ABSTRACT

DAVIS, SARAH E. The Analysis of Effluent and the Impact of Salinity and Anaerobic Stress on Creeping Bentgrass. (Under the direction of Thomas W. Rufty).

Rapid urban development and population growth have put a strain on freshwater supplies across the United States. When freshwater shortages occur, landscapes and golf courses can reduce demand by using alternative irrigation sources such as effluent (treated wastewater). In addition to water conservation, irrigating golf courses with effluent may help to minimize environmental pollution. When used for irrigation, turfgrasses, other plants, and soil microorganisms assimilate 'pollutants' such as nitrogen and phosphorus. The goal of this research is to explain the process of effluent production and to examine several potential problems that may arise with effluent application to turfgrasses.

Three questions were addressed. The first was 'what is the chemical composition of effluent?'. The second question was 'is there potential for direct salt damage to bentgrass?', and the third was 'how susceptible is bentgrass to anaerobic soil conditions?'. The analyses of effluent from different sources showed that nutrient levels were highly variable at a particular location and between locations. Based on several experiments in hydroponics, it seems that bentgrass would not be damaged by salt effects resulting directly from effluent additions. It is conceivable that damage could occur if salt accumulates during intense drought. Results also suggest that there is little likelihood that bentgrass would be severely damaged directly by anaerobic conditions if effluent is added to putting greens.

The Analysis of Effluent and the Impact of Salinity
and Anaerobic Stress on Creeping Bentgrass

by
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BIOGRAPHY

Sarah Elizabeth Davis was born in Raleigh, North Carolina. She has lived here with her parents, Bill and Kyra, and sister, Joanna. Sarah attended Millbrook High School where she participated on the cheerleading and women's golf teams. After high school, she signed as the first golfer on the newly re-established varsity Women's Golf Team at NC State. She graduated *cum laude* with a Bachelor of Science in Environmental Technology in December of 2004. During her time as a student-athlete, she balanced school and golf with a desire to give back to the community. She was a recipient of the ACC Top Six Volunteer award in 2003. As graduation was drawing near, she luckily met Dr. Tom Rufty in October of 2004. He offered her a great opportunity to attend graduate school and combine her environmental background with her interest in the golf industry.

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EFFLUENT USE ON BENTGRASS

INTRODUCTION

Rapid urban development and population growth have put a strain on freshwater supplies across the United States. When freshwater shortages occur, landscapes and golf courses can reduce demand by using alternative irrigation sources such as effluent (treated wastewater). Some state and local governments require the use of effluent for irrigating landscapes and golf courses to facilitate water conservation. In addition to water conservation, irrigating golf courses with effluent may help to minimize environmental pollution. When used for irrigation, turfgrasses and soil microorganisms assimilate ‘pollutants’ such as nitrogen and phosphorus. This natural filtration is a more acceptable alternative to surface water or ocean dumping. As an added benefit, the effluent acts as a source of fertilization for these plants. The goal of this research is to explain the process of effluent production and to examine several potential problems that may arise with effluent application to turfgrasses.

BACKGROUND

Effluent Production and Regulation

Urban water reuse has been practiced since the eighteenth and nineteenth centuries, but to cut costs, many communities cut corners with the water cycle and dumped raw or nearly raw sewage back into local waters. As a result of direct dumping into surface waters, these water bodies became unsafe for aquatic organisms due to increased algal growth and decreased dissolved oxygen (DO) levels. In the 1920s, biological oxygen demand (BOD) tests, which measure the relative polluting strength of different organic substances (Stoddard 2002), were implemented. The Federal Water Pollution Control Act was passed in 1948 to "enhance the quality and value of our water resources by establishing a national policy for the prevention, control, and abatement of water pollution". The Water Quality Act of 1965 made water quality standards state and federally enforceable.

In 1970, the Clean Water Act (CWA) was passed which distinguished two sets of water users; potable water supply users and other water resource users (such as fisherman, boaters, swimmers, and the wildlife living in these waters). The CWA recognized the need to keep both sets of users satisfied by having all components of the urban water cycle functioning efficiently and safely. The CWA increased federal support for upgrading publicly owned treatment facilities and requiring secondary treatment of wastewater which uses microorganisms to break down organic matter. Prior to the CWA, there were close to 5,000 systems serving 56 million people that provided only primary treatment, which utilizes gravitational settling to separate solids from the raw sewage (Stoddard 2002).

