PATHOGEN REMOVAL FROM WASTEWATER BY A DUCKWEED POND

by

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STATEMENT BY AUTHOR

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ABSTRACT

Duckweed plants (*Lemna* spp) are increasingly being used to improve the quality of wastewater in many parts of the world. We investigated a duckweed (*Lemna gibba* L.)-covered pond for its ability to remove *Giardia*, *Cryptosporidium*, enteroviruses, coliphages, and enteric indicator bacteria from unchlorinated secondary effluent. *Giardia* cysts and *Cryptosporidium* oocysts were reduced by 98 and 89 percent, respectively; total coliforms by 61 percent; fecal coliforms by 62 percent; and bacteriophages by 40 percent. The results indicate that the larger organisms (parasites) settled to the bottom of the pond, while the removal of bacteriophages by the pond was not as effective. There was a significant correlation between the removal of *Giardia* cysts and *Cryptosporidium* oocysts by the pond ($p < 0.001$). Influent turbidity and parasite removal were also significantly correlated (*Cryptosporidium* and turbidity, $p = 0.05$; and for *Giardia* and turbidity, $p = 0.01$). However, there appeared to be no correlation between the removal of these parasites and effluent turbidity.

CHAPTER 1

INTRODUCTION

Artificial or constructed wetlands are increasingly viewed as a viable alternative for municipal wastewater treatment before reuse or discharge. In developed countries they are providing secondary and tertiary treatments before reuse or where additional treatment is needed before discharge. In developing countries they can provide a low cost system for the treatment of domestic sewage. They can also be used to provide habitat for wildlife. Recently, attention has been focused on the capability of wetland systems to efficiently remove a wide variety of waterborne pollutants at a considerable savings in capital and energy cost when compared with conventional treatment processes (Gersberg et al., 1987; Karpiscak et al., 1993; Jewel, 1994;). The ability of wetland and aquatic systems to improve the quality of wastewater is well documented (Gersberg et al., 1987; Hammer, 1989; Gilles, 1990; Dortch, 1992; Millin and Heritage, 1992; Mitsch and Gosselink, 1993; Karpiscak et al., 1995; Falabi et al., 1996). The fate of enteric viruses and indicator bacteria in wetland wastewater treatment systems have also been studied recently (Gersberg, et al., 1987; Reed et al., 1995). The use of free-floating aquatic plants in wetland treatment facilities is also increasingly being practiced (Brix, 1993; Hancock and Buddhavaraqu, 1993; Karpiscak et al., 1995; Reed et al., 1995). Different studies have shown that aquatic plants such as water hyacinth (*Eichhornia crassipes* L.), water lettuce (*Pistia stratiotes* L.), cattail (*Typha* spp), and duckweed (*Lemna* spp) are

capable of reducing biochemical oxygen demand (BOD), total suspended solids (TSS), nitrogen, and phosphorus concentrations in wastewater (Dewedar and Bahgat, 1995; Karpiscak et al., 1995). Little or no information is available on the fate of pathogenic protozoan parasites and enteric viruses from secondary sewage effluent applied to constructed wetlands. The removal of pathogenic organisms in aquatic or wetland systems could be the result of several factors, including natural die-off, sedimentation, predation, as well as adsorption (Reed et al., 1995). These factors, in turn, are likely influenced by detention time and seasonal variability.

Pathogens in Domestic Wastewater

Enteric pathogens are those most commonly associated with waterborne disease in the United States (Gerba and Rose, 1993). These organisms include bacteria, viruses, and parasites. Enteric pathogens may be excreted in large numbers by infected individuals and are almost always present in sewage (Gerba and Rose, 1993). Some animals such as beavers and cattle may also excrete these organisms in their feces, directly contaminating water supplies. Many of these excreted microorganisms are capable of surviving for a long period of time in the environment. These microorganisms can also survive conventional wastewater treatment, particularly the viruses and parasites, in concentrations capable of causing disease. Enteroviruses and oocysts of some pathogenic protozoa are somewhat more resistant to disinfection by chlorine, chloramine, or ozone and occasionally active virus particles or live oocysts are recovered from water treated to meet fecal coliform standards (Sobsey and Olson,

1983). Viruses and protozoan parasites now make more than 40 percent of the cases of illness associated with waterborne outbreaks and most of the outbreaks caused by protozoan parasites are associated with disinfected water (Gerba and Rose, 1993).

Bacterial Pathogens

Wastewater bacteria have been characterized and belong to the following groups: (1) gram-negative facultatively anaerobic bacteria (*Aeromonas, Plesiomonas, Vibrio, Enterobacter, Escherichia, Klebsiella,* and *Shigella*); (2) Gram-negative aerobic bacteria (e.g. *Pseudomonas, Alcaligenes, Flavobacterium,* and *Acinetobacter*); (3) Gram-positive spore-forming bacteria (e.g. *Bacillus*); and (4) non-spore-forming gram-positive bacteria (e.g., *Arthrobacter, Corynebacterium, Rhodococcus*). The diseases caused by bacteria found in wastewater include typhoid fever (caused by *Salmonella typhi),* bacillary dysentery (caused by *Shigella),* gastroenteritis (caused by *Escherichia coli, Yersinia enterocolitica, Campylobacter jejuni),* cholera (caused by *Vibrio cholerae*), tuberculosis (caused by *Mycobacterium tuberculosis*), leptospirosis (caused by *Leptospira*) (Sobsey and Olson, 1983).

Viral Pathogens

Enteric Viruses. Water and wastewater can become contaminated by enteric viruses. These viruses enter into the human body orally, multiply in the gastrointestinal tract, and are excreted in large numbers in the feces of infected individuals. Enteric viruses, pathogenic to humans that are found in aquatic environments, include enteroviruses

(poliovirus, coxsackievirus, echovirus, hepatitis A), reovirus, rotavirus, adenovirus, Norwalk agent (calicivirus) and astrovirus. Enteric viruses are responsible for a broad spectrum of diseases that range from skin rash, fever, respiratory infections, and conjunctivitis to gastroenteritis and paralysis. Their presence in a community's wastewater reflects virus infections among the population. From epidemiologic standpoint, enteric viruses are mainly transmitted by person-to-person contact (Bitton, 1994). However they may also be transmitted directly by water (drinking water, swimming, aerosols), or indirectly through contaminated food (e.g., vegetable, shellfish).

Enteric viruses are harbored by all warm blooded animals. These viruses are excreted in fecal material and can find their way into the aquatic environment. Most viruses are highly host specific and only the enteric viruses of humans appear to offer the greatest health concern for waterborne transmission (Gerba and Rose, 1993; Gerba and Rose, 1990). Human enteric viruses are able to exist for extended periods in the environment and many may survive conventional water and wastewater treatment. Enteric viruses are small, ranging in size from 20 nm to 85 nm in diameter. There are more than 110 types of human enteric viruses including enteroviruses, hepatitis A virus (HAV), Norwalk virus, reovirus, rotavirus, and adenovirus. Enteroviruses include polioviruses, coxsackieviruses, and echoviruses. Viruses are obligate intracellular parasites made up of a core of nucleic acid (RNA or DNA) surrounded by a protein coat. They can cause diseases such as paralysis, meningitis, respiratory illness, and diarrhea.

Protozoan Parasites

The major waterborne parasites that cause waterborne illness include *Giardia lamblia, Cryptosporidium, Entamoeba histolytica,* and *Naegleria.* Most protozoan parasites produce cysts that are able to survive outside their host under adverse environmental conditions. Encystment is triggered by factors such as lack of nutrients, accumulation of toxic metabolites, and host immune response. Domestic wastewater is a significant source of *Giardia* and wild and domestic animals constitute important reservoirs of *Giardia* cysts. In the case of *Giardia,* under appropriate conditions, a new trophozoite is released from the cysts (excystment). The major waterborne diseases caused by protozoa include giardiasis, amoebic dysentery, amoebic meningoencephalitis, intestinal ulcer, watery diarrhea, and low grade fever (Sobsey and Olson, 1983).

***Giardia* and *Cryptosporidium*.** The protozoan parasites of primary concern in drinking water and wastewater are *Giardia lamblia* and *Cryptosporidium parvum* (Center for Disease Control), 1978; Rose et al. 1989; Abbaszadegan et al. 1991; Rose et al., 1991; American Public Health Association (APHA), 1992a; Calderon and Craun, 1994). These organisms cause diarrhea or gastroenteritis of varying severity, and many waterborne outbreaks have been attributed to each of these agents. *Giardia* is the most frequently identified etiologic agent in waterborne outbreaks (CDC, 1978; Hibber and Hancock, 1990; Calderon and Craun, 1994). From 1965 through 1990, 111 waterborne outbreaks and more than 26,000 cases were reported (APHA, 1992a).

Cryptosporidium is a protozoan of increasing concern as a human pathogen). It causes a cholera-like diarrhea that is self-limiting in immunocompetent individuals but it may be prolonged and life threatening in immunodefficient persons. *Cryptosporidium* has been associated with traveler's diarrhea and water has been implicated as the vehicle of transmission in several outbreaks (APHA, 1992a). *Giardia* and *Cryptosporidium* are known to be resistant to commonly used water disinfectants and are found in high numbers in wastewater effluent (CDC,1978; Calderon and Craun, 1994; Owens et al., 1994). The water resistance of protozoan parasites to disinfectants and removal by water treatment processes makes them more difficult to control than waterborne enteric bacteria.

Helminth Parasites

Helminth parasites found in wastewater include *Teania* spp, *Ascaris lumbricoides (*roundworms), *Toxocara canis,* and *Trichuris trichiura* (Bitton, 1994). They are excreted in feces and spread by wastewater, soil, or food. Their ova are very resistant to environmental stresses and to chlorination in wastewater treatment plants. Helminth parasites cause a number of diseases including infection of the gastrointestinal tract and ocular damage.

Conventional Wastewater Treatment Technologies

Conventional wastewater treatment is accomplished by physical, chemical, and biological processes. Pretreatment with conventional processes is usually advisable

before discharge into a wetland because of the potential solids or oxygen demand overload that might create nuisance conditions within a wetland receiving raw or inadequately treated wastewater.

Primary Treatment

Primary treatment consists of screening, grit removal, and primary sedimentation. Screening and grit removal may be referred to as "preliminary treatment" because they remove larger solids from the wastewater and the heavier mineral solids that might otherwise erode mechanical equipment such as pumps, valves, and aerators.

Grit in raw wastewater primarily consists of inorganic and organic solids that enter the collection system and include materials such as sand, gravel, seeds, coffee grounds, and other minimally decomposable organic solids. Because grit is more seattleable than more highly decomposable organic solids, it should be removed in the front end of the treatment plant to protect mechanical equipment from abrasion and prevent sedimentation in pipelines and basins. An alternative to screening in preliminary treatment is the use of a comminutor or grinder to reduce the physical size of wastewater solids.

Secondary Treatment

Secondary treatment generally consist of the removal of additional wastewater solids and dissolved organic matter through microbial uptake and growth. Thus

secondary treatment is essentially a biological process in which bacteria and fungi are encouraged to grow in lagoons, mixed tanks, and ponds or a fixed surfaces. The principal secondary treatment technologies are facultative ponds, aerated lagoons, aeration basins with solids recycling (activated sludge), trickling filters, and rotating biological contactors (Kadlec and Knight, 1996). Secondary treatment is the minimum level of municipal and industrial treatment that is required in the United States of America before discharge to most surface receiving waters. Secondary treatment requires a treatment that will produce a minimum reduction of 85 percent in 5-day biochemical oxygen demand (BOD_5) and total suspended solids (TSS) less than 30 mg/liter (Kadlec and Knight, 1996).

