conditions of the vegetated gravel bed are close to plug flow behavior.

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DISCLAIMER

The American Water Works Association Research Foundation and the other agencies listed above not had opportunity to review and comment on this paper, therefore, none of these agencies necessarily endorse the findings presented here.

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Yahya, M. T., Galsomies L., Gerba C. P. and Bales R. C. (1993) Survival of bacteriophages MS-2 and PRD-1 in ground water. *Wat. Sci. Tech.* **27**, 409-412.

Table 1.- Flow rates at the inlet and outlet for Summer and Spring tracer experiments

Parameter	Summer 1997		Spring 2000	
	Inlet	Outlet	Inlet	Outlet
Mean ($m^3 h^{-1}$)	0.78	0.45	0.90	0.66
Standard Deviation ($m^3 h^{-1}$)	0.33	0.36	0.11	0.17
Coefficient of variation (%)	42.47	79	11.68	24

Table 2.- Wetland characteristics and Br^- breakthrough curve parameters

Source	Dimensions (m) LxWxH	Vegetation	Hydraulic loading rate (mm day^{-1})	Effective porosity (%) t_d/t_n x 0	Dimensionless arriving time (t_{10}/t_d)	Dimensionless variance (σ_θ^2)
Tanner (1998)	9.5 x 2 x .4	Bulrush[2] Palla[3]	74			
A				17.5	0.35	0.3
B				21.3	0.53	0.18
C				35.4	0.62	0.11
Bavor *et al.* (1988)[1]		Schoenoplectus				0.19
Schierup *et al.* (1990)[1]						0.46 ± 0.03
CERF	61 x 8 x 0.9	*Multi-species*	43	26	0.58	0.18

[1] Cited by Kadlec and Knight 1996

[2] *Schoenoplectus tabernaemontani*

[3] *S. validus*

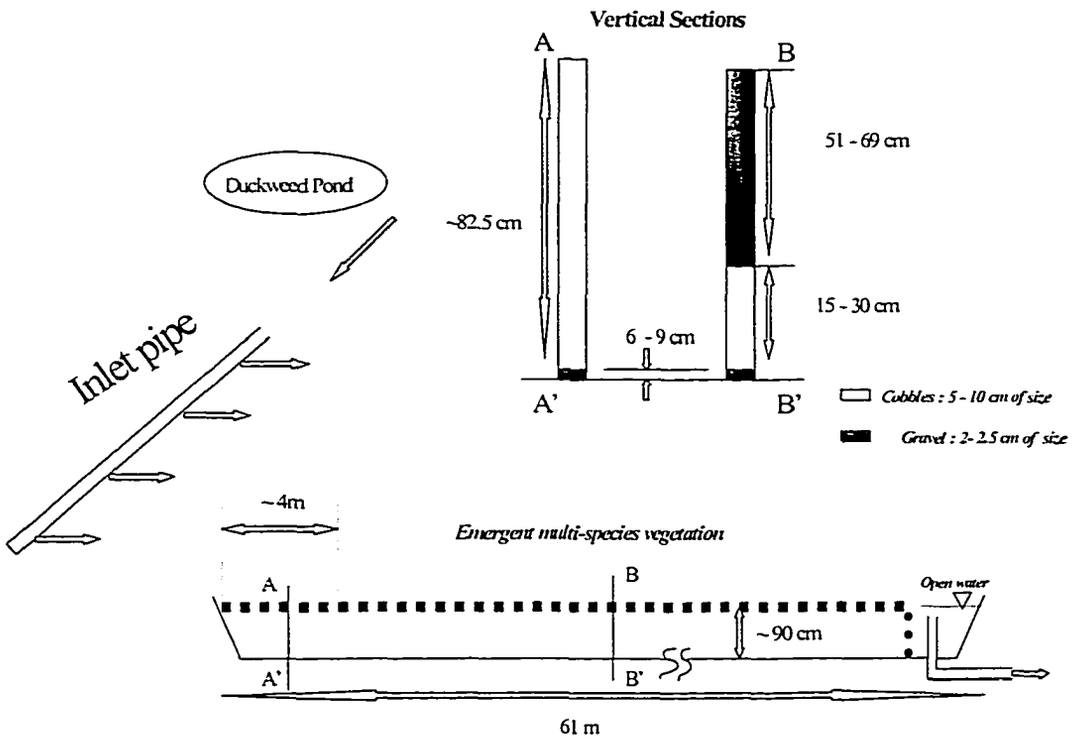

Figure 1. Schematic representation of the vegetated-gravel bed where the tracer study was conducted. The vertical sections (A-A' and B-B') show gravel and cobble layers stratification in the wetland. The influent water is distributed along the inlet by the inlet pipe.

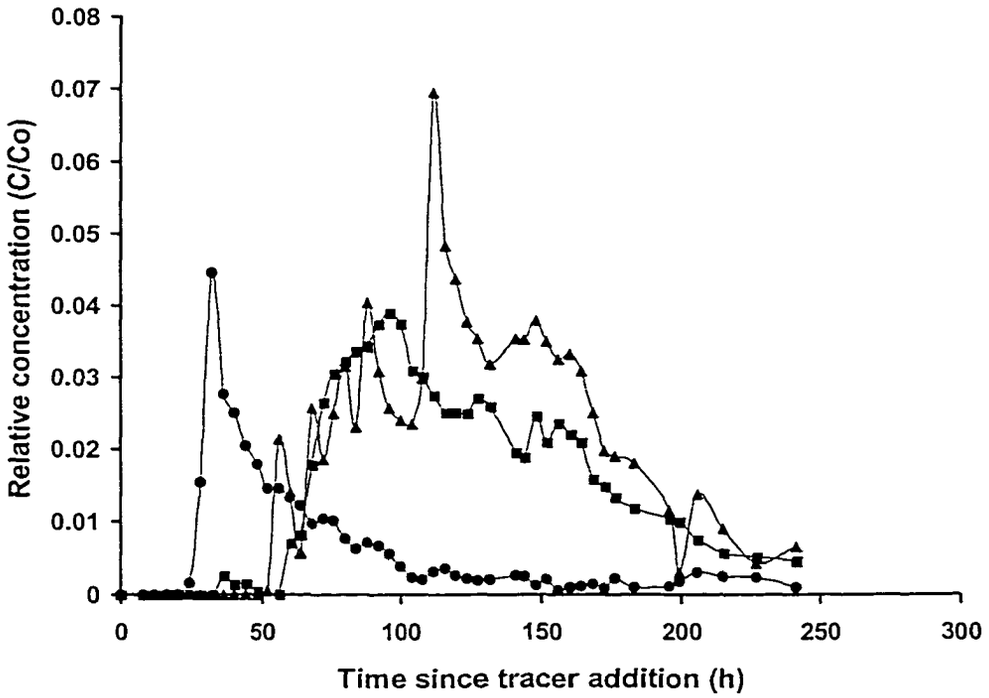

Figure 2. Relative Concentration distribution of Br^- at 20 (●), 40 (■), and 56 m (▲) from the inlet, Summer 1997, expressed by the ratio of the observed (C) and input concentration (Co).

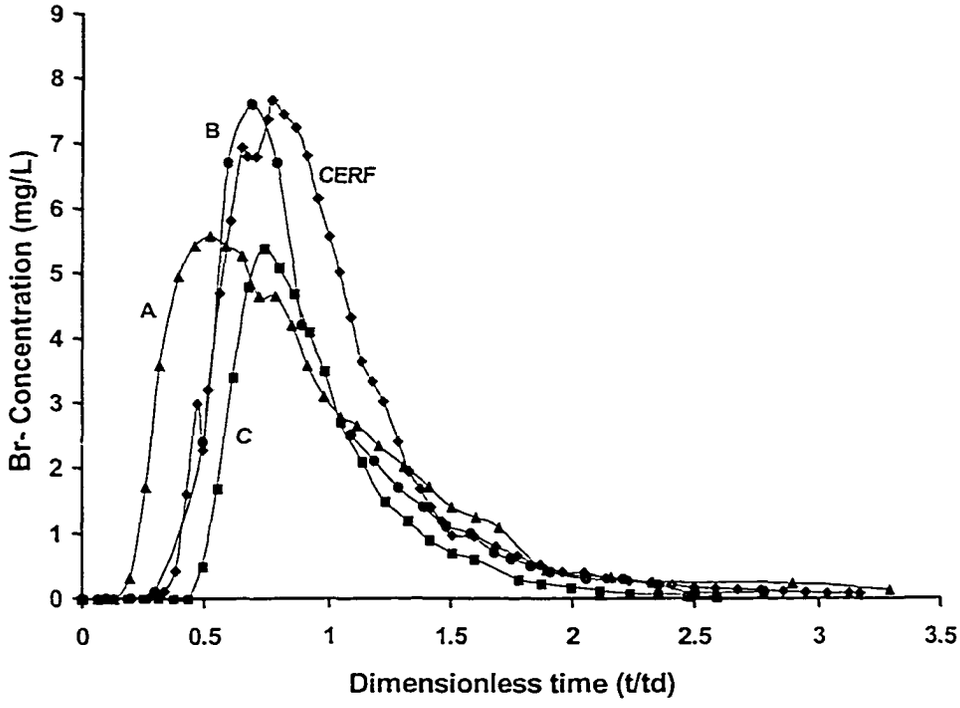

Figure 3. Detention time distribution from CERF and previous reported studies by Tanner *et al.* (1998). Curves A, B, and C were normalized on time with information provided by Tanner (2000).