In 1972, the Federal Water Pollution Control Act Amendments targeted pollutants discharged from point sources and restructured authority for water pollution control to the administrator of the United States Environmental Protection Agency (US EPA), while recognizing the primary enforcement rights and responsibilities of the states. All public treatment plants were mandated to employ full secondary treatment of wastewater by July 1, 1977, with the goal of zero discharge into navigable waters by 1985. There have been delays, and water pollution control efforts are constant and ongoing. As a result of rapid industrialization and population growth, the volume of sewage has increased and many new substances have been found in effluents that do not degrade through secondary treatment. Thus, treatment must improve and new alternatives for dispersal continue to be explored (Stoddard 2002).

The EPA regulates waste treatment plant discharge through the National Pollution Discharge Elimination System permit program which controls water pollution by regulating point source pollutants going into surface waters. The EPA and the US Agency for International Development published the Guidelines for Water Reuse in 1992. However, these are non-binding and each state has its own regulatory powers and mandates. As of 1992, 18 states had enforceable regulations, 18 states had unenforceable guidelines, and 14 had nothing in place creating inconsistencies in state-to-state water regulation (Crook, 1994).

In North Carolina, permits for the discharge of wastewater are controlled by the Department of Environment and Natural Resources. However, since wastewater reuse is a relatively new concept for North Carolina, there are not many well-developed regulations. For example, the following requirements for golf course irrigation are from the NC Administrative Code Section T15A:02H.0200 last revised in 1987:

- treatment process should produce effluent with monthly average TSS < 5mg/L, daily maximum of TSS < 10mg/L, maximum fecal coliform less than 1/100mL before discharging to a 5 day detention pond
- no public access to a 5 day pond
- size of irrigation pond that follows the 5 day pond shall be justified using a mass water balance for worst case conditions on record
- signs shall be posted at the pro shop stating that the course is irrigated with treated wastewater
- 100ft vegetative buffer zone between the edge of spray influence and nearest dwelling
- time of spraying shall occur between 11:00 pm and 3 hours prior to course opening

- rate of application is site-specific, but not to exceed 1.75 in/wk
- design of a sprinkler system – piping is a separate system; with no cross-connections to a potable water supply (no spigots on distribution site either)
- available means to prevent improperly treated wastewater from entering the 5 day pond

Water Reuse

Irrigation and thermoelectric power are the largest consumers of freshwater in the US. Irrigation accounts for 42% of the total use of potable water, while residential use accounts for 40%. Approximately 60% of residential use is for toilet flushing and outdoor use (Lazarova et al. 2005). Since irrigation uses such a large percentage of freshwater, it is important to find more efficient and conservative irrigation practices. Currently, the states most actively using reclaimed water are: California, Florida, Arizona, Nevada, Washington, Hawaii, and Texas.

In the past, reclaimed water was released into local surface waters without considering recycling it for other uses. Presently, there are more stringent standards for wastewater discharge and reuse. Reuse applications, in most cases, require advanced tertiary treatment. Degree of treatment increases with degree of public access and concern for public health. Irrigation of street medians, residential landscaping, and golf courses expose the public to reclaimed water and its aerosols, therefore tertiary treatment with high levels of disinfection is expected to limit exposure to potential pathogens.

There are two perspectives on reuse applications: the disposal issue and the water resource issue. The disposal issue relates to disposal of the excess effluent once it has gone through the wastewater treatment process. Water quality requirements for disposal of effluent into surface waters are much higher than requirements for land applications of effluent. Water bodies are extremely sensitive environments that are subject to eutrophication if effluent is not properly treated. As a water resource issue, reclaimed water can be used to lessen the demand on potable water without compromising environmental quality. Effluent is a less expensive source of irrigation water for golf courses and landscape plants that currently utilize potable water.

Effluent Storage

Storage of effluent is a necessity because the daily peak flows tend to be in the morning and evening, while peak irrigation demands tend to be at night during low flows. Municipal treatment plants commonly experience higher flows during winter months, but irrigation is limited due to limited uptake by slow growing or dormant plants and low evapotranspiration. Storage equalizes daily variations in flow from the treatment plant and stores excess when average wastewater flow exceeds irrigation demands (Pettygrove and Asano 1985).

Adequate storage is essential in the event of a disruption in a treatment operation or irrigation system. The most common forms of storage are open reservoirs or deep stabilization ponds. These storage ponds are lined with clay, concrete, or a synthetic rubber to limit water loss and migration of nitrates into groundwater (Hammer and Hammer 2004). They can also provide extra holding time if there are treatment issues and the effluent needs to be tested. Oxygen demands, suspended solids, nitrogen, and microorganisms can be reduced during storage (Pettygrove and Asano 1985), therefore providing additional treatment. However, close monitoring and proper maintenance is required because bacterial re-growth or contamination can occur over time (Lazarova et al. 2005).