Tertiary Treatment

Any treatment beyond secondary is referred to as tertiary treatment. Tertiary treatment also called advanced treatment, usually involves some type of physical chemical processes, such as coagulation, with alum, lime, iron salts or polyelectrolyte and/or passage through activated carbon or resins to remove residual organics. Coagulation seems to be a highly effective method for the removal of enteroviruses from wastewater (Gerba, 1981). Tertiary treatment may also involve processes which remove nitrogen or pathogenic microorganisms.

Treatment wetland and Aquatic Plant Systems

Wetlands: Definition

There are many terms and definitions used to describe wetlands. Wetlands definition often includes three main components: (1) wetlands are distinguished by the presence of water, either at the surface or within the root zone; (2) wetlands often have unique soil conditions that differ from adjacent uplands; and (3) wetlands support vegetation adapted to the wet conditions (hydrophytes), and conversely are characterized by an absence of flooding-intolerant vegetation (Mitsch and Gosselink, 1993). Although the idea of shallow water at saturated conditions, unique wetland soil, and vegetation adapted to wet conditions are fairly straightforward, combining these three factors to obtain a precise definition is difficult because of a number of characteristics that distinguish wetlands from other ecosystems yet make them less easy to define (Mitsch and Gosselink, 1993): although water is present for at least part of the time, the depth and duration of flooding vary considerably from wetland to wetland and from year to year; wetlands are often at the margin between deep water and terrestrial uplands and are influenced by both systems; wetland species (plants, animals, and microbes) range from those that have adaption to live in either wet or dry conditions (facultative) to those adapted to only a wet environment (obligate), making difficult their use as wetland indicators; wetland location can vary greatly, from inland to coastal wetlands and from rural to urban regions; wetlands vary widely by size, ranging from small prairie potholes of a few hectares in size to large expanses of wetlands several hundreds in square kilometers area; wetland condition, or the degree

to which the wetland is influenced by humans, varies from region to region and from wetland to wetland.

According to the United States scientific definition - Fish and Wildlife Service presented in a report entitled *Classification of Wetlands and Deepwater Habitat of the United States* (Mitsch and Gosselink, 1993), wetlands are defined as lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water. Wetlands must have one or more of the following three attributes: (1) at least periodically, the land supports predominantly hydrophytes, (2) the substrate is predominantly undrained hydric soil, and (3) the substrate is non-soil and is saturated with water or covered by shallow water at some time during the growing season of each year. This scientific definition is still one of the most widely accepted in the United States.

The International Union for the Conservation of Nature Resources (IUCN) in the Convention on Wetlands of International Importance Especially as Waterfowl Habitat, better known as *Ramsar Convention*, adopted the following definition of wetlands (Mitsch and Gosselink, 1993): areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish, or salt including areas of marine water, the depth of which at low tide does not exceed 6 meters.

A wetland definition that will prove satisfactory to all users has not yet been developed because the definition of wetlands depends on the objectives and the field of interest of the user (Mitsch and Gosselink, 1993).

Terms and Categories of Wetlands

Various terms are used to describe particular kinds of wetlands. These include bog, bottomland, fen, marsh, mire, pothole, swamp, wet meadow, wet prairie, etc. Treatment wetlands are usually categorized by origin, hydraulic type, and vegetation type (Brown, 1994).

Origin of wetlands can be natural, constructed, or hybrid. A natural treatment wetland is a preexisting wetland that is incorporated into the treatment system. A constructed wetland is a completely artificial wetland built specially for wastewater treatment. A wetland system of hybrid origin has both natural and constructed wetlands as part of the treatment.

The hydraulic type of wetland can either be free water surface (FWS) or subsurface flow (SF). The term subsurface flow is a generic term that includes all types of systems where the wastewater is below the ground level. Other names or types of SF systems include vegetative submerged bed, reed bed, root zone method, rock reed filter, rhizome method, and microbial rock plant filters (Brown, 1994). The media used in SF wetlands can vary from soil, sand, or peat to large (100 to 150 mm) diameter rock or crushed stone. Hybrid systems have both FWS and SF wetlands as part of the treatment system.

Vegetation categories include marsh, forest, and floating aquatic plants. Categorization by vegetation type is not meant to define the plant species, but rather to indicate the predominant type of plant community. Plants of several categories can be present in wetland treatment systems. Marsh wetlands are characterized by emergent

aquatic species such as cattails (*Typha* spp), bulrush (*Scirpus* spp), and common reed (*Phragmites australis*). Forest wetlands are characterized by trees such as cypress (*Taxodium* spp), willow (*Salix* spp), and red maple (*Acer rubrum*). Floating aquatic plant wetlands are characterized by plants such as duckweed (*Lemna* spp) and water hyacinth (*Eichhornia crassipes*) (Brown, 1994).

Constructed Wetland and Aquatic Plant Systems for Wastewater Treatment

Constructed wetland and aquatic plant systems are becoming popular for wastewater treatment around the world. This interest is due to their low construction and maintenance costs and their appeal as natural treatment systems (Brown, 1994). Wetlands are engineered and constructed for four principal reasons: (1) to compensate for and help offset the rate of conversion of natural wetlands resulting from agriculture and urban development (constructed habitat wetlands); (2) to improve water quality (constructed treatment wetlands); (3) to provide flood control (constructed flood control wetlands); and (4) to be used for production of food and fiber (constructed aquaculture wetlands) (Kadlec and Knight, 1996). Constructed wetlands have been used to treat a wide variety of waters including domestic (ranging from individual home to municipal systems), industrial, agricultural, mine drainage, landfill leachate, and urban stormwater.

Wetland Treatment Systems. Constructed wetland treatment systems use rooted, water-tolerant plant species and shallow flooded, or saturated soil conditions to provide

various type of wastewater treatment. While there are many types of naturally occurring wetlands, only those types with plant species that are adapted to continuous flooding are suitable for receiving continuous flow of wastewaters. Also due to their regulatory status, discharges to natural wetlands must receive a high level of pretreatment (minimum of secondary) (Kadlec and Knight, 1996).

Constructed wetlands mimic the optional treatment conditions found in natural wetlands, but provide the flexibility of being constructible at almost any location. They can be used for treatment of primary and secondary wastewaters as well as waters from variety of other sources including, stormwaters, landfill leachate, industrial and agricultural wastewaters, and acid-mine drainage. Surface flow wetlands (natural and constructed) are densely vegetated by a variety of plant species and typically have water depths less than 0.4 m (Kadlec and Knight, 1996). Open water areas may be incorporated into the design to provide for optimization of hydraulics and for wildlife habitat enhancement.

Subsurface flow (SF) wetlands use a bed of soil or gravel as a substrate for growth of rooted wetland plants. Pretreated wastewater flows by gravity, horizontally through the bed substrate where it contacts a mixture of facultative microbes living in association with the substrate and plant roots. SF wetland bed depth is typically less than 0-6 m, and the bottom of the bed is sloped to minimize overland water flow. Typical plant species used in SF wetlands include common reed (*Phragmites australis*), cattail (*Typha* spp), and bulrush (*Scirpus*. spp).

Wetlands have been found to be effective in treating biochemical oxygen

demand, suspended solids, nitrogen, and phosphorous, as well as reducing metals, organics, and certain pathogens. Effective wetland performance depends on adequate pretreatment, conservative constituent and hydraulic loading rates, collection of monitoring information access system performance, and knowledge of successful operation strategies. The most common difficulties experienced in big wetland treatment systems have been related to maintaining partially aerated soil conditions. When the systems are overloaded by oxygen-demanding constituents or are operated with excessive water depth, highly reduced conditions occur in the sediments, resulting in plant stress and reduced removal efficiencies for BOD and ammonia nitrogen. A common problem encountered in SF constructed wetlands is inadequate hydraulic gradient and resulting surface flows.

Natural wetlands, when available, are typically the least expensive treatment alternative, requiring minimal capital expenditures for pumps, pipes and water distribution structures in addition to the cost of the land itself. However, pretreatment and operational monitoring cost are typically higher for discharges to natural wetlands.

Floating Aquatic Plant Systems. Pond can be covered with floating aquatic plants to provide wastewater treatment. Typical plant species that have been used in large-scale applications are water hyacinths (*Eichhornia crassipes*) and duckweed species (*Lemna*, *Spirodella*, and *Wolffia*).

Floating aquatic plant treatment systems are functionally different from facultative pond because the photosynthetic component (floating aquatic plants as

opposed to submerged planktonic algae) is releasing oxygen above the water surface, effectively reducing atmospheric oxygen diffusion. Consequently, floating aquatic plant systems are oxygen deficient, and aerobic processes are largely restricted to the plant root zone.

The majority of the water column in floating aquatic plant systems is generally anaerobic, with the degree of oxygen depletion dependent on the organic loading rate. Treatment occurs in floating aquatic systems through three primary mechanisms: (1) metabolism by a mixture of facultative microbes in the plant roots suspended in the water column and in the detritus at the pond bottom; (2) sedimentation of wastewater solids and of internally produced biomass (dead plants and microbes); and (3) incorporation of nutrients in living plants and subsequent harvest. Floating aquatic plant systems are typically effective at reducing concentrations of biochemical oxygen demand and total suspended solids. But these systems also have some potential weaknesses that have limited their use. Since these systems depend on one or just few plant species for colonization of the pond surface, they are susceptible to catastrophic events which can kill part or all of these populations during a short time period. For example water hyacinths are easily killed by cold weather and attacked by numerous plant pest species.

Duckweed is less sensitive to cold weather and pests than is water hyacinth, but it can also be killed by winter conditions. When plant cover is lost in a floating aquatic plant system, treatment effectiveness may be seriously impaired for a period of weeks or months as new plants are established.

A second potential problem with floating aquatic plant systems results from harvesting biomass for nutrient removal and for maintenance of plant growth at an optimum rate. These plants are more than 95 percent water when harvested, so drying is required, and once dried there is typically a significant residual solids disposal problem although uses such as biofuels or as soil admendments have been proposed.

Duckweeds (*Lemna*, *Spirodella*, and *Wolffia*)

Duckweeds have been investigated much less than water hyacinths for use in wastewater treatment (Brix, 1993). These are small, green, freshwater plants with a leaflike frond a few millimeters in width and a short root, usually less than a centimeter in length (Reed et al. 1995). Duckweeds are the smallest and the simplest of the flowering plants and have one of the fastest reproduction rates. Each frond is capable of producing 10 to 20 more during its life cycle (Hillman and Culley, 1978). Fresh weight measurements have shown that duckweed is about 95 percent water. On average the duckweeds grown on wastewater are composed of 38.7 percent crude protein, 4.9 percent fiber, 15 percent ash, 35 percent carbohydrate, 5.9 percent nitrogen (as N) and 1.37 percent phosphorus (as P) on the dry weight basis. Several nutritional studies have confirmed the value of duckweed as a food source for a variety of birds and animals (Hillman and Culley, 1978). As noted above, duckweeds are more cold tolerant than water hyacinths and are found throughout the world.

Performance Expectations of the Duckweeds in Wastewater Treatment Processes

Duckweeds play a less direct role in the treatment process as they lack extensive root systems and therefore provide a smaller surface area for attached microbial growth. Thus, the main use of duckweeds is in recovering nutrients from secondary treated wastewater (Brix, 1993). A dense cover of duckweeds on the water surface inhibits both oxygen diffusion in the water and photosynthetic production of oxygen by phytoplankton because of the poor light penetration. Consequently, the water becomes largely anaerobic, which in turn favors denitrification. Because of the decrease light penetration, growth of phytoplankton is restricted and consequently the production of suspended solids.