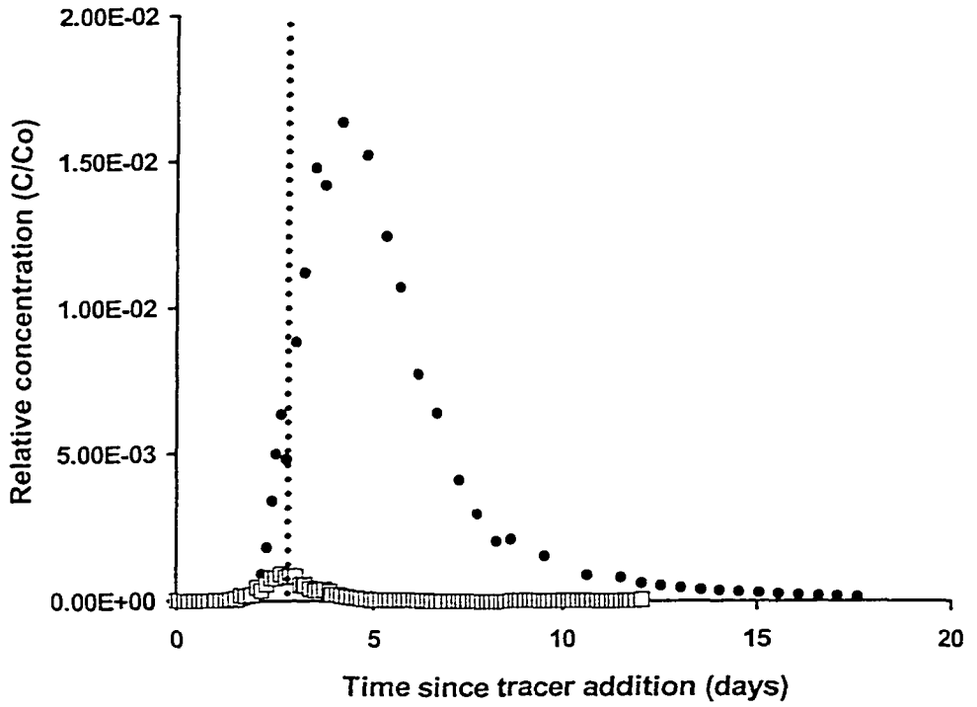

Figure 4. Relative concentration distribution for Br^- (●) and PRD1(■) during the spring tracer experiment expressed by the ratio of observed (C) and input concentration. The dashed line shows the time at 10 percent Br^- mass recovery.

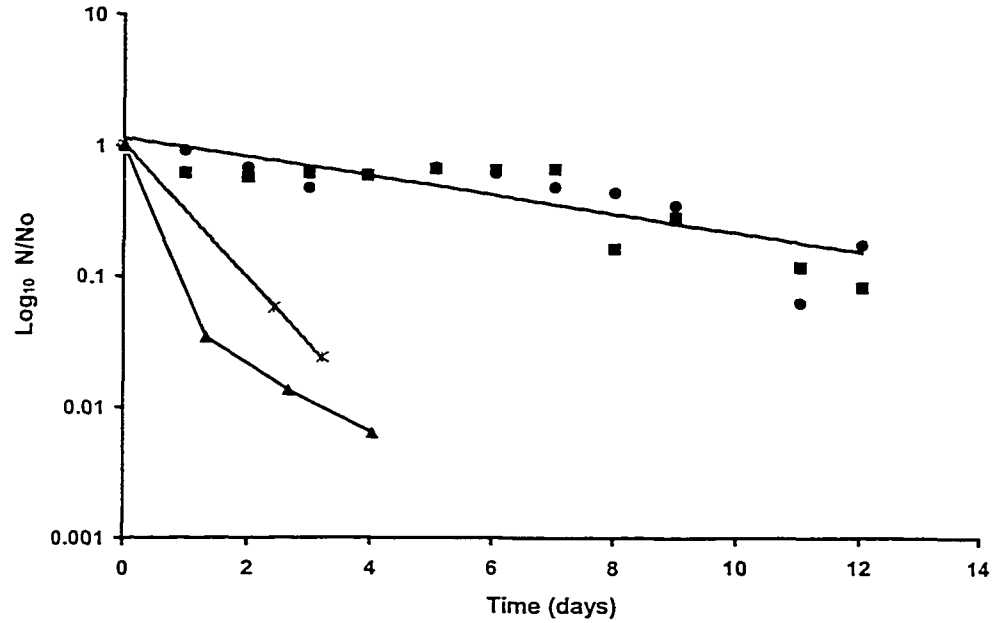

Figure 5.- Inactivation and removal performance of PRD-1 in the summer of 1999 (removal ▲ at 20, 40 and 56m) and spring of 2000 (removal ◆ at 36 and 56 m; inactivation at 36 ■ and 54 m ●) tracer experiment.

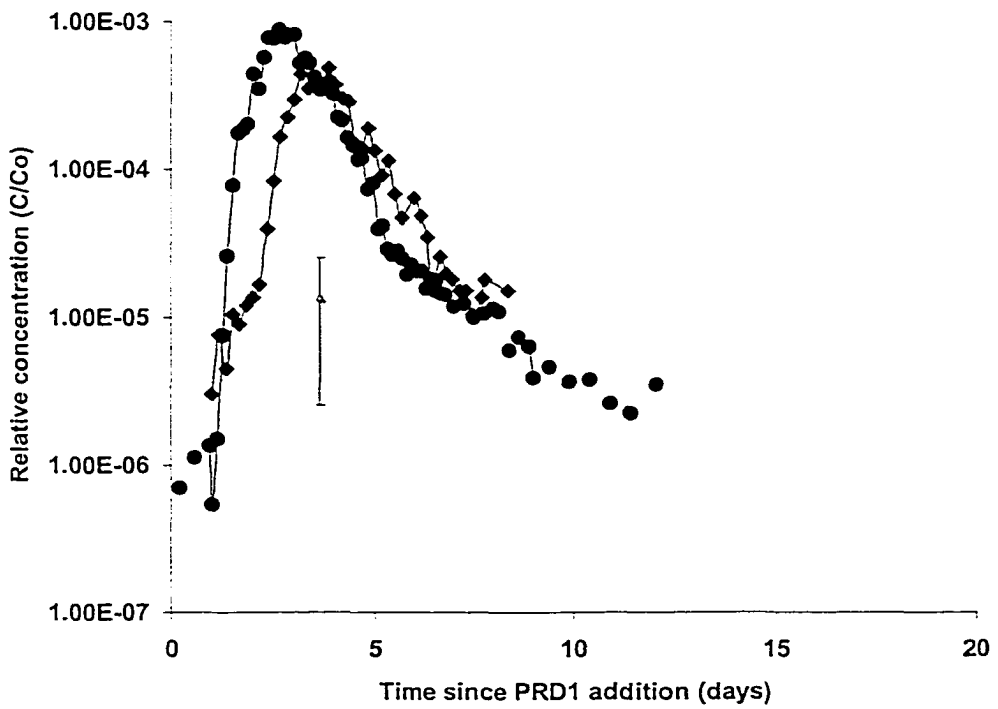

Figure 6. Detention time distribution of PRD-1 during the summer (♦) and spring tracer experiment (●). The error bars represent the range of phage background concentration in the influent water infecting the PRD1 host.

APPENDIX C

REMOVAL OF CHEMICAL AND MICROBIAL INDICATORS OF POLLUTION IN A SURFACE FLOW CONSTRUCTED WETLAND

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Removal of Microbial and Chemical Indicators of Pollution in a Surface Flow Constructed Wetland

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Abstract- Spatial distribution of physical, chemical, and microbial indicators of pollution was determined in a constructed wetland operated with chlorinated secondary effluent and backwash water. Samples were collected before and after vegetation removal. The system studied consisted of two densely vegetated settling basins (0.35 ha), an artificial stream, and a 3-ha surface flow wetland, planted with bulrush (*Scripus* spp.) and cattail (*Typha domingensis*). The average inflow of secondary effluent was 1.9 m^3 min^{-1} while the inflow during backwash water ranged from 0.21 to 0.42 m^3 min^{-1}. The system was able to reduce TSS and BOD_5 to tertiary effluent standards, however, monitoring of chloride revealed that evapotranspiration is concentrating chemical and microbial pollutants. Coliphage removal during backwash operation was 93 and 46 percent of the influent load at the end of the system during 1999 and 2000, respectively. During periods when secondary effluent entered the system, coliphage removal was 65 percent. After vegetation removal, pH and coliphage density increased significantly ($p<0.05$) at the outlet of the wetland. The findings of this study suggest that coliform growth or animal fecal contributions are occurring in the system.