The Treatment Process

(primary, secondary, and tertiary steps; numbered paragraphs refer to Figure 1)

Designs of treatment facilities vary, but there are several major steps in the treatment of wastewater that they all have in common. First, large debris is removed at the bar screens and sent to a landfill. The remaining wastewater is pumped to the next stage: grit and grease removal. Here the flow is slowed down, allowing sand and grit to settle and be removed. At the same time, grease floats to the surface and is pumped to digesters. Next, the wastewater is screened through primary microscreens. The remaining water continues on to biological treatment (secondary treatment). The bacteria use dissolved organic solids as food and the converted solids settle out. Then the clarifiers remove any remaining bio-solids; secondary microscreens may also be used to remove suspended solids. Next, short wave ultra-violet radiation is used to eliminate any bacteria, viruses, and other microorganisms. Finally, during tertiary treatment, chlorination kills the remaining pathogens, and effluent is then suitable for use or release back into surface waters.

Primary treatment

(1) Screens are installed at the entrance of a wastewater treatment plant to protect mechanical equipment and prevent larger objects from getting into primary settling tanks. The large debris is removed and incinerated or sent to a landfill. Smaller plants usually dispose of screenings by burial under 6 ft of cover to avoid fly and odor problems. Larger plants often use incinerators, sometimes mixed with dewatered sludge.

Removing grit from wastewater influent prevents wear of pumps and other mechanical equipment. Grit is the heavy mineral material in raw sewage that may contain sand, gravel, silt, cinders, broken glass, and seeds. Grit settles more rapidly than organic or putrescible material. Therefore, differential sedimentation (gravity settling) is a good way to separate grit from organic matter. There are also velocity-controlled grit channels, which are long, narrow sedimentation basins.

(2) Aerated grit chambers are extensively used at larger treatment plants. An aerated chamber can also be used in addition to grit channels for chemical addition, mixing, and flocculation. Wastewater is freshened by the air through bubbling, thereby reducing odors and removing some BOD. By controlling air supply, these chambers can remove grit of any specified size.

(3) To eliminate problems associated with collection, removal, and storage, wastewater treatment plants install sewage grinders, or 'comminutors'. Comminutors are located between the grit chamber and the primary settling tanks. These devices continually intercept, shred, or grind large floating material in the waste flow into small pieces which acts to reduce odors, flies, and unsightliness.

(4-5) Detritus tanks remove a mixture of grit and organic matter. First, the grit is washed of organic matter, then the grit is removed and organic matter is discharged back into a collection tank. Grease makes up 10% of organic matter in domestic waste and includes a variety of materials (fats, waxes, free fatty acids, Ca and Mg soaps, mineral oils, etc).

Skimming tanks facilitate grease removal and sedimentation, and help improve BOD reduction. Air is released either through pressure-type units or vacuum-type units, and the tiny air bubbles then attach themselves to solid particles and carry them to the surface. The grease and scum can then be removed by hand or mechanically using a circular radial arm skimmer, which rotates, moving the scum up onto a ramp and into a scum trough. The scum is then combined with primary sludge and is digested, incinerated, or buried.

(6) Sedimentation, sometimes called clarification, is generally used in combination with coagulation and flocculation. There are three types of clarifiers (also called settling or sedimentation basins). Horizontal-flow clarifiers have basins designed to keep velocity and flow distribution as uniform as possible to assure suspended material settles. Usually made of steel or concrete; the bottom of the basins slope to make sludge removal easier. Solid-contact clarifiers bring incoming solids in contact with a suspended sludge layer near the bottom. Liquid rises upward while an interface retains solids below. These clarifiers have a reduced retention time for equivalent solids removal in horizontal-flow clarifiers. Inclined-surface clarifiers use inclined trays to divide the depth into sections, therefore reducing settling time. These clarifiers provide large surface area, reducing clarifier size.

(7) When solids settle to the bottom of a basin, a sludge layer develops. Small wastewater treatment plants can manually remove the sludge by draining the basins and flushing the sludge to a hopper and draw-off pipe. However, mechanical removal is

typically used for sludge removal. Sludge is then pumped to discharge treatment facilities.

(8) The next operation, flotation, separates solid or liquid particles from a liquid by adding gas (air). Rising bubbles adhere to particle structure of the suspended solids. This decreases specific gravity relative to the liquid and allows separation. There are two methods of flotation, but only dissolved-gas flotation is used in wastewater treatment. Performance of the flotation system depends on the pressure of the gas released. The pressurization system generally consists of a pressurization pump, retention tank, and a gas supply. The pump increases wastewater pressure. The retention tank provides adequate time for gas to transfer into liquid and also releases excess gas. Three different types of pressurization systems can dissolve gases. Full-flow pressurization transfers gas to the total-feed flow. Partial-flow, when effective, can reduce costs of the pressurization system. In a recycle-flow system, a portion of the clarified flotation effluent is recycled to the pressurization system. The recycled flow becomes the carrier of the dissolved gas later released for flotation. This system is favored when dissolved air flotation is used for thickening biological sludges.