Duckweed systems are capable of high levels of BOD and TSS removal. Wolverton and McDonald (1979) have reported on the performance of a duckweed-covered basin (following an aerated cell) near Biloxi, Mississippi. The organic loading on the basin was about 24 kg/ha.d (21 lb/ac.d). The final effluent from the basin contained 15 mg/L of BOD. The detention time in this basin was 22 days. A study conducted by Karpiscak et al. (1995) showed an average reduction of 52 percent in BOD by a 7 day-detention-time duckweed pond, the pond used in the present study.

Duckweed based systems are susceptible to changing environmental conditions: high winds may pile the duckweed into thick mats and eventually completely sweep the plants from the water surface. To prevent the plants from being blown around, floating booms or cells are usually used to contain the plants.

Objectives of the present study

The objective of this study was to evaluate the ability of an aquatic system covered with duckweed to remove human pathogenic enteric viruses (enteroviruses) and protozoan parasites (*Giardia* and *Cryptosporidium*) from secondary effluent. Physical-chemical data, such as temperature, turbidity, and pH were measured to determine if microbial removal was related to any of these parameters. Samples were also collected to determine the removal of total and fecal coliform bacteria, and coliphages to assess if they could be used to predict the removal of the pathogens by the system.

CHAPTER 2

MATERIALS AND METHODS

Constructed Wetland Facility

The Constructed Ecosystem Research Facility (CERF) was originally conceived in 1983 and began operation in January 1989 (Karpiscak et al, 1995). CERF is located adjacent to the Roger Road Wastewater Treatment facility operated by Pima County in Tucson, Arizona. The facility is operated by The University of Arizona's Office of Arid Land Studies for the Pima County Wastewater Management Department (PCWWMD). There are six raceways (ponds) and receives primary and/or secondary unchlorinated wastewater from the Roger Road facility. The CERF has six raceways which are lined with 30 mil plastic and have a total surface area of about 0.33 ha. Raceway #1 through #5 (Subsurface and surface flow wetlands) are planted with various plant species, such as cattail (*Typha domingensis*), bulrush (*Scirpus olneyi*), giant reed (*Arundo donax*), black willow (*Salix nigra*), cottonwood (*Populus fremontii*), and water hyacinth (*Eichhornia crassipes*). Raceway #6 (aquatic system) is the duckweed (*Lemna gibba* L.)-covered pond used for this study (Figure 1).

The duckweed raceway is 65 m long, 11.9 m wide and 2.6 m deep, with an average influent flow rate of 55 liters per minute (Karpiscak et al. 1995). The depth of water during the period of this study was 0.9 m. The average detention time during the period of the study was estimated at 6 days.

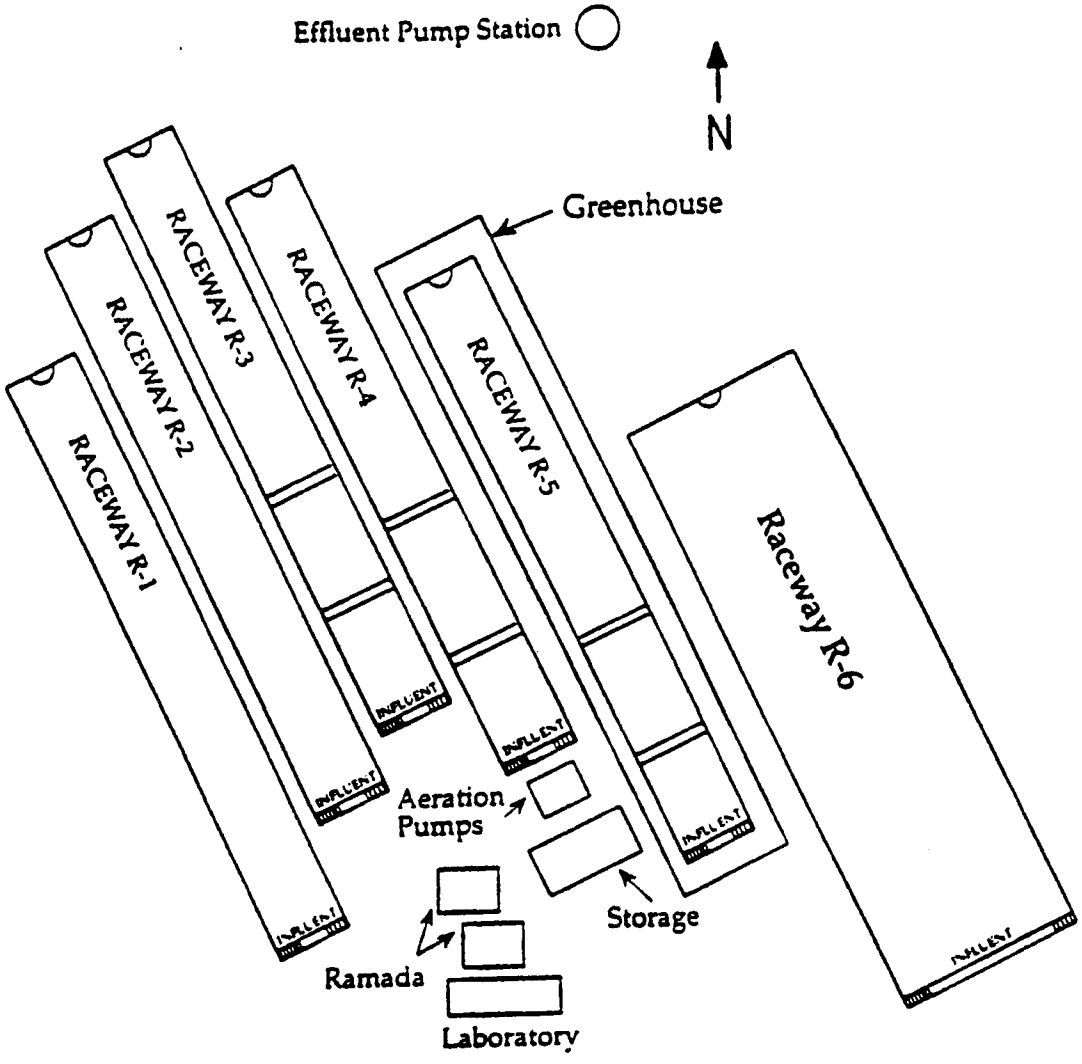

Figure 1: Layout of Constructed Ecosystem Research Facility

Sample Collection and Processing

Water samples of influent (wastewater entering the pond) and effluent (wastewater existing the pond) were collected from June 1994 through May 1996 from the duckweed-covered pond. These samples were analized for *Giardia* cysts and *Cryptosporidium* oocysts (1-10 liters), total and fecal coliforms (50 ml), coliphages (50 ml), and enteric viruses (100-300 liters). The samples were usually collected once a month in sterile plastic bottles and transported on ice to the laboratory for analysis. The samples for total coliform and fecal coliform bacteria were processed within 6 hours and coliphages samples within 72 hours. Parasite samples were concentrated within 48 hours and enterovirus samples were eluted from the filters within 72 hours.

Giardia and *Cryptosporidium* Detection

Giardia and *Cryptosporidium* were detected simultaneously using an immunofluorescent method with slight modification. One to four liter volumes of influent and effluent wastewater were collected directly from the pond in sterile plastic bottles. The bottles were kept at 4°C and shipped to the laboratory for further analysis. The immunofluorescent method includes three major steps: parasite concentration into a pellet, pellet floatation to clarify the samples, and antibody staining for the detection of parasite using a microscope with fluorescent light. *Giardia* cysts and *Cryptosporidium* oocysts were concentrated from the water samples by centrifugation at 1050 g for 15 minutes. Centrifugation was performed using 750-ml centrifuge bottles and a swinging bucket rotor centrifuge (Jouan, Inc. Winchester,

VA). Without disturbing the pellet, the supernatant in the bottles was aspirated from the tube. The pellet was resuspended and diluted in eluting solution using a vortex mixer. The eluting solution is a mixture of 100 ml of 1 percent sodium dodecyl sulfate: SDS (1 g of SDS mixed with 100 ml of distilled water), 100 ml of 1 percent of polyoxyethylene-sorbitan monooleate also called Tween 80 (1 ml of Tween solution mixed with 100 ml of distilled water), 100 ml of 10x phosphate buffered saline: PBS (80 g of NaCl, 2 g of KH_2PO_4, 29 g of $Na_2HPO_4\,12H_2O$ or 12.72 g of Na_2HPO_4, and 2 g of KCl mixed with one liter of distilled water, pH 7.4), 0.1 ml of antifoam A (Sigma Chemicals CO, ST Louis, MO) and 700 ml of distilled water, pH 7.4 (APHA, 1992). No more than 1 ml of packed pellet was processed per floatation tube. The suspension was processed with Sheather's floatation solution (500 g of sucrose, 320 ml of distilled water and 9.7 ml of liquid phenol) with a specific gravity of 1.24. As a result, *Giardia* cysts and *Cryptosporidium* oocysts were separated from some of the particulate matter. The separated material was then distributed in a monolayer on 25 mm-diameter cellulose acetate filters with 0.2-μm pore size (Costar Corp. Commence Circle, Pleasanton, CA). The filters were labeled with fluorescent antibodies (Hydrofluor™ Combo Meridian Diagnostics,Inc., Cincinnati, OH) by an indirect staining procedure. These fluorescent antibodies are specific for both *Giardia* cysts and *Cryptosporidium* oocysts. During each assay, positive and negative controls were done to ensure that assay reagents worked properly. The filters were then examined microscopically. Cysts and oocysts were identified according to specified criteria: immunofluorescence, size, shape, and internal morphological characteristics

(APHA, 1992a). The results were reported as the total number of *Giardia* and *Cryptosporidium* per liter of sample.

Detection of Enteroviruses

Enteroviruses were concentrated from the secondary effluent water by adsorption-elution onto positively charged MK filter (Zeta Plus, CUNO Inc., Meriden, CI) with 5-μm pore size (Ma et al., 1994). Wastewater at the site was pumped through a MK filter contained inside a filter housing with the aid of a Homelite P100 waterburg gasoline pump (Homelite Textron, Charlotte, NC). A flow meter placed after the filter allowed a determination of the quantity of water filtered. Volumes of 90 to 387 liters of wastewater were filtered at each collection. After the desired volume was processed, the filters were placed in a plastic Ziploc^{R} bag and shipped on ice to the laboratory for further analysis. The filters were processed within 72 hours after collection. Viruses adsorbed to the MK filter were eluted by passage of about a liter of 3 percent beef extract through the filter (Becton Dickinson Microbiology Systems, Cockeysville, MD) by applying air pressure. The 3 percent beef extract (BE) was prepared by mixing 15 g of BE, 3.75 g of glycine (Bio-Rad Laboratories, Richmond, CA) with one liter of distilled water at pH 9.4 - 9.5. The eluate was frozen at minus 70°F if the samples were not to be reconcentrated the next day. In case the reconcentration was done the following day, the eluate was placed in a refrigerator at 4°C. The frozen eluate was thawed at 37°C for further analysis. After the elution step, the samples were reconcentrated and clarified. The eluate was

reconcentrated by precipitation of the proteins and viruses by adjustment of the pH to 3.5 by addition of 1N HCl. The eluate was then centrifuged at 2000 g for 30 min using a swinging bucket rotor centrifuge (Jouan, Inc. Winchester, VA). The pellet obtained after centrifugation was resuspended in 0.15M sterile sodium phosphate, dibasic solution ($Na_2HPO_4 .7H_2O$: 40.2 g of sodium phosphate dissolved in 1000 ml at pH 9.0 - 9.5). The suspension was clarified using Freon (1,1,2 trichloro- 1, 2, 2,- trifluoroethane, Fisher Scientific, Pittsburgh, PA). An equal volume of Freon was thoroughly mixed with the virus suspension and then centrifuged for 15 min. The top layer containing the viruses was collected with a pipette and placed in a 50 ml flat top cap disposable polypropylene centrifuge tube (Corning Costar Corporation, Cambridge, MA). The extract was then treated with various antibiotics: Penicillin (20,000 U/ml), Streptomycin (20,000 U/ml), Gentamicin 100 µg/ml, Mycostatin, and Kanamycin (20,000 U/ml) (Life Technologies, Gaithersburg, MD). The extract containing the antibiotics was incubated for 30 min in a water bath. The sample was then dispensed in 20-ml vials, labelled, and stored in a freezer for cell culture assay.