INTRODUCTION

Numerous countries are actively using constructed wetlands for wastewater treatment because they have been effective in reducing biochemical oxygen demand (BOD_5), total suspended solids (TSS) and coliform bacteria (Geller, 1997; Green *et al.*, 1997; Mashauri *et al.*, 2000; Perkins and Hunter, 2000). These ecosystems are being used to treat a wide variety of wastewater in many climatic zones and compared to traditional wastewater treatment facilities, their operation costs and trained personnel demands are noticeably lower.

Total and fecal coliforms have been reduced in constructed wetland with efficiencies over 90 percent. These efficiencies, however, can be decreased significantly depending on the hydraulic load (Green *et al.*, 1997; Tanner *et al.*, 1998) and vegetation density (Gersberg *et al.*, 1989). Moreover, coliform densities may be considerably higher in the outflow of wetlands if they attract abundant wildlife or receive additional fecal contamination (Kadlec and Knight, 1996).

Scarce data has been published about removal of viral indicators from wastewater and particularly from backwash water in constructed wetlands. The available information reveals that viral removal has been studied under various approaches such as the ratio of outflow and inflow load of indigenous coliphage (Karpiscak *et al.*, 1995), survival tests (Vinluan, 1996), and tracer studies (Chendorain *et al.*, 1998). Although these studies have

shown that virus removal may be as high as 99.9 percent of the influent load, higher concentrations at the outlet have been observed in non-vegetated wetlands (Gersberg *et al.*, 1989). Other studies have suggested that virus populations decline rapidly with the distance (Scheuerman *et al.*, 1989).

This paper reports the findings of a monitoring program for coliphage, and total and fecal coliforms as well as physical and chemical pollution indicators in a constructed wetland which was designed to remove suspended solids from backwash water before aquifer recharge. The wetland received secondary and backwash water at variable flow rates for 8 months prior to vegetation removal and backwash water for 8 months following removal.

MATERIALS AND METHODS

The Research Site

Research was conducted at the Sweetwater Wetland and Recharge Facility in Tucson, AZ. The system consists of two polishing subsystems, east and west. These facility has been in operation since 1997 and were designed to remove suspended solids from wastewater produced during periodic backwashing of pressure mixed media filters used to treat secondary effluent at the City of Tucson Reclamation Plant. Residual chlorine in secondary

effluent at the pressure mixed media filters is on average 1 mg L^{-1}. The backwash water produced during washing does not receive additional chlorine.

Wetland influent water is introduced into a splitter structure that usually diverts the inflow in equal parts to each of the treatment subsystems. The water then enters a pair of settling basins with a combined area of 0.35 ha. These basins are densely vegetated with bulrush (*Scirpus* spp.) in the East subsystem, and cattail (*Typha domingensis*) in the West subsystem. After passing through the settling basins the water enters large surface flow wetland cells. However, in the East subsystem water flows briefly through a small artificial stream before entering the wetland cell. Each of the wetland cells is about 3 ha in area and contains open water areas (1.2 m deep) and densely vegetated shallow zones (0.3 m deep). The dominant plants in both the large wetland cells are species of bulrush (*Scirpus* spp.) and cattail (*Typha domingenses*).

Sampling Sites and Analysis

From February to September of 1999 and again in 2000, water samples were collected in 1-L bottles monthly at the backwash splitter box, outlet of the south settling basin, both ends of the stream, and outlet of the wetland cell in the East subsystem (Figure 1). Concurrently water temperature was taken with a standard mercury thermometer at the site of sampling. Measurements of biochemical oxygen demand (BOD_5), total suspended

solids (TSS), sulphate (SO_4), chloride (Cl^-), turbidity, pH, coliphage, and total (TC) and fecal (FC) coliforms were conducted in the laboratory.

Physical and Chemical Analysis

The 5-day incubation method found in Standard Methods for the Examination of Water and Wastewater was used to determine BOD_5. Determination of TSS was made by filtering a known volume of sample through a precleaned and preweighed glass fiber filter, drying the filter for at least 24 hours at 100 °C, reweighing the filter, and calculating the concentration. Sulfate was determined by adding $BaCl_2$ to a know volume of sample and measuring the absorbance at 420 nm in a HACH DR/2000 spectrophotometer (Loveland, CO). If the sample was at all colored, a "blank" sample (without the addition of $BaCl_2$) was read and the absorbency subtracted from that reading made in the sample with the $BaCl_2$ added. The absorbency was compared against a standard curve. Chloride was determined using a chloride-specific electrode method. One ml of Ionic Strength Adjuster (ISA) Solution (5 M $NaNO_3$) was added to 50 ml of room-temperature sample. The Cl^-specific electrode attached to a pH meter (Corning, model M220, New York) is placed in the solution and the mV of the sample solution read and compared against a standard curve. Turbidity was measured by using a portable turbidimeter (HACH, model 2100P, Loveland, CO), pH with a pH meter (model 8005, West Chester, PA) and total and free chlorine (Cl_2) using the DPD method (HACH Spectrophotometer, model DR/2000, Loveland, CO).

Coliforms and Coliphages

Coliform bacteria were analyzed within 4 h of sampling by membrane filtration using mEndo Agar Les and mFC culture media (DIFCO, Detroit, MI) for total coliforms (TC) and fecal coliforms (FC), respectively. The membrane filters were 47 mm diameter with a porosity of 0.45 μm (Milipore, Molsheim, France). Sample volumes of 0.1, 1, and 10 ml were assayed and incubated at 37 and 44.5 °C for TC and FC, respectively. The colony formed units (cfu) developed after 24 h of incubation were enumerated.

Coliphage was enumerated by the double layer agar method described by Adams (1959). A 1-ml aliquot from *Escherichia coli* ATCC 15597 (ATCC) culture, previously incubated at 37 °C for 24 h in Trypticase Soy Broth (DIFCO, MI), was combined with 1 ml of collected sample in a test tube containing molten overlay agar. The agar was poured onto a layer of Tripticase Soy Agar (DIFCO, MI), and incubated at 37°C for 18 h in order to enumerate the coliphage plaque forming units (pfu).

Statistical Analysis

The statistical analysis was conducted by using SYSTAT 9 (SPSS Inc., Chicago ILL). Tests to determine significant difference between observations of BOD_5, Cl^-, SO_4, T°, pH, and turbidity, at the sampling sites during backwash operation periods, were conducted by ANOVA analysis.

The high sensibility of the arithmetic mean to extreme values observed for TSS and microbial indicators during backwash operation suggested that the median may represent better the central tendency of data sets collected during the study. Therefore, TSS concentrations and microbial populations were statistically analyzed comparing the median observations between sites and backwash periods to reduce extreme value effects. The ten variables studied were compared to each other by Pearson Correlation Analysis.

For the second and third period of sampling, TC, FC, and coliphage concentrations in the collected samples were represented statistically in box plots. In these figures, the horizontal line shows the median, ends of the box the 25th and 75th percentiles, error bars the 10th and 90th percentiles, and extreme values are depicted by circles. A nonparametric Mann-Whitney U-test was used to compare the median observations between sampling sites and backwash periods.

RESULTS

Sampling

Based on the type of effluent entering the system and vegetation removal, the sampling program was divided into three major sampling periods: **1) Secondary effluent period:** from February 12 to March 18 of 1999, the system was receiving chlorinated secondary effluent at an average rate of 1.84 m^3 min^{-1} to facilitate a tracer study. The

detention time of the wetland cell determined at the end of the test was 7.3 days (Vidales *et al.*, 2000). During this time, the influent water was introduced into the splitter box and flowed into the stream without entering the east pair of settling basins. Periodically, the basins were flooded with backwash water to keep them wet. On March 18, the system was returned to normal operation averaging approximately the same flow rate as was used during the tracer study. The influent water thus flowed from the splitter box passing through both settling basins as well as into the east polishing wetland cell. A mixture of chlorinated secondary and backwash water was introduced into the system until March 23 of 1999.

2) Backwash period before vegetation removal: only backwash water was received by the east subsystem from March 23 to September 21 of 1999. From March 23 to June 30, the average influent rate was 0.42 m^3 min^{-1} with a coefficient of variation of 24 percent. This influent rate was reduced to 0.21 m^3 min^{-1} from the end of Jun through September 21 when the east subsystem started to be drained for vegetation removal. The theoretical detention time during this period was in a range of 25 to 50 days.

3) Backwash period after vegetation removal: After vegetation removal during the Winter of 1999, the system was return to normal operation on February 2000 at an average inflow rate of 0.32 m^3 min^{-1} of backwash water. These rates were calculated with information provided by Tucson Water for the period of February 17 to September 10 of 2000. The theoretical detention time was estimated to be between 28 and 38 days.