Wastewater treatment facilities are frequently using chemicals in gravity and flotation clarification applications to reduce suspended solids in the effluent. Ferric chloride, alum, lime, and other polymeric compounds are added to form stable floc particles or break oil emulsions. The chemicals are usually added during pretreatment before the flotation unit. Full and partial-flow systems are rarely used with chemically treated wastewater because it degrades in the pressurization systems and does not re-form in the flotation unit.

Foam formation collects suspended solids and oils and separates them from the wastewater. Air is diffused into the bottom section of the fractionation unit. Foam is generated and lifted upward by the gas in the unit. This process discharges the foam and collapses it by heating it or spraying it with previously collapsed foam. The effluent is discharged near the bottom of the fractionation unit.

(9) Sludge pumping is an important next step in wastewater treatment operations. Raw sludge is obtained from a primary clarifier, and requires further treatment such as aerobic or anaerobic digestion, incineration, or wet combustion. It dewaters (the removal of water from a waste product) relatively well in a centrifuge because it consists mostly of large solid particles. Activated sludge is a result of the overproduction of microbial organisms in the activated sludge process. It is a light material composed of bacteria, rotifers, protozoa, and enough filamentous organisms to make concentration difficult. As a result, wastewater treatment facilities use thickeners to concentrate the sludge. Raw sludge is moved to digesters, while activated sludge is returned and periodically discharged.

(10) Solids removed from wastewater typically contain large amounts of biologically degradable matter. Digestion converts the odorous sludges to relatively inert material that can be rapidly dewatered without unpleasant odors (Hammer and Hammer 2004). Digestion can be aerobic or anaerobic, but both involve microorganisms that use the sludge as a food source. Anaerobic digestion is a bacterial process involving two steps. Organic compounds are broken down into organic acids, which are then converted to

methane and carbon dioxide gases. This process substantially decreases the weight of solids that require further processing or dispersal. The gases produced during anaerobic digestion can be used for heating and incineration (Liu and Liptak 2000).

Aerobic digestion can be used in municipal treatment facilities, but is mainly used for industrial treatment. In aerobic digestion, sludge can be stabilized by long-term aeration. Aerated digesters are equipped with diffused or mechanical aerators. Gates or pipes are used to draw off supernatant after gravity settling of the stabilized solids when aerators are turned off. The supernatant is returned to the treatment process and the gravity-thickened sludge is removed (Hammer and Hammer 2004). The end product of aerobic digestion is biologically stable and has a higher fertilizer value than sludge from anaerobic digestion. Fewer operational problems occur with aerobic digestion because the process is much simpler, however higher power costs are involved.

Secondary treatment

(11) In the activated-sludge process, clarified wastewater discharged from the primary clarifier is delivered into the aeration basin where it is mixed with an active mass of microorganisms (activated sludge). Organic matter is aerobically degraded into carbon dioxide, water, new cells, and other end products. Aeration maintains the aerobic environment in the basin and keeps reactor contents completely mixed. After a specific treatment time, the mixed liquor passes into the secondary clarifier. Here, sludge settles and a clarified effluent is produced for discharge. The process recycles a portion of the settled sludge back to the aeration basin to maintain the necessary activated-sludge

concentration. The process also wastes a portion of the settled sludge to aid in effective BOD removal.

An extended aeration process is a modification of the conventional activated-sludge process that is generally used in small installations such as schools, resorts, and small rural communities. The main advantage of the extended-aeration process is the amount of excess biological solids produced is minimized or eliminated. Although excess sludge in the extended-aeration process is significantly reduced, secondary clarification is needed to remove the accumulated non-biodegradable portion of sludge and the influent solids that are not degraded or removed (Liu and Liptak 2000).

(12) Trickling filters use an attached-growth biological process for effluent treatment. The attached-growth process is when microorganisms remove nutrients and dissolved material from the wastewater, using them for food. As the biological material grows, it can no longer stay attached to the media and falls to the bottom of the tank. Wastewater is distributed from the top of the filter and moving it in between the spaces of the film-covered medium. As the wastewater moves through the filter, the organic matter is adsorbed onto the film and degraded by a mixed population of aerobic microorganisms. The oxygen required for organic degradation is supplied by circulating air through the filter induced by natural draft or ventilation. At the bottom of the filter is a porous underdrain system that collects treated effluent and circulates air. The filter recirculates and mixes a portion of the effluent with the incoming wastewater to reduce its strength and provide uniform hydraulic loading (Metcalf and Eddy 1991). As the film thickens, it may become anaerobic or the adhesive ability of the microorganisms may be reduced.