Water concentrates were assayed for enteroviruses using the Buffalo Green Monkey (BGM) kidney cell line. Samples (1-3 ml) were inoculated onto 75 cm^2 cell culture flasks and observed for cell destruction (CPE, cytopathic effect) for 14 days. The number of viruses was determined by a MPN (most probable number method) (APHA, 1992).

Detection of Indicator Bacteria

Total and fecal coliforms were detected by membrane filtration on selective media: mEndo agar (DIFCO Laboratories, Detroit, MI) for total coliforms and mFC agar (DIFCO Laboratories, Detroit, MI) for fecal coliforms according to the Standard Methods (APHA, 1992b). The samples were diluted using a Tris-Buffered saline solution which was prepared by mixing 1600 ml of distilled water, 63.2 g of Trizma Base: Tris[hydroxymethyl]amino methane (Sigma Chemicals CO, ST Louis, MO). Ten-fold dilution of the samples was done: 0.3 ml of the original or diluted samples was added to 2.7 ml of Tris-buffered saline to obtain the different dilutions. Various dilutions of the samples were filtered through 0.45 μm pore size filters (Gelman brand GN-6, Gelman Sciences, Ann Arbor, MI). The filters were then placed on the selective medium and incubated for 24 h at 37^0C. The results were expressed as number of colony forming units (CFU) per 100 ml of wastewater.

Detection of Coliphages

Coliphages were detected by the double layer agar method described by Adams (1959). The host bacteria used for the assay was *Escherichia coli*, strain ATTC 15597 (American Type Culture Collection, Rockville, MD). Wastewater samples were filtered through 0.22-μm pore size filters (Gelman Sciences, Ann Arbor, MI) to remove bacteria that can interfere with the visualization of the coliphage plaques. The filters were treated with 3 ml 1.5 percent beef extract (Becton Dickinson Microbiology Systems, Cockeysville, MD) to avoid phage adsorption to the filters. The filtered

samples (1 ml) to be assayed and 1 ml of the host strain culture were added to the previously melted soft top agar (Tryptic Soy Broth + 1% agar). The top agar was melted by placing the tubes containing the top agar inside a steamer for 20 min. The top agar with the host strain and the sample was then overlaid onto the bottom agar (Tryptic Soy Broth + 1.5 % agar) in a petri dish (9 cm in diameter). All samples were assayed in triplicate. The agar plates were allowed to solidify and then incubated at 37^0C. Plaque enumeration was determined after 18 h of incubation.

Determination of Physical/Chemical Parameters

The influent and effluent water turbidity was determined by using a turbidimeter 2100P (Hach Company, Loveland, CO). The water pH was determined by using both pH indicator strips (EM Science, Gibbstown, NJ) and Corning pH meter, model 345 (Corning Inc., Corning, NY). The water temperature was determined using a water thermometer.

Statistical Analysis of the Data

The percent removal of the studied microorganisms was calculated using the following formula:

Percent removal = $(N_{influent} - N_{effluent}) \times 100/N_{influent}$

Where, $N_{influent}$ = Number of microorganisms in the influent wastewater

$N_{effluent}$ = Number of microorganisms in the effluent wastewater

Cysts, oocyst, fecal coliform, total coliform and coliphage percents removal

were transformed for analysis using $\log_{10}(y+1)$ where y = number of microorganisms. Arithmetic averages were calculated and correlation coefficients were developed for turbidity, temperature, and each of the microorganisms studied by using Microsoft Excel version 5.0. The average percent removal of microorganisms was calculated by using the above formula. The values of $N_{influent}$ and $N_{effluent}$ used, were the average numbers of microorganisms in the influent and effluent.

CHAPTER 3

RESULTS

During the period of this study, 21 samples were collected and analyzed for various microorganisms. Figures 1-7 present the data for each of the microorganisms studied and a summary is shown in Table 1. Decreases were observed for all microorganisms after the wastewater had passed through the duckweed pond. In general, the number of the microorganisms in the incoming wastewater (influent) was higher than in the outgoing (effluent) wastewater (Figures 1-6, Appendix Tables 1-5).

Table 1. Average densities and removal of microorganisms by the duckweed pond

Microorganisms	Influent	Effluent	Percent removal	Size of organisms (μm)
Giardia (per liter)	15.6	0.35	98	8 -12
Cryptosporidium (per liter)	1.58	0.17	89	2-6
Total coliforms (per 100 ml)	4.24 x 10^6	1.65 x 10^6	61	1.1-1.5
Fecal coliforms (per 100 ml)	1.77 x 10^6	5.97 x 10^5	62	1.1-1.5
Coliphages (per ml)	1233	742	40	0.045-0.065

Removal of *Giardia* and *Cryptosporidium*

During the period from July 1994 through December 1995, the number of *Giardia* cysts in the influent to the duckweed pond ranged from 2 to 28 per liter, while no more than 1 cyst per liter was detected in the effluent (Figure 2, Appendix Table 1). The percent reduction ranged from 50 to 99 percent with no apparent seasonal trend in the removal. There was an increase in the number of *Giardia* cysts in the influent during the fall and winter months of September through April 1995 (Figure 2). *Giardia* cysts were detected in all 17 influent samples and in 14 out of 17 effluent samples. During the period of this study, the average number of *Giardia* cysts in the duckweed pond was 15.6 cysts per liter in the influent and 0.35 cyst per liter in the effluent. The average removal rate was 98 percent (Appendix Table 1).

Cryptosporidium sp. concentrations ranged from 0 to 3 oocysts per liter in the influent, while no more than one oocyst per liter was detected in the effluent (Figure 3, Appendix Table 2). The observed percent reduction ranged from 0 to 99 percent. On average, *Cryptosporidium* oocysts decreased by 89 percent with an average number of 1.58 oocyst per liter in the influent and 0.17 oocyst per liter in the effluent. Oocysts appear to be slightly more prevalent during the months of July through October of 1994 in the influent (Figure 3). In the five instances when no oocysts were found in the influent, there were none observed in the effluent.