Initial Period of Secondary Operation

BOD_5, TSS, and Turbidity

Two samples were collected per sampling site during February and March of 1999. The influent concentrations of BOD_5 and TSS were 29 and 21 mg L^{-1}, respectively. Both concentrations decreased 69 percent after filtration through the settling basin (Figure 2). In the stream, BOD_5 was around 7 mg L^{-1} and remained at this level through the system reaching 8 mg L^{-1} at the outlet end of the wetland cell for a total removal of some 72 percent. TSS mean concentration was lowered slightly to 5 mg L^{-1} in the stream and was below the detection limit (5 mg L^{-1}) of the analytical method used in the wetland outflow. Turbidity was very closely related to BOD_5 and TSS distribution in the system. The turbidity of the water entering the system was 17.9 NTU which fell to 7.8 in the settling basin and 7.6 NTU in samples from the inlet end of the stream, and then was reduced to 2.8 NTU at the end of the wetland cell for an average removal efficiency of 84 percent. Pearson correlation coefficients comparing turbidity to BOD_5 and turbidity to TSS concentrations in effluents from all sampling sites were 0.93 and 0.97, respectively.

Cl^-, SO_4^{2-}, pH, and Temperature

The average concentration of Cl^- was 126 mg L^{-1} in samples collected from the splitter box (Figure 3) decreasing to116.5 mg L^{-1} at the outlet of the wetland. A similar overall trend was observed for SO_4^{2-} with the mean concentration decreasing from 128 mg

L^{-1} at the splitter box, to 122.5 mg L^{-1} at the outlet of the wetland. However, the concentration of SO_4 was higher at the outlet of the settling basin, and at the outlet end of the stream. Pearson correlation analysis revealed a correlation coefficient of 0.76 comparing average concentrations of SO_4^{2-} to Cl^-.

Water pH was considerably lower at the outflow from the settling basin than at the splitter box (Table 1a). In samples collected from both ends of the stream, water pH increased from 7.5 to 7.8 reaching 7.6 at the outlet of the wetland which was lower than the original 7.8 at the splitter box. The mean water temperature at the splitter box was 22.7 °C decreasing to 20.2 °C at the outlet of the settling basin, 18 °C at the end of the stream and 10.5 °C at the outflow from the wetland (Table 1).

On February 19, total and free Cl_2 concentrations were 1.19 and 0.14 mg L^{-1} , respectively, in water from the splitter box . Both concentrations were below the detection limit in samples from the other sampling sites. On March 20, Cl_2 was not detected at any sample point within the east system.

Indicator Microorganisms

The average TC density in water samples from the splitter box was 2.56 $\log_{10}$ increasing to 4.21 $\log_{10}$ in the settling basin and decreasing from 4.07 to 3.81 $\log_{10}$ at the outflow point of the stream (Figure 4). Treatment in the wetland resulted in a reduction of approximately one order of magnitude. The incoming FC population observed at the splitter

box was 1.81 $\log_{10}$ gaining about two $\log_{10}$ in the settling basin. In the stream, the inflow and outflow concentrations were 3.63 and 3.07 $\log_{10}$, respectively. At the same time, at the wetland outlet, the observed FC population was 2.35 $\log_{10}$. Coliphage load in the incoming water was 3.7 $\log_{10}$; however, the highest concentration of 3.86 $\log_{10}$ occurred in the settling basin outflow. At both ends of the stream, coliphage concentrations were 3.9 and 3.78 $\log_{10}$ which decreased to 3.23 $\log_{10}$ at the outlet of the wetland for an average removal of 65 percent.

Backwash Operation Before Vegetation Removal

BOD_5, TSS, Temperature, and Turbidity

Concentrations of TSS, and BOD_5 as well as turbidity detected during backwash operation for the last 6 months of 1999 are reported in Figure 5. ANOVA analysis showed that the influent turbidity of 155 NTU decreased significantly ($p<0.05$) to 25 NTU at the outlet of the settling basin, and 33 NTU at the wetland outflow.

During this backwash period, BOD_5 concentrations demonstrated inconsistency because of the high variability in the water quality. The most critical situation was with samples from the settling basin where only four samples out of eight gave valid results. The mean BOD_5 concentration in water entering the system was 127 mg L^{-1} which decreased significantly ($p<0.1$) to 51 mg L^{-1} in samples collected from the settling basin. In the stream,

the BOD_5 concentration went from 37 to 28 mg L^{-1} and then diminished to 14 mg L^{-1} in the outflow of the wetland.

Similar to BOD_5, the mean water temperature also varied along the system. For example, it was 27.7 °C in the entering water, 25.4 °C in the settling basin, and 19.8 °C in the wetland outflow (Table 1b).

Since the arithmetic mean is highly sensitive to extreme values as shown by the TSS concentration detected at the outlet of the settling basin on May 21 of 1999 (Figure 5), the median of the observed concentrations at each sampling site was chosen as a better representation of their central tendency. The noparametric Mann-Whitney U-test revealed that the median influent concentration of 159 mg L^{-1} diminished significantly ($p<0.05$) in samples from the settling basin, where the estimated median was 8.5 mg L^{-1}. This concentration was 5 mg L^{-1} in the water from the stream and increased to 6 mg L^{-1} at the end of the wetland cell. Based on the observed concentrations, the TSS was removed, on average, more efficiently than BOD_5 and turbidity. Finally, the observed removal efficiencies in the system for these three parameters were about 96, 84, and 76 percent, respectively.

Cl^-, SO_4^{2-}, and pH

From April to September of 1999, ANOVA analysis established that the average influent level of Cl^- increased, no significantly ($p<0.05$), from 141 to 164 mg L^{-1} in the

outflow of the settling basin (Figure 6). This concentration declined to 142 and 155 mg L^{-1} at in and out of the stream; however, at the outlet of the wetland, an increase of 40 mg L^{-1} over the former concentration was statistically significative ($p<0.05$).

Initial concentration levels of sulphate coming into the wetland facility averaged 144 mg L^{-1} which was decreased significantly ($p<0.05$) in the settling basin (Figure 7). Meanwhile, in the stream, concentrations of SO_4^{2-} reached an approximated level of 125 mg L^{-1} increasing to 146 mg L^{-1} at the end of the polishing wetland. In the same effluents, water pH ranged from 7.4 to 7.5 at the splitter box, settling basin, and stream; whereas, at the outflow of the wetland, pH decreased, not significatively ($p<0.05$), to 7.35.

Indicator Microorganisms

Both total and fecal coliforms followed a similar distribution pattern during backwash operation in 1999. The median concentration of TC in the influent water was 3.5 $\log_{10}$ increasing significantly ($p<0.05$) to 4.5 $\log_{10}$ in water samples collected from the settling basin (Figure 8). Practically, no TC removal occurred in the stream and outlet of the polishing wetland where a concentration of 4.6 $\log_{10}$ was observed. Similar to TC, FC populations also increased, significatively ($p<0.1$), about an order of magnitude in the settling basin (Figure 9). The FC density was stabilized at some 4.0 $\log_{10}$ until the end of the stream reaching 4.3 $\log_{10}$ in the outflowing water from the system.

The median coliphage load in backwash water at the splitter box was 4.2 $\log_{10}$ decreasing significatively ($p<0.1$) to 3.7 $\log_{10}$ after passing through the settling basin (Figure 10). The coliphage concentration decreased to 3.4 $\log_{10}$ at the end of the stream and 3.1 $\log_{10}$ in the outflow of the wetland cell, for a total removal of 9 2 percent.

Backwash Operation After Vegetation Removal

BOD_5, TSS, Temperature, and Turbidity

The third study started on February 2000, after vegetation removal, with 100 percent backwash water entering the system. During this period, the average BOD_5 load in the incoming backwash water was 145 mg L^{-1} (Figure 11). The ANOVA test showed that the 38 mg L^{-1}-concentration observed at the outflow from the settling basin was statistically lower ($p<0.05$) than the entering BOD_5 load. Little, if any, BOD_5 removal occurred within the stream since the observed concentrations were 37 and 31 mg L^{-1} at the inlet and outlet, respectively, decreasing significatively ($p<0.05$) to 23 mg L^{-1} in the outflow of the wetland for a total system removal of 84 percent.

The amount of total suspended solids showed a distribution similar to that of BOD_5 concentrations and turbidity during this third sampling period. The influent concentration of 123 mg L^{-1} was lowered significantly ($p<0.05$) to 7.9 mg L^{-1} in the settling basin effluent. This BOD_5 level increased slightly to 11.6 mg L^{-1} after passing by the stream and wetland for a removal efficiency of 90 percent.

Influent turbidity was reduced by the settling basin from 128 to 57.9 NTU, remaining without change until the outlet of the stream. At the outlet of the wetland cell, turbidity was about 33 NTU.

Similar to turbidity, the mean water temperature tended also to decrease from the inflow to the outflow of the system. Influent water temperature averaged 29.3 °C decreasing to 25.6°C at the outlet of the settling basin. Additional decreases were observed in the stream (25 °C) and again in the wetland outflow (23.4 °C).

After vegetation removal, only at the outlet of the settling basin, turbidity increased significatively ($p<0.05$). Similarly, the ANOVA test showed that the mean concentration for BOD_5 observed at the outlet of the wetland was significantly higher than the one observed under backwash operation before vegetation removal. In contrast, the nonparametric Mann-Whitney U-test revealed that the median TSS concentration before and after vegetation removal were statistically ($p<0.05$) similar.