Once the thick film is removed, a new film starts to grow on the medium surface, signaling the beginning of a new growth cycle (Characklis and Marshall 1990).

The microorganism population in a trickling filter consists of aerobic, anaerobic, and facultative bacteria, fungi, algae and protozoa. Higher forms such as worms, insect larvae, and snails are also present. The most important microorganisms are the facultative bacteria. Fungi in the trickling filter are responsible for waste stabilization, which is especially important in wastewater with low pH levels. Algae provide oxygen during the daytime (through photosynthesis) to the percolating wastewater. Protozoa are responsible for keeping the bacterial population in check. Changes in organic loading, hydraulic retention time, pH, temperature, air availability, and wastewater composition, can cause microbial populations to vary throughout the filter (Liu and Liptak 2000).

(13) Rotating biological contractors are attached-growth biological processes that consist of basins with large circular disks mounted on horizontal shafts that rotate partially submerged through the wastewater. Rotation provides a way to remove excess bacterial growth on the disk surfaces, maintaining suspension of sloughed biological solids in the wastewater. Final clarifiers remove sloughed solids.

(14) Stabilization ponds are sometimes used in small communities because they provide a low-cost treatment option. The pond is shallow and natural processes like wind or heat create a mixing effect, although, mechanical aeration is sometimes used. Stabilization ponds can be aerobic, anaerobic, or aerobic-anaerobic. Aerobic ponds treat soluble organic waste from treatment plants. Anaerobic ponds are used when rapid stabilization

of nutrients and microbial activity in waste is required. It is common for wastewater treatment plants to use these ponds along with aerobic-anaerobic ponds to treat domestic and some industrial waste. In aerobic and aerobic-anaerobic ponds, bacteria oxidize organic matter, which produces ammonia, carbon dioxide, sulfate, and water by aerobic metabolism. Algae use these products during the day and produce oxygen, which is then used by bacteria to decompose the remaining organic matter. Anaerobic ponds treat wastewater with high solids concentration. These ponds are deeper and maintain lower temperatures for anaerobic conditions. Precipitation and anaerobic metabolism convert organic wastes to carbon dioxide, methane, and other gases.

In aerated lagoons, oxygenation of wastewater is usually achieved through surface, turbine, or diffused aeration which keeps the contents of the lagoon suspended. Depending on retention time, the aerated effluent may contain one-third to one-half the value of the incoming BOD. In anaerobic lagoons, acid-producing bacteria reduce sulfate compounds to hydrogen sulfide. The hydrogen sulfide produces a terrible odor unless sulfate concentrations are maintained below 100mg/l. Aside from the odor, other important factors when dealing with aerated lagoons include BOD removal, the contents of the effluent, temperature effects, and oxygen requirements.

Anaerobic treatment is also an effective means of treating organic wastes, where facultative and anaerobic microorganisms convert organic matter into gaseous products such as methane and carbon dioxide. There are some major advantages of the use of anaerobic treatment over aerobic treatment. Biomass yield is much lower, saving costs in sludge removal and disposal. In addition, the methane produced is an economically valuable product. The savings from lower sludge production, electricity conservation, and methane production range from \$0.20 to \$0.50 per 1000 gal of domestic sewage treatment (Jewell 1987). The reduction of sludge and aeration energy consumption each result in savings that are greater than the cost of the energy required by the anaerobic process (Jewell 1987). Also, much of the energy requirement for the anaerobic process can be achieved through exhaust gases. Disadvantages to anaerobic treatment include possible odors from the creation of hydrogen sulfide, higher detention times, and more difficult operation.

(15) The next process, disinfection, should kill all pathogenic organisms, bacteria, and viruses to acceptable levels prior to discharge. Primary treatment removes up to 75% of bacteria through settling alone, but further removal through disinfection is required, and there are various methods by which this may be accomplished. Chemical agents such as chlorine and its compounds, ozone, bromine, iodine, phenols, and alcohols can be used. Heat and UV light are also effective physical disinfection agents. However, the amount of remaining suspended matter in the effluent can reduce the efficiency of UV radiation. Using heat and UV light for large quantities of effluent can be costly and may not be practical in all treatment facilities.

Effectiveness of chlorine, the most common type of disinfection, relies on pH, temperature, and contact time. As an active element, chlorine reacts with many compounds in the wastewater to form new, less effective compounds. When chlorine gas is added to water, hydrolysis and ionization occur. This creates hypochlorous acid (HOCl) and hypochlorite (OCl^-), which is free available chlorine that is kills pathogens and acts as an oxidizing agent to change the character of the harmful chemicals. Disinfection by chlorine also controls taste and odor.