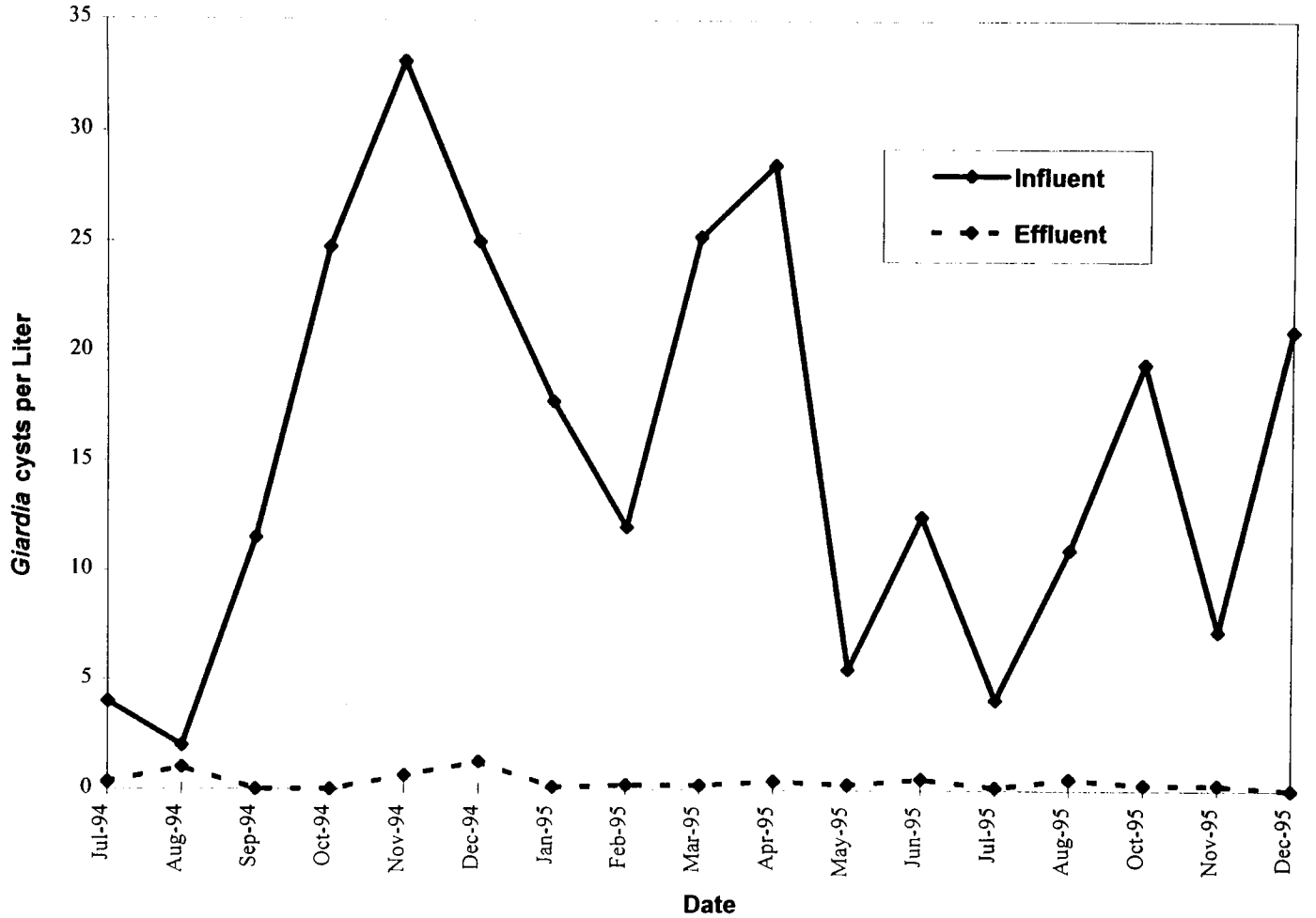

Figure 2. Reduction of *Giardia* cysts by the duckweed pond

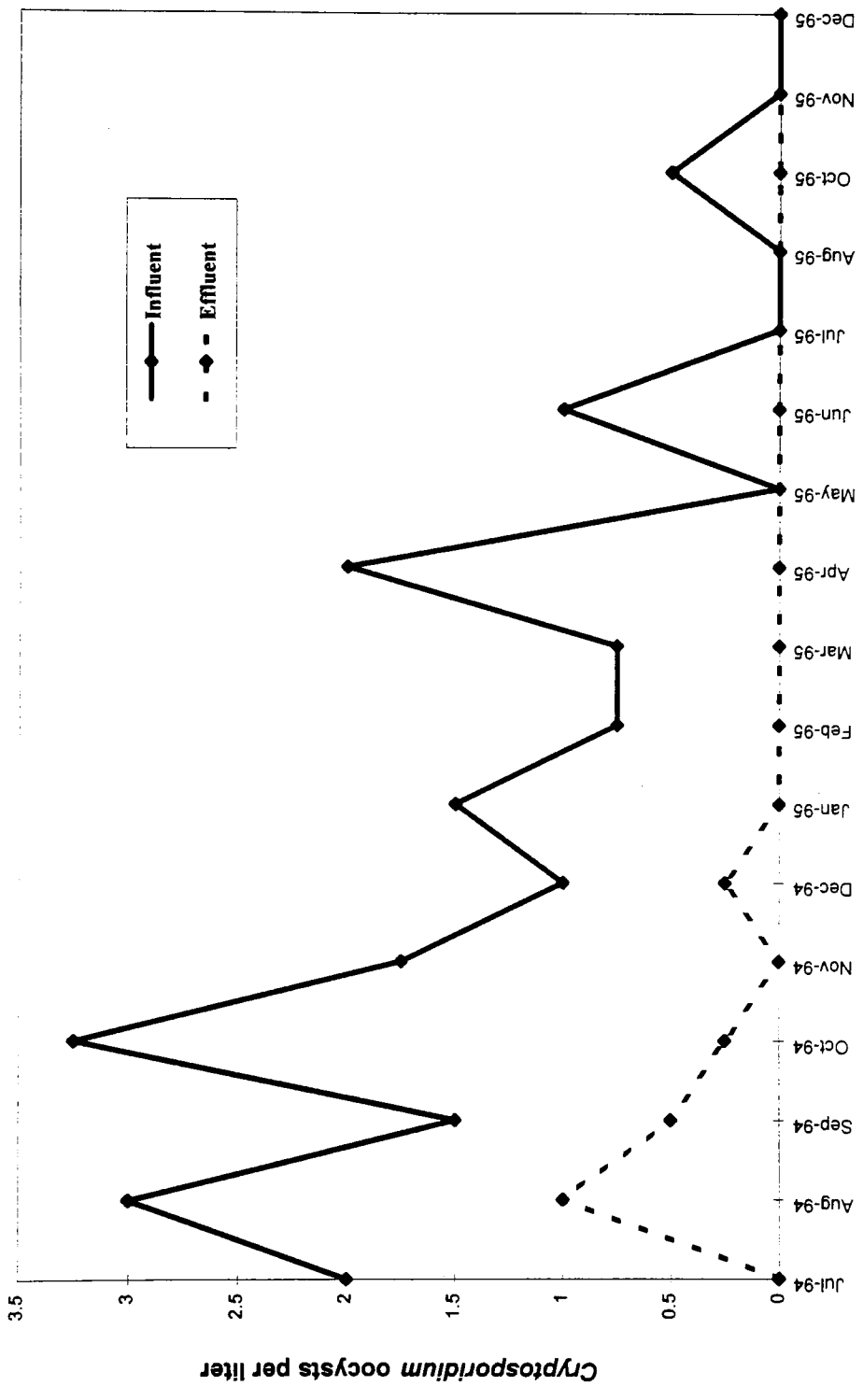

Figure 3. Reduction of *Cryptosporidium* by the duckweed pond

Removal of Coliform Bacteria

Total coliform bacteria in the pond ranged from 1.5 x 10^5 to 8.75 x 10^6 colony forming units per 100 ml in the influent, and from 8.5 x 10^4 to 3.8 x 10^6 colony forming units per 100 ml in the effluent. The reduction in total coliform bacteria by the duckweed pond ranged from 22 to 97 percent (Figure 4, Appendix Table 3). The number of fecal coliforms per 100 ml wastewater ranged from 1.3 x 10^5 to 5.3 x 10^6 in the influent and from 6.5 x 10^4 to 1.2 x 10^6 in the effluent. The percent removal ranged from 40 to 85 percent (Figure 5, Appendix Table 4).

Coliphage Removal

Coliphage (Plaque Forming Units) ranged from 653 to 1987 per liter in the influent and from 69 to 1454 per ml in the effluent. The reduction for coliphage from influent to effluent was between 5 and 94 percent (Figure 6, Appendix Table 5).