Cl^-, SO_4^{2-}, and pH

The average load of Cl^- concentration entering the system was 117 mg L^{-1} which was similar to the outflow of the settling basin (Figure 6). Concentration of Cl^- was 107 and 115 mg L^{-1} in the inflow and outflow of the stream, respectively, reaching 137 mg L^{-1} at the outlet of the wetland.

The average SO_4 concentration in the incoming water was 130 mg L^{-1} which decreased to 115 mg L^{-1} at the end of the stream (Figure 7). Between sampling sites, the difference of SO_4^{2-} concentrations was not statistically significative ($p<0.05$). However, the mean concentration of 148 mg L^{-1} observed at the end of the wetland cell was statistically ($p<0.05$) higher than the latter concentration.

The pH readings obtained in water samples from the splitter box to the stream were about 7.4. Average pH increased to 7.7 in the wetland cell. Statistically, these results were similar to pH readings obtained during the first period of backwash water except for those observed at the outlet of the wetland. Similarly, there was no statistical evidence to indicate that vegetation removal from the system caused a significative change ($p<0.05$) in SO_4^{2-} concentrations at the monitoring sites. In contrast, Cl^- concentrations were significantly lower ($p<0.05$) after vegetation removal.

Indicator Microorganisms

The spatial distribution of TC and FC populations along the system are reported in Figure 8 and 9, respectively. The median TC density in the influent water was 3.8 $\log_{10}$. This concentration increased significatively ($p<0.05$) to 4.5 $\log_{10}$ in the outflow of the settling basin, stream, and outlet of the wetland. Likewise, FC concentrations increased significantly ($p<0.05$) from 3.4 $\log_{10}$ at the splitter box to 4.0 $\log_{10}$ in samples collected from

the settling basin. The stream did not reduce FC populations significantly ($p<0.05$). At the outflow from the wetland, the median population was 3.7 $\log_{10}$.

The nonparametric Mann-Whitney U-test revealed that TC and FC densities were statistically constant ($p<0.05$) during both backwash operational periods. Therefore, vegetation removal did not cause a significant change of coliform distribution in the system.

A statistical representation of coliphage concentrations after vegetation removal can be appreciated in Figure 10. During this period, an influent coliphage population of 3.8 $\log_{10}$ was reduced significantly ($p<0.05$) to 3.5 $\log_{10}$ in the settling basin. A similar median concentration was also observed in the wetland outlet for a removal efficiency of 46 percent. The nonparametric Mann-Whitney U-test showed that the differences found during the two backwash periods (Figure 10) were not statistically different for most points in the system. However, after vegetation removal, a significant increase ($p<0.05$) of coliphage densities was observed at the end of the wetland cell.

Correlation Analysis

Correlation analyses were conducted to determine the relationship among the 10 variables measured during the current study. Corresponding Pearson coefficients often showed no correlation between the paired variables thus only those with correlation coefficients over 0.5 are reported. The Pearson correlation coefficient comparing TSS to turbidity in the incoming and stream water was about 0.8. At the other three sites, these

coefficients were significantly low suggesting no correlation between both variables. When coliphage densities were compared to their paired TSS correlation coefficients in the order of 0.65, 0.68, 0.41, and 0.61 were estimated at the splitter box, settling basin, stream, and outlet of the wetland. Also, FC populations and TSS concentrations were compared yielding a negative correlation of -0.6 in samples from the splitter box, -0.8 in the settling basin effluent, -0.57 and -0.68 in collected samples from both ends of the stream, and -0.44 in the wetland outflow. Conversely, BOD_5 concentrations were positively correlated to TSS levels in collected samples from the sampling sites. At these locations, the correlation coefficients were 0.17, 0.94, 0.55, 0.30 and 0.71 from the splitter box to the wetland outflow. When comparing water pH to Cl^- concentrations, the correlation coefficients were ranging from 0.57 to 0.77 in the splitter box, settling basin, and stream outflow, respectively. However, this coefficient was 0.23 at the inlet of the stream and -0.71 at the wetland outlet.

DISCUSSION

Pollution Indicators

A significant increase of turbidity, BOD_5, and TSS concentrations was observed when the water entering the wetland was switched from secondary effluent to backwash water. Observed reductions of turbidity, BOD_5, and TSS occurred mostly in the settling basin where removal of turbidity ranged from 56 to 83 percent, BOD_5 from 60 to 75 percent,

and TSS form 62 to 94 percent. A higher removal of TSS and turbidity was observed during operation with backwash water whereas higher BOD_5 removal occurred during the initial two month period when secondary effluent was entering the system. These parameters showed a similar evolution in the system; however, when their data sets obtained at each sampling location were compared to each other a small correlation was often observed. Apparently, BOD_5, TSS, and turbidity levels depend on other factors that are affecting their interrelationship (Kadlec and Knight, 1996). For example, turbidity may be affected by the color produced in water by impurities like algae or sediments from reductive environments (Maier, 2000).

At the wetland outlet, reduction of BOD_5 are comparable to results reported by Vrhovsek *et al.* (1996) who found a 89 percent reduction in a subsurface flow wetland operated at a BOD_5 loading rate of 962 mg L^{-1}. Removal of BOD_5, and TSS found in the current study are in general agreement with wetland systems operating across the USA (Kadlec and Knight, 1996).

The wetland system receiving secondary effluent for the initial two-month period met on average the Arizona Department of Environmental Quality tertiary standards for BOD_5 and TSS of 10 mg L^{-1}. This level was only slightly exceeded in TSS concentration at the outlet end of the system for the period of backwash operation following vegetation removal. In contrast, the average BOD_5 outflow concentration was over the accepted value

of 10 mg L^{-1} but without exceeding the recommended level of 30 mg L^{-1} for secondary effluent, during both backwash operation periods. Chloride is considered highly stable in most terrestrial environments. In wetlands, its total mass is approximately constant (Kadlec and Knight, 1996) because its incorporation in plant tissues is negligible (Hayashi *et al.*, 1998). Consequently, Cl^- has been used as a conservative tracer to estimate evapotranspiration in wetland ecosystems (Hayashi *et al.*, 1998). Loses of Cl^- were not evident during the initial period of secondary effluent. Probably, the increase of detention time during backwash water operation promoted the evaporitic enrichment of Cl^- in the outflowing water from the polishing wetland. In general, Cl^- concentrations at the wetland outlet were lower in 2000 than in 1999 backwash operation; such a condition appears to be induced by a lower influent concentration. The difference of Cl^- concentration between both ends of the polishing wetland was 26 and 19 percent during backwash operation before and after vegetation removal, respectively.

Sulphate is an essential nutrient for plants; thus it can be retained by plant uptake in terrestrial environments; however, it is rarely a limiting factor for plant growth in wetlands (Kadlec and Knight, 1996). The electron acceptor of sulfur-reducing bacteria in anaerobic environments is SO_4^{2-} (Maier, 2000) where its presence and a high organic content induces the production of hydrogen sulfide. Probably, this microbiological process was responsible for the reduction of SO_4^{2-} in the settling basin, mostly observed during 100 percent

backwash water operation (Figure 7). Similar to Cl^-, a significant increase of SO_4^{2-} occurred by an apparent evaporitic enrichment at the outflow of the wetland. Comparing both sampling periods, vegetation removal from the system did not produce a significant change of SO_4^{2-} during backwash operation. However, SO_4^{2-} behavior was noticeably impacted by evaporation as a consequence of the longer detention time during backwash operation (Figure 3 and 7).

After vegetation removal, water pH was statistically higher at the outflow of the wetland cell. Probably, vegetation removal may have favored a higher light penetration in the shallow areas promoting algae proliferation and a higher pH in the outflow of the wetland cell (Parhad and Rao,
1972; Kadlec and Knight, 1996).

Indicator Microorganisms

Removal efficiency over 90 percent of total and fecal coliforms in surface flow wetlands have been commonly reported (Gersberg *et al.*, 1989; Kadlec and Knight, 1996; Karpiscak *et al.*, 1995; Perkins and Hunter, 2000; Steen *et al.*, 1999; Vrhovsek *et al.*, 1996). In this study, the high densities of both total and fecal coliforms observed at the settling basin and outlet of the east polishing basin are unclear. Growth or recovery of injured bacteria can occur if the amount of organic matter and temperature are elevated (Gerba, 2000). Studies conducted in soils have found evidences of coliform regrowth associated

with inputs of nutrients in the environment coinciding with elevated conditions of temperature and moisture (Van Donsel *et al.*, 1967). Coliform bacteria such as *Klebsiella*, *Enterobacter*, and *Citrobacter* have shown the ability to proliferate during wastewater treatment (Niemi *et al.* cited by Elmund *et al,* 1999). *Klebsiella* was found at high densities in the outflowing water from a treatment facility receiving municipal wastewater (Elmund *et al.*, 1999) which was attributed partially to an increase of carbohydrates in wastewater. In water reservoirs, natural or artificial, animal fecal material have been reported as a possible source of high densities of total and fecal coliform bacteria (Have, 1973; Kadlec and Knight, 1996; Moorhead *et al.*, 1998). During operation of the east polishing subsystem, the settling basin has shown a high ability to reduce BOD_5 and TSS from wastewater. Thus, it appears that the amount of organic matter introduced into the settling basins are playing an important role in the multiplication or recovery of total and fecal colifroms.