Tertiary treatment

Advanced treatment makes the effluent clean enough for reuse or nonpolluting discharge and further removes nutrients, trace organics, and dissolved minerals. Many advanced treatment plants also include a nitrogen removal system. This tertiary treatment removes almost all solid and organic contaminants from wastewater, producing practically drinkable water. It is possible that in the future, tertiary treatment may be the answer for areas with scarce drinking water sources.

(16) The hydrolysis-adsorption process uses lime to hydrolyze large organic molecules into smaller molecules that can be adsorbed by activated carbon. The hydrolysis of large molecules into smaller ones allows the carbon to adsorb more of the organic molecules and lowers the detention time. This process removes approximately 90% of BOD and 97% of the phosphorus. The hydrolysis-adsorption process can actually produce drinking quality water (Liu and Liptak 2000) which only requires a longer contact time in the carbon adsorbers.

(17) Ammonia stripping is a desorption process used to lower the ammonia content of wastewater. It is usually easier and less expensive to remove nitrogen in the form of ammonia than to convert it to nitrate-nitrogen before removing it (Culp et al. 1978). Ammonia reacts with water to form ammonium hydroxide. Lime is then added to raise

the pH, which converts ammonium hydroxide ions to free ammonia gas. The free ammonia is then stripped from the water and released into the atmosphere (US EPA 2000).

(18) Effluent from anaerobic treatment will contain nitrogen in the form of ammonium (NH_4^+). During nitrification, the ammonium is first oxidized by chemotrophic bacteria. Then, activated sludge containing high concentrations of nitrifiers is settled in a clarifier and can either be returned to an aeration tank for reuse, or disposed of. While nitrification reduces effluent ammonia, high amounts of nitrate is released, resulting in the need for denitrification.

Denitrification is used to remove nitrate-nitrogen. This process reduces nitrate or nitrite to dinitrogen gas using facultative heterotrophic bacteria (Hammer and Hammer 2004). Since dinitrogen gas occurs naturally in the atmosphere, the process of denitrification converts nitrate or nitrite pollutants into a harmless gas. This process is becoming more popular as a final removal of nitrogen pollutants before discharge back to local waters.

(19) Chemical precipitation is a process that removes additional suspended solids that have made it through secondary treatment. These solids may be metals, inorganics, fats, oils, greases, and other organic substances. The precipitation of certain chemical agents causes these solids to flocculate and settle. Precipitation occurs through the use of a coagulant, an agent which causes smaller particles suspended in solution to move together to form larger particles (US EPA 2000). Some typical coagulants include lime, ferrous sulfate, alum, ferric chloride, or polymers.

(20) Enhanced filtration is another difference between primary or secondary treatment and tertiary treatment. Improvements in filter design are also included in tertiary treatment. Multi-layer filters enhance filter capacity due to more layers or deeper coarse layers where more settling can occur. Filtration efficiency can be improved by adding chemical coagulants like alum, iron, or polyelectrolytes. Adding coagulants can be costly due to chemical costs, and shorter filter runs.

LAND TREATMENT OF EFFLUENT AND ITS HAZARDS FOR TURFGRASS

The need to eliminate direct discharges of effluent to surface waters is leading to increased land applications, especially in areas with turfgrasses. Land applications treat effluent by using plant cover, soil profiles, and geologic materials to remove certain wastewater pollutants (Gohill 2000). As wastewater moves through the soil, the nutrients or pollutants are removed physically, chemically, and/or biologically by vegetation, roots, and microorganisms. This is particularly important for nitrogen, which is the main driver of eutrophication in most US coastal areas (Scavia and Bricker, 2006). Land applications of reclaimed water, once filtered through the soil, can replenish subsurface water sources with minimal negative impacts. Furthermore, effluent can be beneficial for turfgrasses, as it can supply nutrients such as nitrogen and phosphorus, potentially reducing chemical fertilizer costs and applications. In order to be most effective, land applications should be conducted on permeable soils that allow complete infiltration of the wastewater.

There are several issues that must be considered before land application with effluent. Land application requires large areas of land year round, and while the supply of wastewater is continuous, the demands for irrigation depend on growing season, application rates, and climate. Frequent applications of wastewater, without flushing with freshwater, can cause excessive salt accumulation at the soil surface. As a result, turfgrass quality may be compromised, soil infiltration rates may be reduced, and the soil microflora may be diminished. Frequent flushing of soils with freshwater can help alleviate problems with salt accumulation. Insect propagation and disease transmission are also potential problems for effluent application on turf. However, most problems should be able to be handled with proper management practices (Gohill 2000).