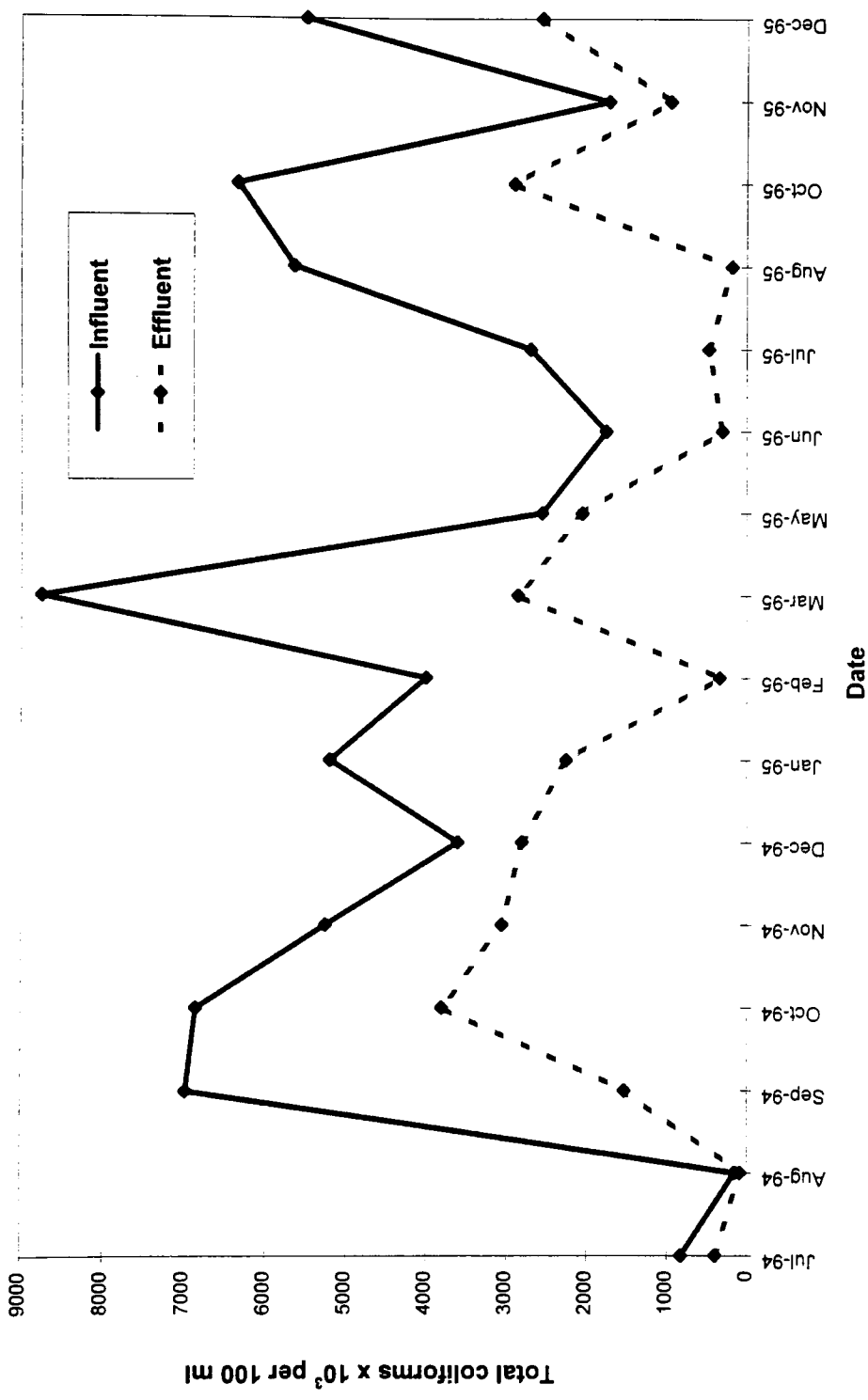

Figure4. Reduction of total coliforms by the duckweed pond

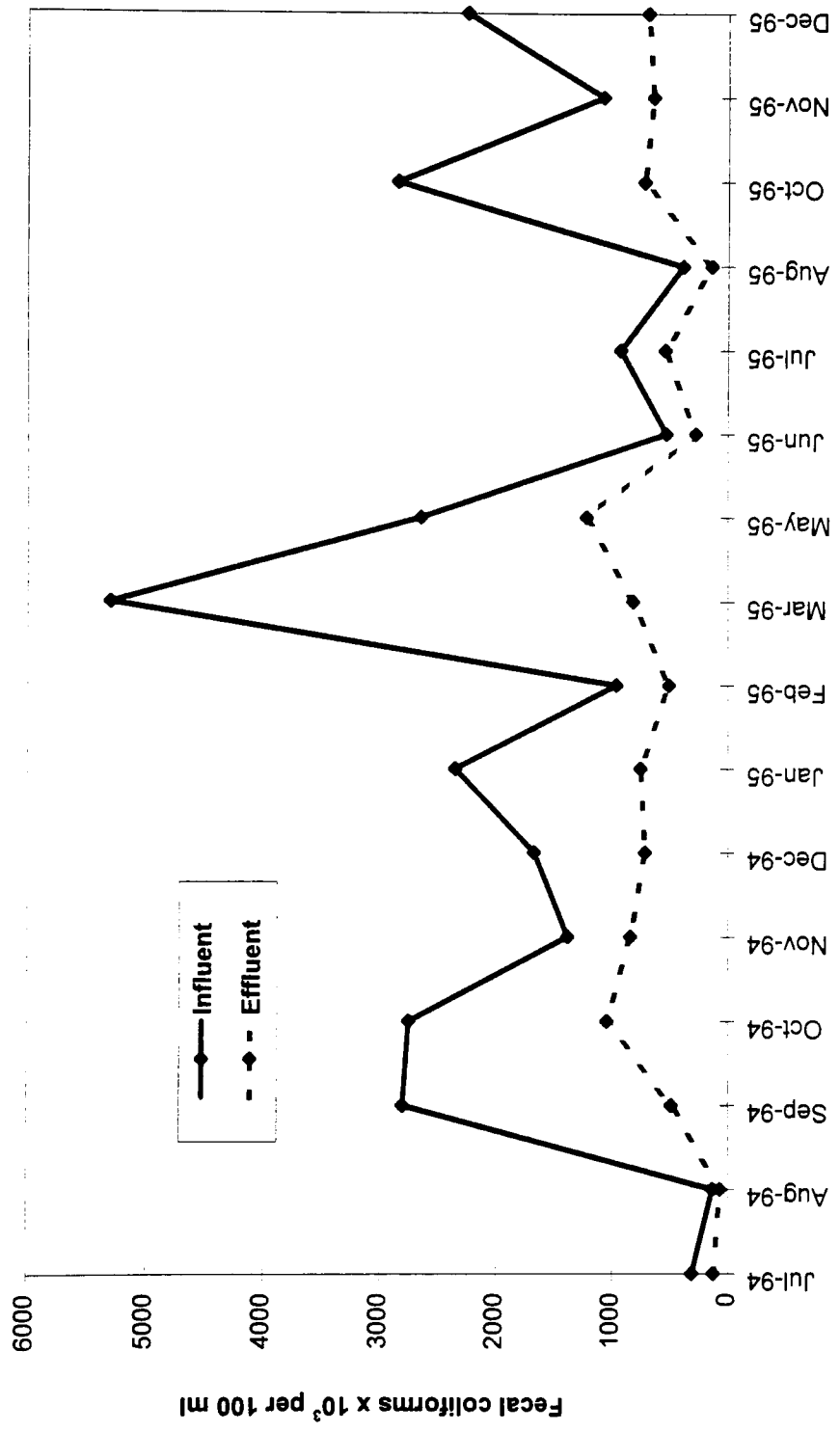

Figure 5. Reduction of fecal coliforms by the duckweed pond

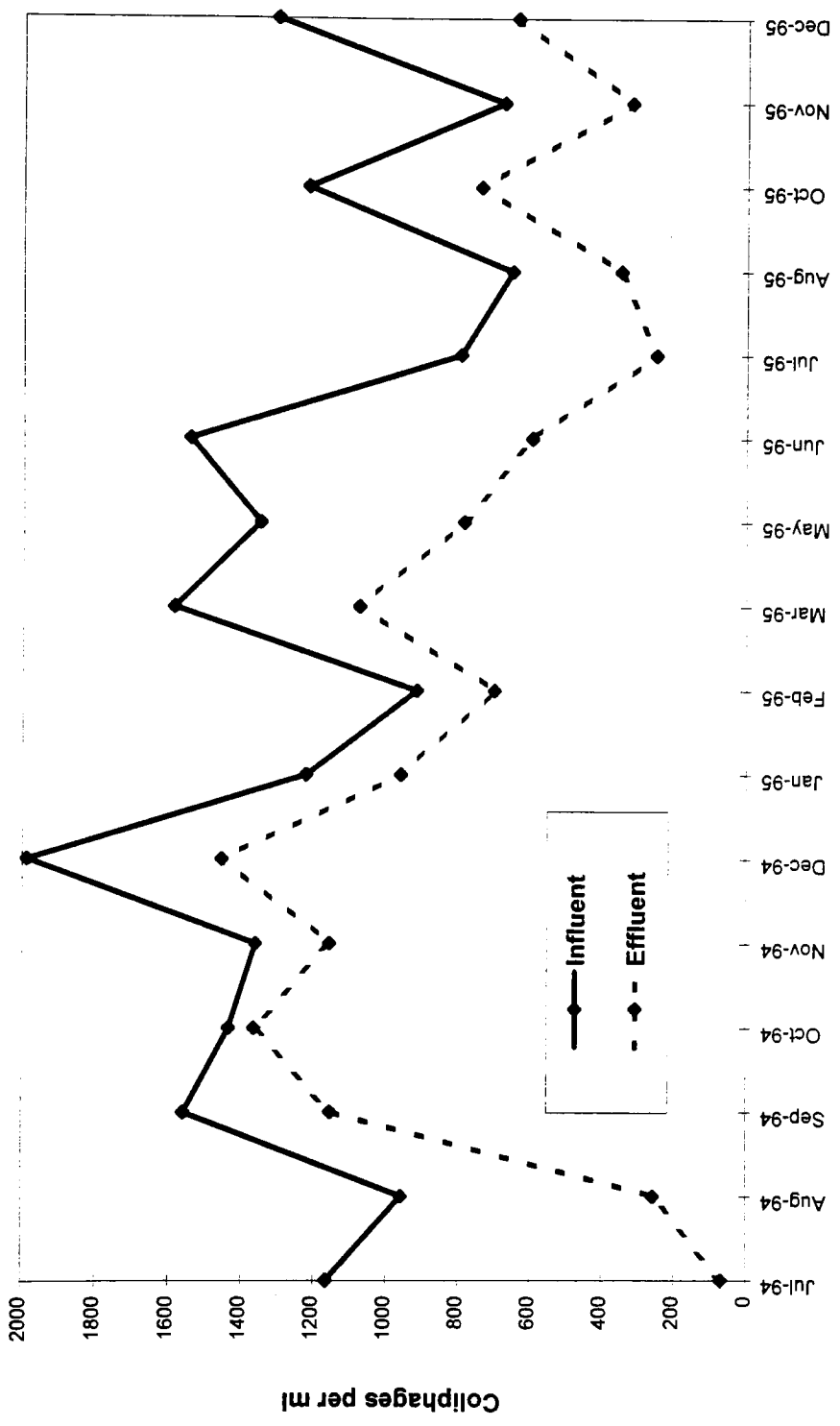

Figure 6. Reduction of coliphages by the duckweed pond

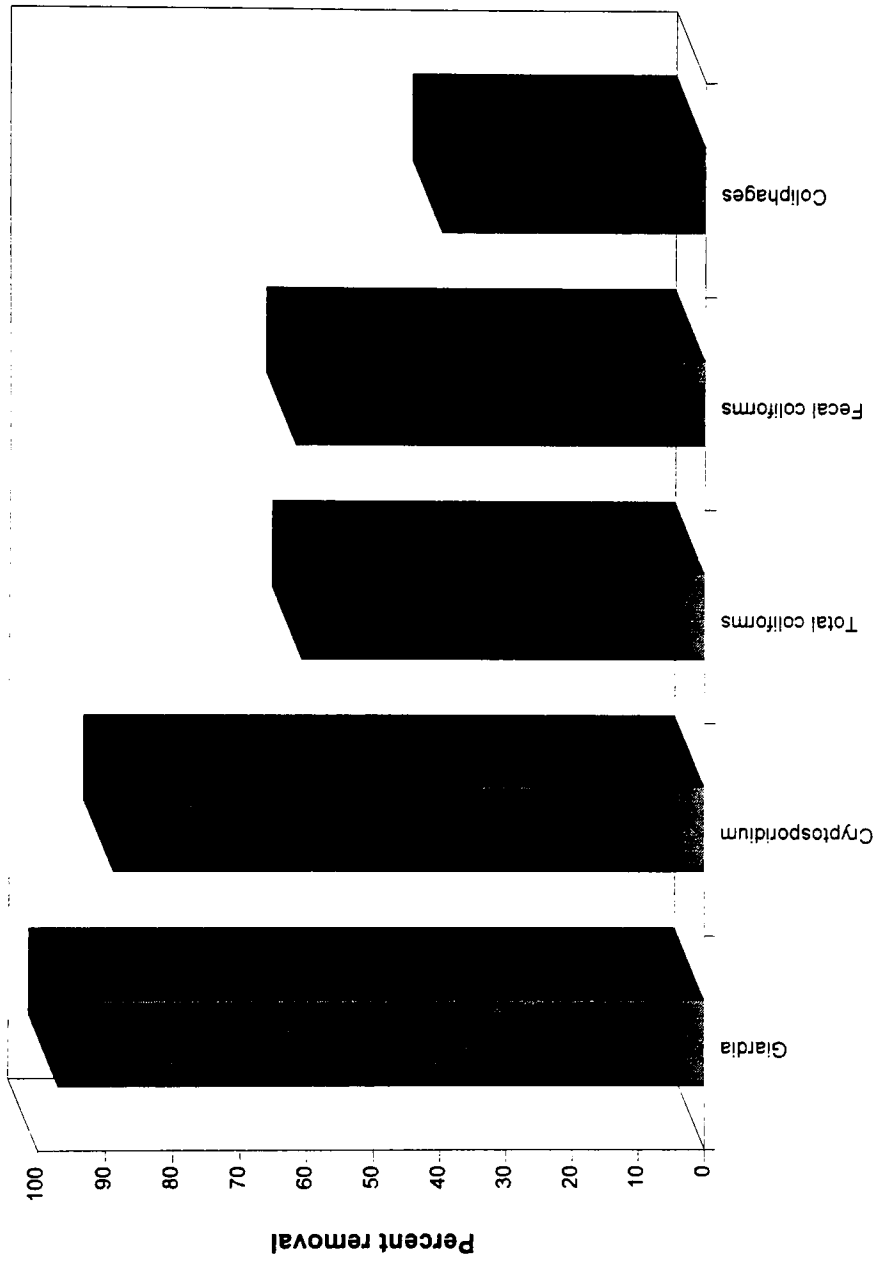

Figure 7. Average removal of the studied microorganisms by the duckweed pond

Enterovirus Removal

No enteroviruses were detected in the influent and effluent of the duckweed pond. Volumes of 90 to 387 liters of wastewater was filtered and about 30 ml of concentrate was obtained. Volumes of 9 to 12 ml of that concentrate was assayed for each sample (Table 1).

Physical/Chemical Parameters

During the period of this study, temperature of the influent to the duckweed pond ranged from 21 to 32°C, and from 11 to 31°C in the effluent. The turbidity values ranged from 3.3 to 23.6 NTU (Nephelometric Turbidity Unit) for the influent and from 10.4 to 74.6 for the effluent. The turbidity removal by the pond ranged from 0 to 49 percent. The influent pH ranged from 6.6 to 8.38 and the effluent pH, from 6.5 to 8.2 (Tables 3 & 4).

Table 2. Densities of enteroviruses in the duckweed pond (July 1995 - May 1996)

Date	Sample volume assayed (liter)		Enteroviruses (liter)		
	Influent	Effluent	Influent	Effluent	Percent removal
7/17/1995	50.5	45	0	0	ND
8/1/1995	57.5	56	0	0	ND
12/22/1995	57	54.5	0	0	ND
1/12/1996	49	69	0	0	ND
3/11/1996	68	45.5	0	0	ND
3/31/1996	71.5	110.5	0	0	ND
4/7/1996	50	49	0	0	ND
5/16/1996	189	193.5	0	0	ND

ND: Not determined

Table3. Physical/chemical parameters of the pond influent (July 1994 - May 1996)

Months	Temperature (°C)	pH*	Turbidity (NTU)
Jul 94	32	6.96	6.3
Aug 94	32	7.1	3.3
Sept 94	27	6.85	ND
Oct 94	27	7.1	13.0
Nov 94	22	6.65	13.4
Dec 94	22	6.8	23.6
Jan 95	21	7.92	11.6
Feb 95	21	8.12	16.5
Mar 95	24	8.38	17.4
Apr 95	26	8.14	18.0
May 95	27	8.25	8.2
Jun 95	28	7.37	20.7
Jul 95	30	8.23	6
Aug 95	31	7.58	12.4
Oct 95	29	7.89	14.3
Nov 95	24	8.04	12.7
Dec 95	21	7.61	ND
Jan 96	21	7.71	9.4
Mar 96	24	7.64	17.1
Apr 96	22	7.95	8.8
May 96	30	ND	20.1

ND: Not determined

*: pH from July 1994 through December 1995 was measured with pH indicator strips

Table 4. Physical/chemical parameters of the pond effluent (July 1994 - May 1996)

Months	Temperature (°C)	pH*	Turbidity (NTU)
Jul 94	31	6.96	18.8
Aug 94	31	6.80	17.6
Sept 94	24	6.85	ND
Oct 94	24	7.20	17.9
Nov 94	15	7.80	20
Dec 94	15	6.50	12
Jan 95	18	7.99	13.5
Feb 95	18	7.76	10.9
Mar 95	24	7.93	11
Apr 95	32	8.20	23.1
May 95	30	7.92	17.6
Jun 95	29	7.34	17.2
Jul 95	30	7.86	11
Aug 95	31	7.52	10.5
Oct 95	26	7.78	26.2
Nov 95	15	7.86	41.3
Dec 95	11	7.59	ND
Jan 96	13	7.63	10.4
Mar 96	16	7.55	17.9
Apr 96	15	7.92	34.6
May 96	21	ND	74.6

ND: Not determined

*: pH from July 1994 through December 1995 was measured with pH indicator strips

CHAPTER 4

DISCUSSION

The pond system examined in this study received unchlorinated secondary effluent from the Roger Road Wastewater Treatment Facility in Tucson, AZ. During the period of this study, *Giardia* cysts were detected in all 17 influent samples examined. The influent to the duckweed pond was the unchlorinated secondary effluent from the Roger Road Treatment Facility, while the effluent is the water coming out from the pond after the unchlorinated secondary effuent has passed through it. Only three out the 17 effluent samples from the duckweed pond did not contain *Giardia* cysts. In the effluent, *Cryptosporidium* oocysts were detected in only 4 samples out of a total of 17 analyzed. In the 17 influent samples collected, oocysts were not detected in 5 samples.