At the outlet of the system, it is probable that the high densities of both groups of coliforms are a result of a combination of factors such as: a) total and fecal coliform growth because organic matter contributions from backwash water, b) increase of total and fecal coliform densities by evapotranspiration because of the long detention time in the wetland cell, and c) animal fecal contributions , Sweetwater Wetlands provide habitat for small mammals, birds and waterfowl. All these factors could be offsetting any total and fecal coliform removal within the wetland.

F-specific RNA bacteriophages have been used as potential indicator of human enteroviruses indicator instead of fecal coliforms and fecal streptococci because it has been correlated with human viruses in a wide range of environments (Stetler, 1984; Havelaar *et al.*, 1993). Compared to enteroviruses, the former groups of bacteria have shown lower populations in chlorinated effluents and higher densities in surface water exposed to animal fecal contamination (Havelaar *et al.*, 1993). In the current study, a moderated correlation of coliphages compared to TSS was observed during backwash operation. The recovery of coliphages in samples collected from the wetland outlet revealed a removal of 0.43 log for the initial two-month period, 1.1 log for the second period, and 0.2 log after vegetation removal from the wetland. It is important to point out that the higher coliphage removal coincided with the highest influent concentration. When comparing removal efficiencies of coliphage between sampling periods at each sampling location, the removal difference between backwash periods was statistically significative ($p<0.05$) only at the outlet of the wetland cell. Frequently, coliphage removal have been found over one log reduction (Gersberg *et al.*, 1987, 1989; Chendorain *et al.*, 1998); However, lower coliphage removals have been found by Karpiscak *et al.* (1995) in a duckweed (*Lemna* spp.) system and by Gersberg *et al.* (1989) in nonvegetated wetlands.

CONCLUSIONS

Even, after vegetation removal and backwash water operation, the system was able to decrease BOD_5 and TSS to secondary standards required by the Arizona Department of Environmental Quality. However, little removal was observed in either the stream or the 3 ha surface flow wetland. Based on the results of the current study fecal and total coliform bacteria are doubtful indicators of human fecal contamination because inputs or recovery of both groups of coliforms are occurring in the east polishing system. Apparently, coliphage was a better indicator during the current study although its low level of removal in the wetland cell suggests additional inputs may be occurring from animal sources.

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cities); Water Replenishment District of Southern California; City of Riverside, California; and City of Los Angeles Department of Water and Power.

DISCLAIMER

The American Water Works Association Research Foundation and the other agencies listed above not had opportunity to review and comment on this paper, therefore, none of these agencies necessarily endorse the findings presented here.

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Table 1. Average temperature and pH observed in the samples collected from the east polishing system during the secondary effluent operation (February and March 1999) and the 1999 and 2000 backwash periods.

a)Secondary effluent

Parameter	Sampling site				
	Splitter box	Settling basin	Stream in	Stream out	Wetland outlet
Temperature (°C)	22.75	20.25	19	18	10.5
pH	7.8	7.3	7.5	7.8	7.6

b)Backwash water

Parameter	Sampling site									
	Splitter box		Settling basin		Stream in		Stream out		Outlet	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Temperature (°C)	27.7	29.3	25.4	25.6	24.6	25.9	24.2	25	19.8	23.4
pH	7.42	7.39	7.5	7.3	7.4	7.4	7.5	7.4	7.3	7.7

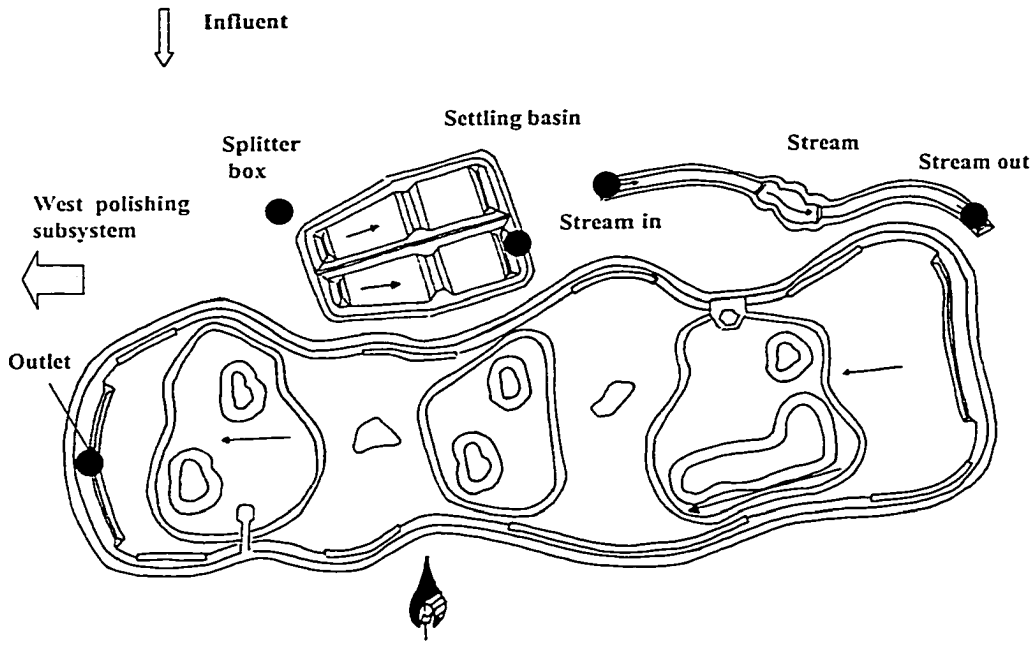

Figure 1. Schematic representation of the east polishing subsystem and location of the sampling sites (●).

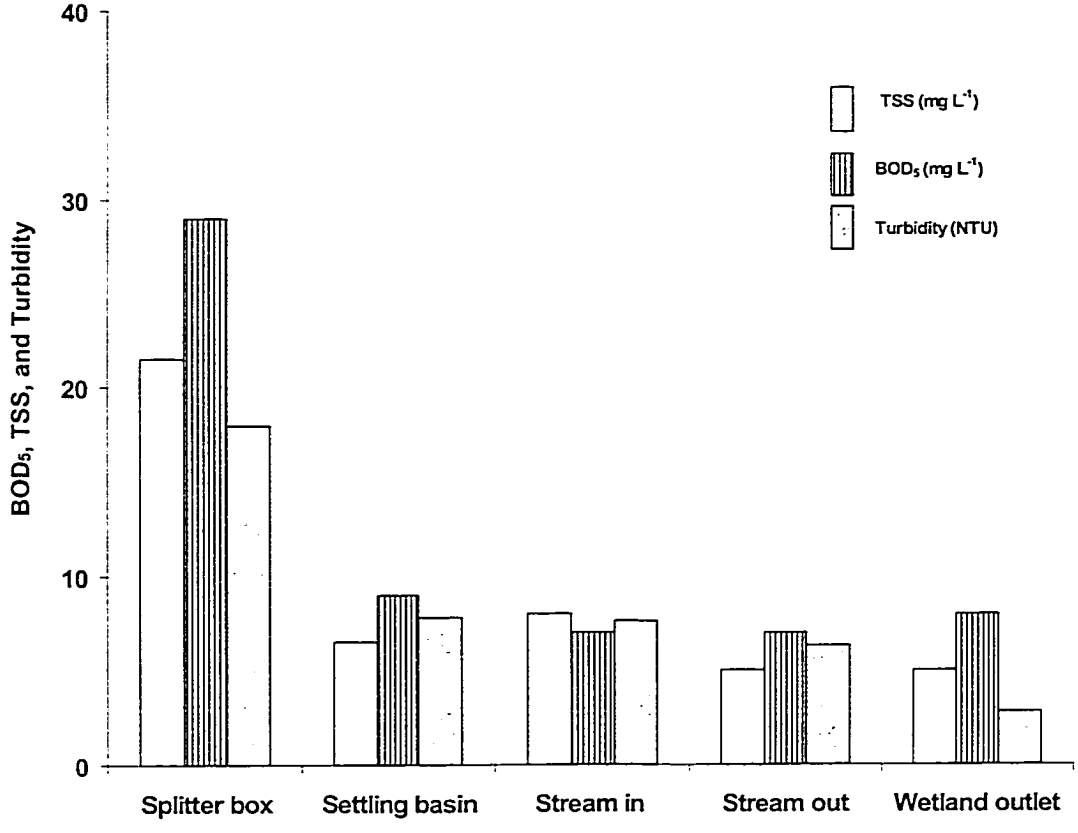

Figure 2. Average concentration of TSS, BOD_5, and Turbidity in the east polishing system during secondary effluent operation (February and March 1999).