When using effluent for golf course irrigation, how much can be applied at one time must be considered. The effluent must be applied at a fairly consistent rate to stay below capacity of the holding ponds, but not so much as to saturate the soil. North Carolina DENR regulations and permitting do not allow the collection of effluent as puddles or standing water. If saturation occurs for extended periods of time, it can inhibit aeration, leach nutrients, induce saline conditions, and pollute ground water. The actual amount of effluent that can be applied must be calculated daily taking into account pET (potential evapotranspiration), recent precipitation, and soil type.

The potential problems are greatest during winter when the turf is dormant and not utilizing as much water or nutrients as during the growing season. Water needed for normal plant growth is equal to the evapotranspiration; more than 99% of water absorbed by plants is lost by transpiration and by evaporation from the plant surface (Lazarova 2005). Because transpiration rates are affected by temperature, wind, and humidity, the potential for transpiration must be calculated on a daily or weekly basis to estimate the amounts of effluent that can be applied.

Bentgrass sensitivity is important to consider when applying effluent to golf courses to avoid potential negative effects on turf health and quality. The greens could be injured by disease or algae due to an over-fertilization effect from the excess nutrients in effluent or the salinity and anaerobic effects that effluent irrigation may cause. Currently, courses in North Carolina are not using effluent on their bentgrass greens due to these uncertainties.

Management Practices

Human exposure is the first concern in management practices when using effluent irrigation. Exposure risk is influenced by the level of treatment the effluent has been through, as well as exposure time. Anyone working in golf course maintenance is likely to come in direct contact with the effluent, or turf recently sprayed with effluent and should wear protective clothing when appropriate. Appropriate signs and color-coded piping should be in place to keep the public, including players and residents, informed.

Fertilizer applications, soil management, and possible sprinkler clogging are other points of concern for management. Effluent can provide some nitrogen, phosphorus, potassium, and other micronutrients, which need to be considered when developing a fertilization program with special attention paid to nitrogen needs of turf during different growth stages. The effluent nutrient concentrations will vary, and need to be monitored. For the management of soil, if there are sodium problems, applying a light application of gypsum should keep it under control. It is also likely that sprinklers may clog due to bacterial or algal growth, or from deposits of suspended solids leading to reduced irrigation efficiency.

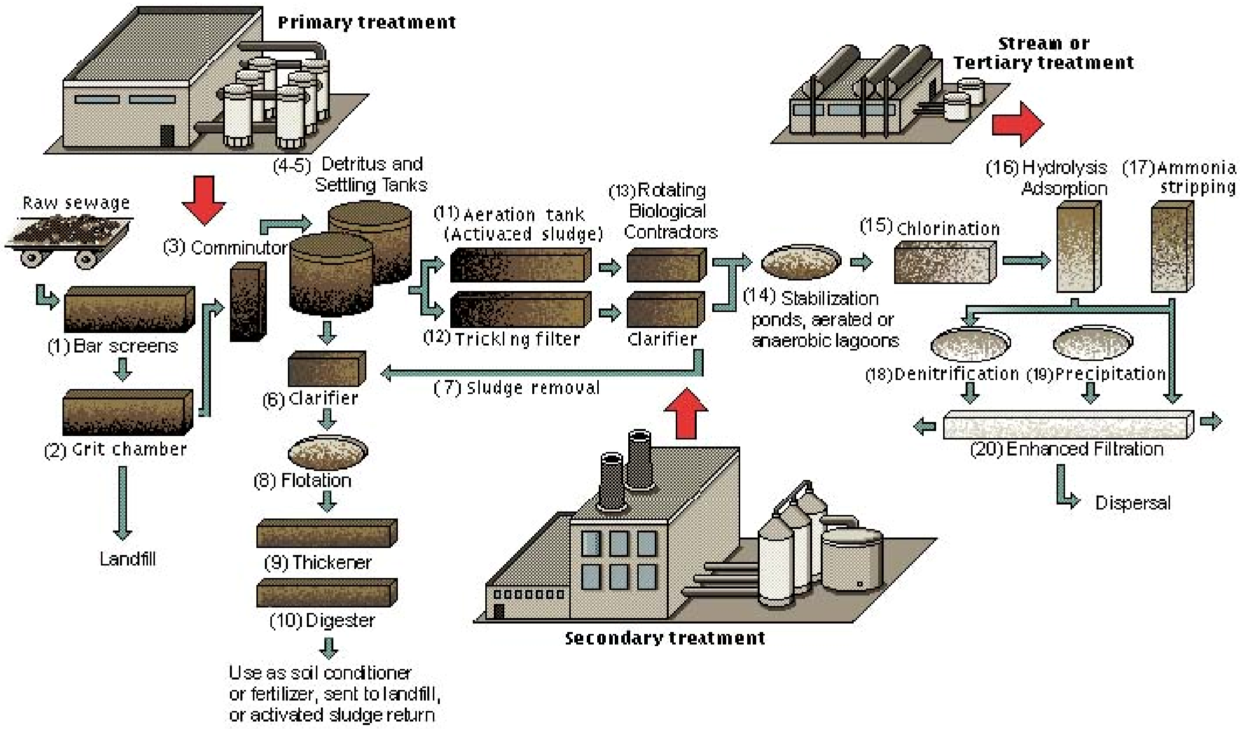

Figure 1. Diagram of a wastewater treatment plant. Adapted from Carroll, 2007.