Grimason et al. (1993) studied the occurrence and removal of *Cryptosporidium* oocysts in Kenyan waste stabilization ponds. Cyst levels that they detected in raw wastewater samples ranged from 212 to 6212 cysts per liter. They also observed that the number of oocysts in the effluent from these ponds ranged between 3 to 230 cysts per liter. No *Cryptosporidium* oocysts were noted in the final effluent from the 11 ponds studied. The minimum detention time for the removal of *Cryptosporidium* oocysts and *Giardia* cysts by the stabilization ponds was 37 days. In the duckweed-covered pond at CERF *Cryptosporidium* oocysts were detected in only 4 out of 17 effluent samples after an estimated 6-day detention time.

Total and fecal coliform bacteria were reduced, on average by 61 and 62 percent respectively in the duckweed pond. This compares to the 91 to 99 percent removal noted by EPA (1983) for fecal coliforms in facultative ponds with detention times of 47 to 180 days.

Coliphage reduction averaged about 40 percent the duckweed pond. Gersberg et al. (1987) found that the total number of the indigenous F-specific bacteriophage (F-specific RNA and F-specific DNA phages) was reduced by about 99 percent after passage through a constructed wetland composed of bulrush planted in gravel with a detention time of 5.5 days. They also added poliovirus to the wetland water during the experiment. Poliovirus was reduced by 99.9 percent. The authors concluded that artificial wetlands may offer an attractive alternative to land treatment systems for reducing the load of disease-causing viruses to the aquatic environment (Gersberg et al., 1987).

Coliphages are found in abundance in wastewater and sewage polluted water and their concentrations exceed those of enteric viruses. Many coliphages are more resistant to inactivation by adverse environments and disinfection than enteroviruses. Gerba (1987) stated that the above characteristics and others make the use of coliphages attractive as indicators of enteric viruses (Gerba, 1987). Because no enteroviruses were found during this study, additional data are needed to determine if coliphages can be used as indicators of enteric viruses (which include enteroviruses) in the duckweed pond.

In the samples analyzed, no enteroviruses were detected (Table 2). About half

of the 30-ml concentrates obtained from the original volumes of water filtered (90 to 387 liters) was analyzed for enteroviruses. The absence of enteroviruses in half of the sample volumes does not imply their absence in the secondary effluent applied to the pond. This finding does indicate the numbers of enteroviruses are probably small and therefore would require larger volumes of samples to detect their presence. Bitton (1994) noted that large volume environmental samples must be collected in order to detect enteroviruses. Conventional treatment is known to remove large numbers of viruses from wastewater. Primary treatment removes 0 to 50 percent of the viruses initially present, while secondary (biological) can be expected to remove 90 to 99 percent (Gerba and Rose, 1990). The duckweed pond investigated in the present study receives secondary effluent. One of the reasons no enteroviruses were detected in the samples analyzed would probably be the low numbers of the latter in the secondary effluent. Another possible explanation may be the low recovery efficiency of viruses using MK filters. Rose et al. (1984) detected from 2 to 600 enteric viruses from 10 liters of unchlorinated secondary effluent using a positively charged filter 1-MDS Virozorb. In that study, up to 100 liters of sample were filtered at each collection. The authors also compared 1-MDS Virozorb with other positively charged filters (50S and 30S Zeta-plus). No statistically significant difference was observed in the recovery rate of enteric viruses among the filters studied. Ma et al. (1994) compared the efficiency of poliovirus 1 (PV1) and coxsackievirus (CB3) recovery from tap water using MK and 1-MDS filters. At high virus inputs (10^6 PFU), the overall recovery of PV1 and CB3 from tap water with the MK filter was less than that achieved with the

1-MDS filter ($p < 0.05$). The recovery of PV1 from tap water with the MK and 1-MDS filters were 73.2% ± 26% (n = 5 trials) and 90.2% ± 5.9% (n = 5 trials), respectively. The recoveries of CB3 with the MK and 1-MDS filters were 32.8% ± 34.5% (n = 4 trials) and 95.8% ± 12.0% (n = 4 trials), respectively. The authors concluded that the MK filter consistently provided lower recovery with wider variability, of PV1 and CB3 from tap water than the 1-MDS filters. Increasing the sample volume or using 1-MDS filters may allow the detection of enteric viruses in the pond influent because the absence of viruses in 45-193 liters (half of volumes processed) of unchlorinated secondary effluent wastewater does not imply their absence in a larger volume.

Pathogen removal in pond systems is believed to be due to natural die-off, sedimentation, and adsorption (Reed et al., 1995). Helminths, ascaris, and other parasitic cysts and eggs settle to the bottom in the quiescent zone of the pond. Duckweeds lack extensive root systems onto which microorganism can become attached, and they also decrease sunlight below the duckweed mat; therefore, the removal of microorganisms in duckweed-covered ponds is likely the result of sedimentation. In this study, the larger the organisms, the greater the percent removal (Table 1). The larger organisms settle more rapidly to the bottom of the pond while the removal of viruses was not as effective. Studies done by Reed et al. (1995) on the removal of fecal coliforms and enteric viruses in multiple-cell pond systems showed a significant reduction in the number of microorganisms after passage through the pond. The removal of enteric viruses by a three-cell facultative pond in Shelby,

MS, was over 99 percent. The detention time was 72 days. The same removal (> 99%) was obtained by the authors with a three-cell facultative pond located in El Paso, TX, with a detention time of 35 days.

Removal or inactivation of indicator and pathogenic bacteria in oxidation ponds is controlled by a variety of factors among which are temperature, sunlight, pH, lytic action of bacteriophages, predation by macroorganisms, and attachment to settleable solids (Bitton, 1994).

Correlation coefficients developed for the data show no associations between the removal of coliphage, coliform bacteria, and *Giardia* (Table 7). There was no correlation between *Giardia*, *Cryptosporidium*, coliform bacteria, and coliphage removal, and the water pH and temperature (Tables 5 & 6). There was a correlation between the removal of total coliforms and *Cryptosporidium* oocysts ($p = 0.10$). However, more data are needed to conclude about the significance of that correlation. *Giardia* cyst and *Cryptosporidium* oocyst removal and influent turbidity were significantly correlated ($p = 0.01$ for *Giardia* and turbidity; and $p = 0.05$ for *Cryptosporidium* and turbidity) (Table 5). The removal of *Cryptosporidium* oocysts and *Giardia* cysts were significantly correlated ($p < 0.001$). Rose et al., (1991)

Table 5. Correlation coefficients for influent turbidity, temperature, pH , and the removal of the microorganisms

	Temperature	Turbidity	pH
Giardia	- 0.45385	0.6540[a]	0.25217
Cryptosporidium	0.35268	0.56477[b]	0.35637
Total coliforms	0.14841	0.06551	0.20686
Fecal coliforms	- 0.02242	0.21234	0.27619
Coliphages	- 0.26900	0.08110	-0.45365

N = 15
[a]: p = 0.01
[b]: p = 0.05

Table 6. Correlation coefficients for effluent turbidity, temperature, pH , and the removal of the microorganisms

	Temperature	Turbidity	pH
Giardia	-0.34026	0.0930	0.41276
Cryptosporidium	-0.28833	0.17971	0.47379
Total coliforms	-0.10590	-0.07474	0.30807
Fecal coliforms	-0.17362	0.14673	0.29312
Coliphages	-0.07493	-0.06333	-0.55122

N = 15

Table 7. Correlation coefficients for coliform bacteria, *Giardia*, *Cryptosporidium*, and coliphage removal

	Giardia	*Cryptosporidium*	Total coliforms	Fecal coliforms	Coliphages
Giardia	1	0.88960	0.11954	0.17711	0.08957
Cryptosporidium	0.88960[c]	1	0.50369[d]	0.19200	0.17584
Total coliforms	0.11954	0.50369[d]	1	0.04102	-0.13034
Fecal coliforms	0.17711	0.19200	0.04102	1	-0.41440
coliphages	0.08957	0.17584	-0.13034	-0.41440	1

N = 15
[c]: $p < 0.001$
[d]: $p = 0.10$

compared the occurrence of *Cryptosporidium* and *Giardia* in surface waters and found that the concentrations of these two parasites were significantly correlated in all the waters analyzed ($p < 0.01$). The recovery rate of *Giardia* and *Cryptosporidium* may be low when the wastewater turbidity is high because visualization of the cysts and oocysts is difficult under the microscope. There was no correlation between the removal of the parasites and the effluent turbidity.

CHAPTER 5

CONCLUSIONS

The duckweed pond was more effective in reducing the number of the protozoan parasites (*Giardia* 98%; *Cryptosporidium* 89%) than that of indicator bacteria (total coliform 61%, fecal coliform 62%). However, the removal of coliphages was not significant and therefore further studies need to be done on the removal of both coliphages and enteroviruses to determine if coliphage can be used to predict the likely reduction of enteric viruses by the duckweed pond.

The removal of microorganisms in the pond appeared to be related to the size of the organisms (Table 1). The percent removal of the larger organisms (parasites) was greater than the percent removal of the smallest (viruses).

No correlation was found between the removal of microorganisms and pH, or the water temperature. *Giardia* and *Cryptosporidium* removal and the influent water turbidity were correlated. The removal of *Giardia* cysts and *Cryptosporidium* oocysts was also significantly correlated. There was a correlation between *Cryptosporidium* and total coliform removal. However, more data are needed to determine its significance. There was no correlation between the removal of parasites and the other indicator microorganisms.

Aquatic systems for wastewater treatment appear to be promising as tertiary treatment systems for enteric pathogens. Additional detention time could increase the removal capability of these systems. Increasing the detention time will slow the flow

rate through the pond and there will more time for the microorganisms and other suspended solids to settle before the water reaches the outlet of the pond.

Wetland and aquatic plant systems can help to reduce the high cost associated with wastewater treatment. They can be used especially in developing countries with less resources where adequate wastewater treatment is lacking.