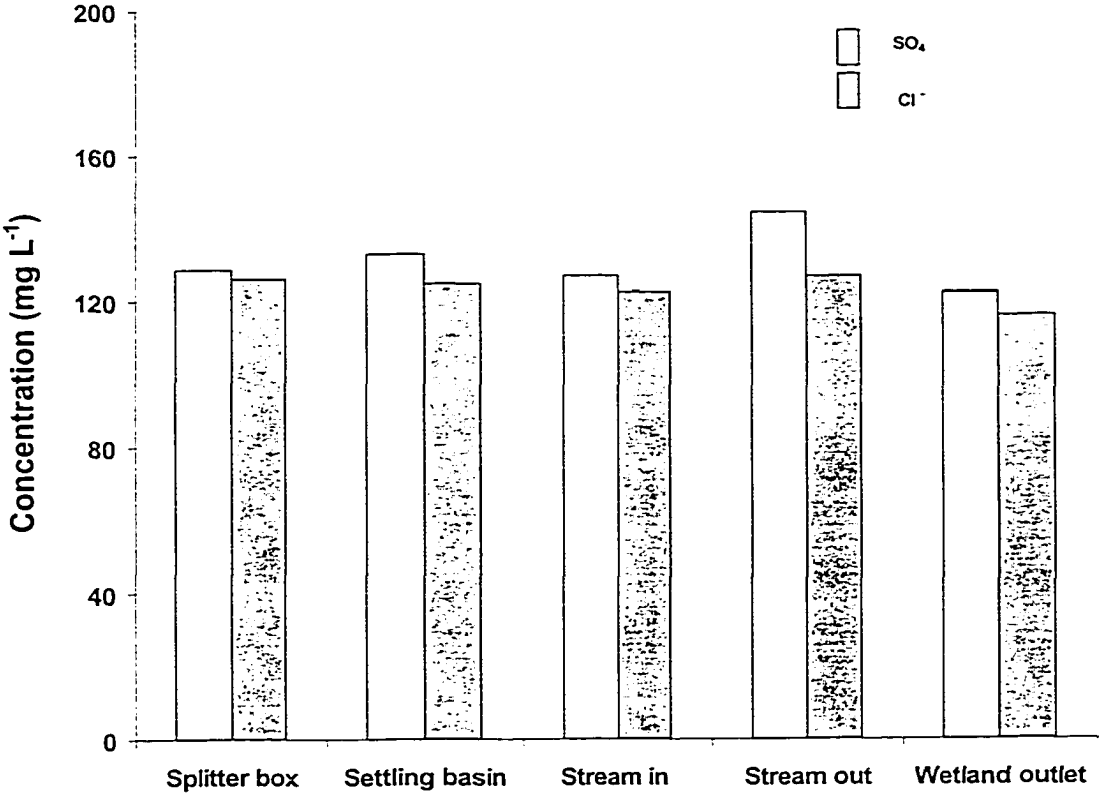

Figure 3. Average concentrations of Cl^- and SO_4 from sampling sites in the east polishing system during secondary effluent operation (February and March 1999).

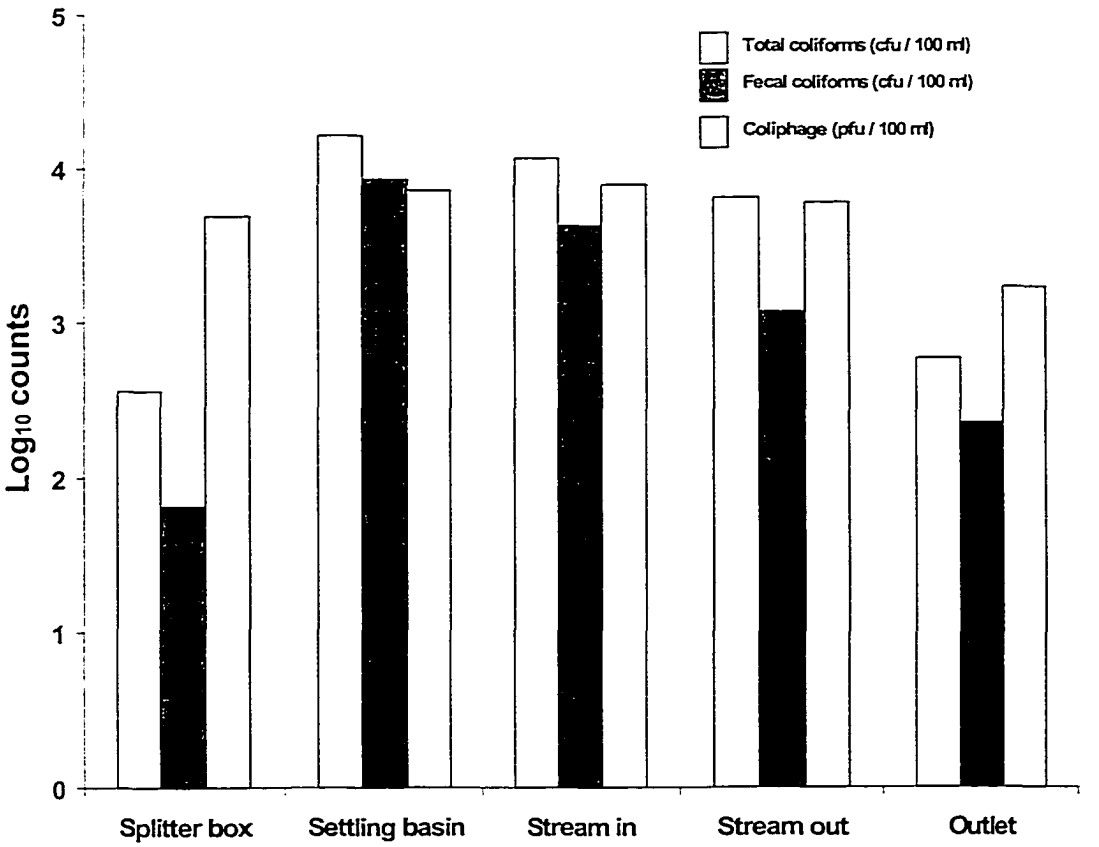

Figure 4. Microbial indicator populations in the east polishing subsystem during secondary effluent operation (February and March 1999).

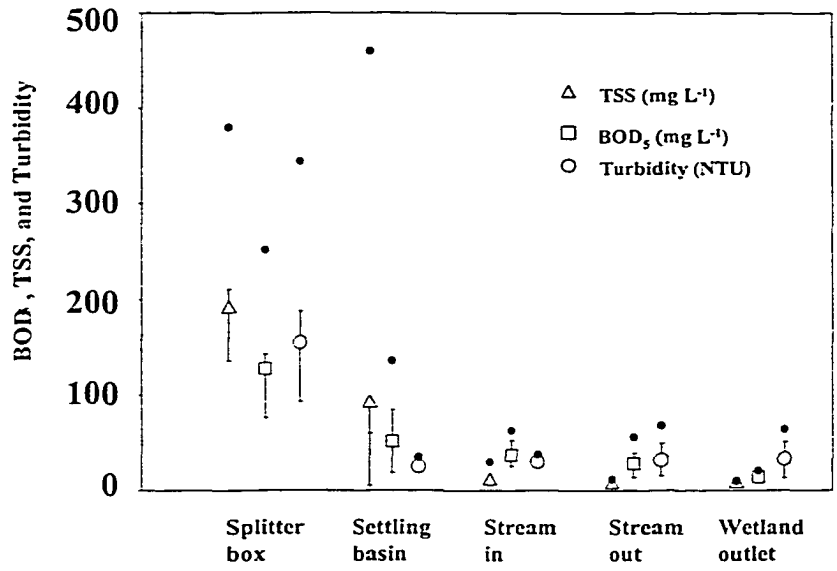

Figure 5. Statistical representation of BOD_5, TSS, and Turbidity observed in the east polishing system during secondary effluent operation (February and March 1999) period of sampling. The symbol represents the mean concentration, error bars the 25th and 75th percentile, and the dot (•) the maximum observation.

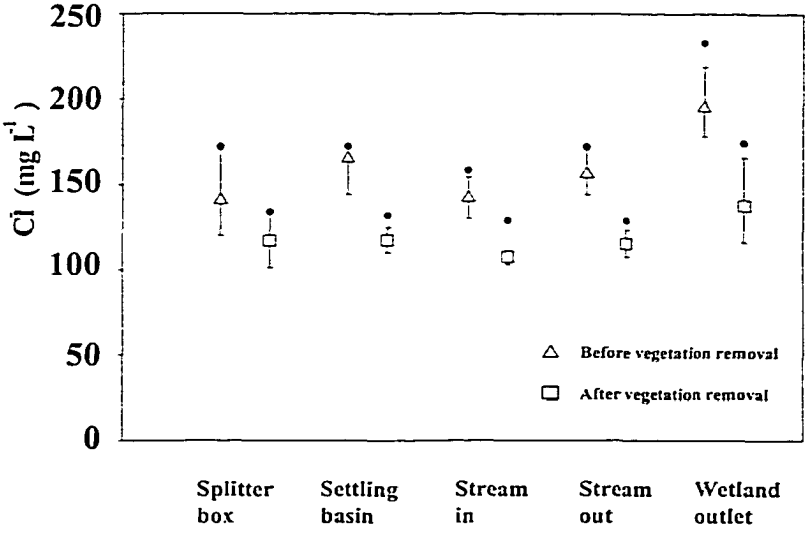

Figure 6. Concentration of Cl^- in the east polishing system during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. The symbol represent the mean concentration, error bars the 25 th and 75th percentile, and the dot (•) the maximum observation.

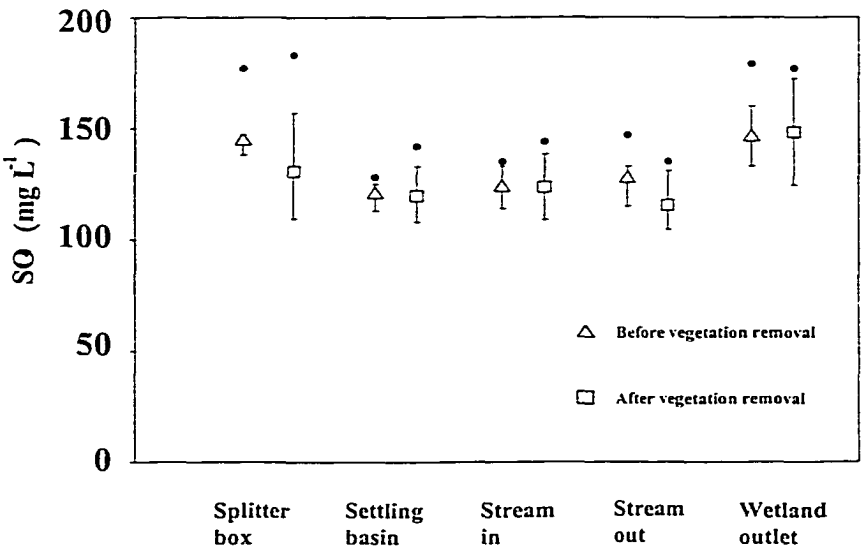

Figure 7. Statistical representation of SO_4 in the east polishing system during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. The symbol represents the mean concentration, error bars the 25 th and 75 th percentile, and the dot (•) the maximum observation.

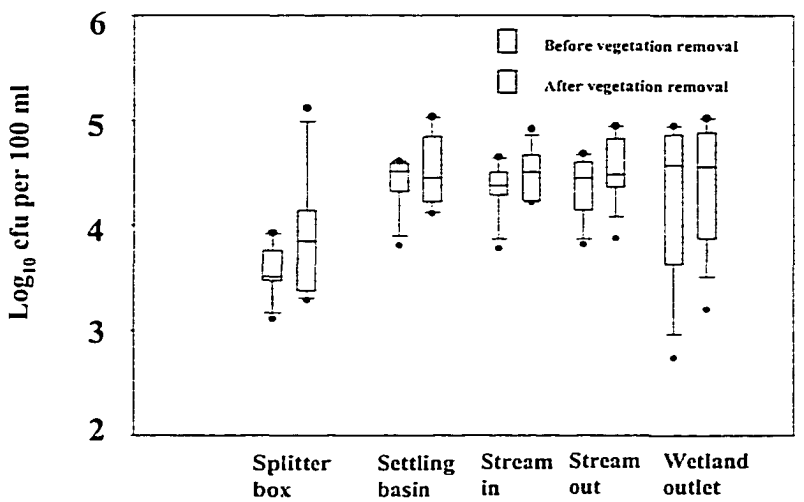

Figure 8. Statistical representation of total coliform concentration observed during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. In the box plot, the horizontal line shows the median, the box ends indicate the 25th and 75th percentile, error bars the 10th and 90th percentiles, and the dots (•) extreme values.

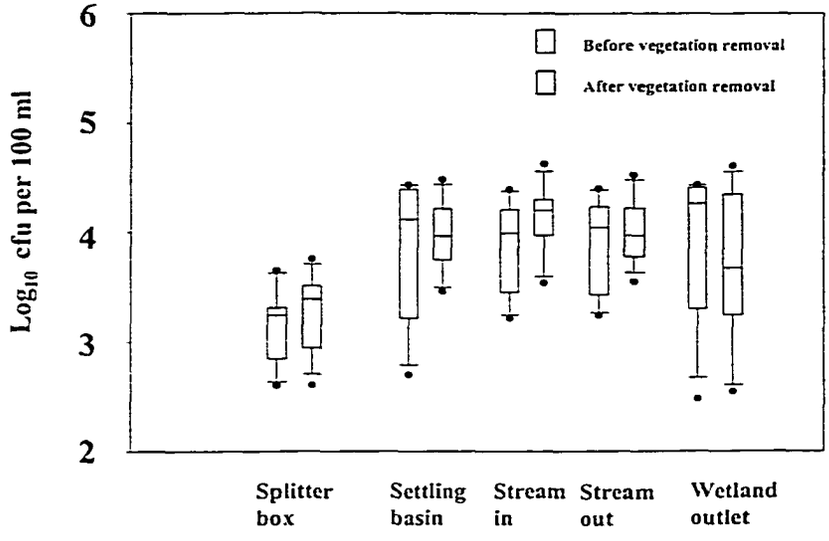

Figure 9. Fecal coliform concentrations in the east polishing system observed during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. In the box plot, the horizontal line shows the median, the box ends indicate the 25th and 75th percentile, error bars the 10th and 90th percentiles, and the dots (•) extreme values.

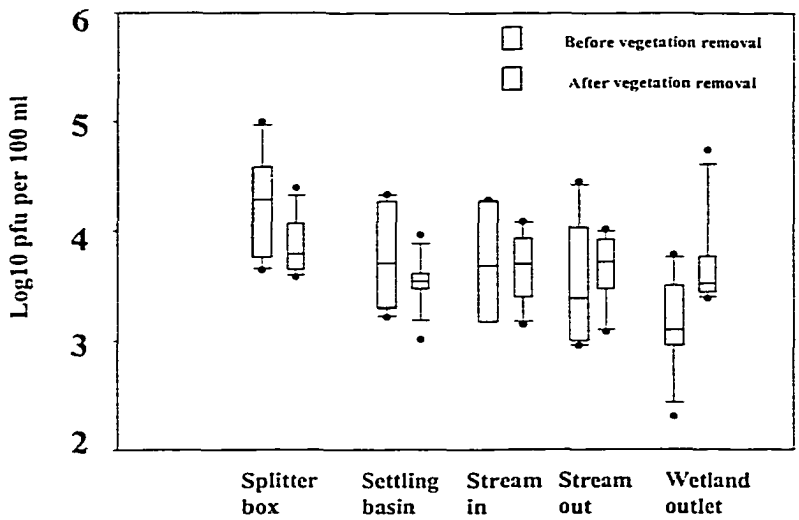

Figure 10. Coliphage concentrations in the east polishing system during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. In the box plot, the horizontal line shows the median, the box ends indicate the 25th and 75th percentile, error bars the 10th and 90th percentiles, and the dots (•) extreme values.

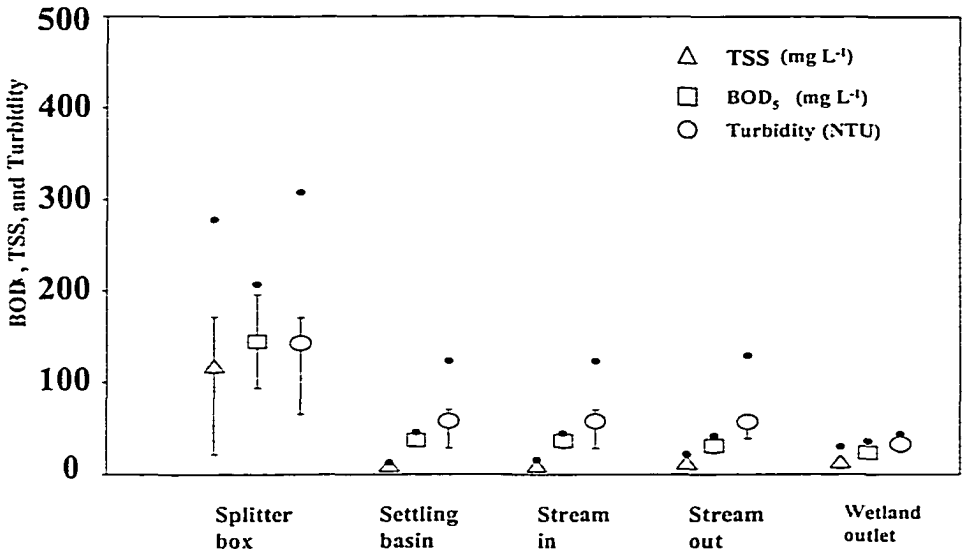

Figure 11. Statistical representation of BOD_5, TSS, and Turbidity observed in the east polishing system during backwash operation before (April through September 1999) and after (February through September 2000) vegetation removal. The symbol represents the mean concentration, error bars the 25 th and 75th percentile, and the dot (•) the maximum observation.