APPENDIX 1: THE DUCKWEED POND DATA

Table 1. *Giardia* cyst reduction by the duckweed pond (July 1994-December 1995)

Date	Sample volume (liter)	*Giardia* cysts per liter		Percent removal
		Influent	Effluent	
Jul 94	1	4	0.33	92
Sep 94	4	11.5	0	> 99.99
Oct 94	4	24.8	0	> 99.99
Nov 94	4	33	0.625	98
Dec 94	4	25	1.25	95
Jan 95	4 - 10*	17.8	0.1	99
Feb 95	4 - 10*	12	0.2	98
Mar 95	4 - 10*	25	0.2	99
Apr 95	4 - 10*	28.5	0.4	99
May 95	4	5.5	0.25	95
Jun 95	4	12.5	0.5	96
Jul 95	4	4	0.125	97
Aug 95	4	11	0.5	95
Oct 95	4	19.5	0.25	99
Nov 95	4	7	0.25	97
Dec 95	4	21	0.35	> 99.99
Average		15.6	0.35	98 ± 12.61

*: Four liters of the influent and ten liters of the effluent were collected

Table 2. *Cryptosporidium* oocyst reduction by the duckweed pond (July 1994 - December 1995)

Date	Sample volume (liter)	*Cryptosporidium* oocysts per liter		Percent removal
		Influent	Effluent	
Jul 94	1	2	0	> 99.99
Aug 94	2	3	1	67
Sept 94	4	1.5	0.5	67
Oct 94	4	3.25	0.25	92
Nov 94	4	1.75	0	> 99.99
Dec 94	4	1.5	0.25	83
Jan 95	4 - 10*	1.5	0	> 99.99
Feb 95	4 - 10*	0.75	0	> 99.99
Mar 95	4 - 10*	0.75	0	> 99.99
Apr 95	4 - 10*	2	0	> 99.99
May 95	4	0**	0**	N/A
Jun 95	4	1	0	> 99.99
Jul 95	4	0**	0**	N/A
Aug 95	4	0**	0**	N/A
Oct 95	4	0.5	0	> 99.99
Nov 95	4	0**	0**	N/A
Dec 95	4	0**	0**	N/A
Average		1.58	0.17	89 ± 13.41

*: Four liters of the influent and ten liters of the effluent were collected
**: Not included in average
N/A: Not applicable

Table 3. Reduction of total coliform bacteria by the duckweed pond (July 1994 - December 1995)

Date	Total coliforms per 100 m		Percent removal
	Influent x 10^3	Effluent x 10^3	
Jul 94	822	397	52
Aug 94	150	85	43
Sept 94	6975	1522	78
Oct 94	6850	3800	45
Nov 94	5250	3050	42
Dec 94	3600	2800	22
Jan 95	5200	2250	57
Feb 95	4000	330	92
Mar 95	8750	2850	67
May 95	2550	2050	20
Jun 95	1750	295	83
Jul 95	2700	470	83
Aug 95	5650	180	97
Oct 95	6350	2900	54
Nov 95	1715	945	45
Dec 95	5500	2550	54
Average	4238	1655	61 ± 23.3

Table 4. Reduction of fecal coliform bacteria by the duckweed pond (July 1994 - December 1995)

Date	Fecal coliforms per 100 ml		Percent removal
	Influent x 10^3	Effluent x 10^3	
Jul 94	310	125	60
Aug 94	130	65	50
Sept 94	2800	485	83
Oct 94	2750	1040	62
Nov 94	1380	835	39
Dec 94	1670	710	57
Jan 95	2350	750	68
Feb 95	960	505	47
Mar 95	5300	815	85
May 95	2650	1220	54
Jun 95	530	275	48
Jul 95	925	540	42
Aug 95	385	140	64
Oct 95	2850	720	75
Nov 95	1075	645	40
Dec 95	2250	690	69
Average	1770	598	62 ± 14.8

Table 5. Reduction of coliphages by the duckweed pond (July 1994 - December 1995)

Date	Coliphages per ml		Percent removal
	Influent	Effluent	
Jul 94	1165	69	94
Aug 94	957	256	73
Sept 94	1557	1153	26
Oct 94	1433	1363	5
Nov 94	1360	1157	15
Dec 94	1987	1454	27
Jan 95	1223	960	15
Feb 95	917	700	24
Mar 95	1587	1077	32
May 95	1350	787	42
Jun 95	1545	597	61
Jul 95	797	250	69
Aug 95	653	350	46
Oct 95	1220	740	39
Nov 95	677	320	53
Dec 95	1307	640	51
Average	1233	742	40 ± 24.2

REFERENCES

Abbaszadegan, M., C. P. Gerba, and J. B. Rose. 1991. Detection of *Giardia* cysts with a cDNA probe and application to water samples. Appl. Environ. Microbiol., 57:927-931.

Adams, M. H. 1959. Bacteriophages. Interscience Publisher, Inc. NY.

American Public Health Association, American Water Works Association, and Water Environment Federation. 1992a. Standard Methods for the Examination of Water and Wastewater, 18th Edition Supplement. Washington, DC.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1992b. Standard Methods for the Examination of Water and Wastewater, 18th ed., Washington, DC.

Bitton, G. 1994. Wastewater Microbiology. John Wiley & Sons Inc., New York.

Brix, H. 1993. Wastewater treatment in constructed wetlands: System design, removal processes, and treatment performance. *In*: Constructed Wetlands for Water Quality Improvement. CRC Press Inc,. Boca Raton, FL, pp. 9-22.

Brown S. D. 1994. Constructed Wetlands in the USA. Water Quality International, 4:24-28.

Calderon, L. R., and G. Craun. 1994. Disease surveillance and outbreak investigations. *In*: Extended Abstracts of the 1994 Water Quality Technology Conference. American Water Works Association, Denver, CA, pp. 68.

Center for Disease Control. 1978. Intestinal parasite surveillance, annual summary 1977. Center For Disease Control, Atlanta, GA.

Dewedar A., and Bahgat M. 1995. Fate of faecal coliform bacteria in a wastewater retention reservoir containing *Lemna gibba* L. Wat. Res., 29:2598-2600

Dortch, M. S. 1992. Literature analysis addresses the functional ability of wetlands to improve water quality. The Wetland Research Program Bulletin, 2:1-4.

Environmental Protection Agency. 1983. Design Manual Municipal Wastewater Stabilization Ponds, EPA 625/1-83-015, Center for Environmental Research Information. Cincinnati, OH.

Environmental Protection Agency (EPA). 1988. Constructed Wetlands and Aquatic Plant Systems for Municipal Wastewater Treatment. EPA/625/1-88/022, Center for Environmental Research Information, Cincinnati, OH.

Falabi, A. J., C. P. Gerba, and M. M. Karpiscak. 1996. Fate of selected pathogens in a duckweed (*Lemna gibba* L.) -covered pond. *In*: 1996 Water Reuse Conference Proceedings, Amer. Water Works Assoc., Denver, CO, pp. 1071-1080.

Gerba, C. P. 1981. Virus survival in wastewater treatment. *In*: Virus and Wastewater Treatment (M. R. Goddard and M. Butler, eds.) , Pergamon Press, New York, pp. 39-48.

Gerba, C. P. 1987. Phage as indicators of fecal pollution. *In*: Phage Ecology. Goyal S. M., Gerba C. P. , and Bitton G. (Eds.). John Wiley and Sons, New York, pp. 197-209.

Gerba, C. P., and J. B. Rose. 1990. Viruses in source and drinking water. *In*: Drinking Water Microbiology. Gordon A. McFeters (Ed.). Springer-Verlag New York Inc., NY, pp. 380-396.

Gerba, C. P., and J. B. Rose. 1993. Estimating viral disease risk from drinking water. *In*: Comparative Environmental Risk Assessment (C. R. Cothern, ed.). Lewis Publishers Inc., Boca Raton, FL, pp.117-135.

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

Gilles, V. 1990. Use of artificial wetlands for the treatment of recreational wastewater. Wat. Sci. Techn., 29:67-74.

Grimalson, A. M., H. V. Smith, W. N. Thitai, P. G. Smith, M. H. Jackson, and R. W. A. Girdwood. 1993. Occurrence and removal of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in Kenyan waste stabilization ponds. Wat. Sc. Tech., 1.27:97-104.

Hammer, D. A. (ed.). 1989. Constructed Wetlands for wastewater treatment: Municipal, Industrial, and Agricultural. Lewis Publishers, Inc., Chelsea, MI.

Hancock S. J., and L. Buddhavaraqu. 1993. Control of algae using duckweed (*Lemna*) systems. pp. 399-414. *In*: Constructed Wetlands for Water Quality Improvement, CRC Press Inc., Boca Raton, FL.

Hibber, P. C., and C. M. Hancock. 1990. Waterborne giardiasis. In: Drinking Water Microbiology. Gordon A. McFeters (Ed). Springer-Verlag New York Inc., NY, pp. 271-293.

Hillman, W. S., and D. C. Culley. 1978. The use of duckweed. Am. Sci., 66:442-451.

Jewel, J. W. 1994. Resource-recovery wastewater treatment. American Scientist, 82:366-375.

Kadlec, R. H., and R. L. Knight. 1996. Treatment Wetlands. CRC Press Inc., Boca Raton, FL.

Karpiscak, M. M., K. E. Foster, S. B. Hopf, and G. W. France. 1993. Treating municipal effluent using constructed wetlands technology in the Sonoran Desert. *In*: K. D. Schmidt (ed.) Proceeding of the Symposium on Effluent Use Management. pp. 45-53.

Karpiscak, M. M., K. E. Foster, S. B. Hopf, J. M. Bancroft, and P. J. Warshall. 1994. Using water hyacinth to treat municipal wastewater in the desert Southwest.Water Resources Bull., 30:219-227.

Karpiscak, M. M., C. P. Gerba, P. M. Watt, K. E. Foster, and J. A. Falabi. 1995. Multispecies plant systems for wastewater quality improvements and habitat enhancement. *In*: Second International Symposium on Wastewater Reclamation and Reuse, Symposium Preprint Book 1. Iraklio, Greece. Int'l Assoc. Water Qual. London, pp. 437-442.

Ma, J., J. Naranjo, and C. P. Gerba. 1994. Evaluation of MK filters for recovery of enteroviruses from tap water. Appl. Environ. Microbiol. 6:1974-1977.

Millin, P. A., and A. Heritage. 1992. Application of constructed wetlands for the treatment of domestic sewage in rural Australia. *In*: CSIRO Division of Water Resources: Research Areas Pertinent to Intensive Industry Waste Management. Division Report 92/4, June 1992, pp. 25-33.

Mitsch, W. J., and J. G. Gosselink. 1993. Wetlands. Van Nostrand Reinhold, New York, NY.

Owens, J. H., R. Miltner, F. W. Schaefer, and E. W. Rice. 1994. Pilot-scale ozone inactivation of *Giardia* and *Cryptosporidium*. *In*: Proceedings of Water Quality Technology Conference. Amer. Water Works Assoc., Denver, CO. pp. 1319-1323.

Reed, S. C., R. W. Crites, and E. J. Midlebrooks. 1995. Natural Systems for Waste Management and Treatment. 2nd ed. McGraw-Hill, Inc., New York, NY.

Rose, J. B., L. K. Riley, and C. P. Gerba. 1989. Evaluation of immunofluorescence techniques for detection of *Cryptosporidium* and *Giardia* from environmental samples. Appl. Environ. Microbiol., 55:3189-3196.

Rose, J. B., S. N. Singh, C. P. Gerba, and L. M. Kelly. 1984. Comparison of microporous filters for concentration of viruses from wastewater. Appl. Environ. Microbiol. 47:989-992.

Rose, B. J., C. P. Gerba, and W. Jakubowski. 1991. Survey of potable water supplies for *Cryptosporidium* and *Giardia*. Environ. Sci. Technol., 25:1393-1400.

Sobsey, M. D., and B. H. Olson. 1983. Microbial agents of waterborne disease. *In*: P. S. Berger and Y. Argaman (eds). Assessment of Microbiology and Turbidity Standards of Drinking Water. U.S. Environmental Protection Agency, Washington, DC, pp. 1-69.

Wolverton, B. C., and R. C. McDonald. 1979. Upgrading facultative wastewater lagoons with vascular aquatic plants. J. Water Pollution Control Fed., 51:305-313.

Zhao K. ,P. R. Scheuerman, and L. Forest. 1996. Use of microbial activity to monitor constructed wetland function. Abstracts of 96th General Meeting of the American Society for Microbiology, Washington, DC, pp. 399.