CHAPTER II

EFFLUENT EFFECTS ON BENTGRASS

INTRODUCTION

The use of effluent on turfgrasses is relatively new in the southeastern U.S. In the transition zone climate of North Carolina, droughts are commonly encountered in summer months when effluent applications are highest; raising the possibility of salt problems even though extensive leaching can occur during most times during the year. Also, during periods of rain, if effluent were to be applied, there is the possibility of anaerobic conditions. Thus, for a given location, the situation can swing from times of salt build-up to times when salt problems would be minimal but water loading would be excessive.

Applications of effluent in the transition zone can be a particular problem for bentgrasses, commonly grown for golf course putting greens. North Carolina is close to the southern limit for growing cool season grasses like creeping bentgrass, as the hot summers cause acute stresses that can directly damage the grass or make it susceptible to other stresses like diseases or those associated with effluent. Because of this, golf course superintendents have experienced a number of problems and have been reluctant to apply effluent to greens. The exact causes for the bentgrass problems, however, have not been defined.

One of the greatest threats with effluent is the possibility of salt damage (Fu et al. 2005, Qian et al. 2007). In arid areas like those in the southwestern U.S., irrigated agricultural land is continually at risk of increased salinization (Tester and Davenport

2003). Similarly, turfgrass managers in those areas are struggling to produce high quality turfgrass. Irrigation water salinity is generally classified as low (<0.25 dS/m), medium (0.25-0.75 dS/m), high (0.75-2.25 dS/m), and very high (>2.25 dS/m) (US Salinity Laboratory 1969), and effluent is usually considered to be high in salinity. Salts generally contained in effluent include combinations of Na, Cl, Mg, Ca, SO_4, NO_3 and bicarbonates HCO_3.

Since reclaimed water is of lower quality than traditional water sources, improvements in management need to be made before the water is safe to use. Leaching programs, improved irrigation systems, detailed soil monitoring programs, and/or the use of salt tolerant turf species are methods that can be implemented to help avoid serious problems when using reclaimed water for irrigation. At low concentrations, dissolved salts such as those mentioned above are beneficial. However, if irrigation water contains high concentrations of these salts, they can build up to levels in the soil that inhibit turfgrass growth (Thomas et al 2006). A leaching fraction can be applied to help alleviate this problem. The leaching fraction is the amount of water that must be applied during irrigation to maintain soil salts below levels that are damaging to the plant. The formula for the leaching fraction is the EC of the irrigation water divided by the EC tolerated by the turf (Stowell and Gelernter 2001). Salt injury can be avoided if the soil salinity is kept below the salinity tolerance level of the turfgrass.

Turfgrass may be more sensitive to salts during establishment because of shorter root systems. Salt levels are generally higher near the soil surface due to evaporation and from fertilizer application. Sodium, specifically, can add to salinity problems because Na^+ effects the dispersion of soil colloids, resulting in loss of soil structure (Marcum

2004). Loss of soil structure increases the chance of soil compaction, which can lead to an anaerobic environment and decreased root growth. Also, plants react differently to salt stresses in different climates. Plants are more sensitive to salinity in hot and dry climates rather than cool, humid climates. This is most likely due to higher evapotranspiration rates in hot, dry climates which lead to increased salt uptake (Hoffman and Rawlins 1971).

High salt levels in effluent can have both direct and indirect effects on the metabolic and growth processes of the plants. Direct stresses include damage to the cytoplasm of plant cells (Tester and Davenport 2003), and ion toxicity and nutrient imbalances (Hu et al. 2005). Of the indirect effects, perhaps the biggest problem is with high soluble salt content in the soil that causes physiological drought. This is when salt levels in soil solution are high enough to cause a negative osmotic effect that limits the ability of the turfgrass to take up water (Carrow and Duncan 1998). Fertilizers only add to the salt concentration and the potential for a water limitation. Whether weakened by direct or indirect effects, turfgrasses will be more susceptible to other stresses such as pathogens, weeds, drought stress, temperature stress, and wear. Turfgrasses should be most susceptible to salt damage during establishment when root systems are still developing.

Bentgrass can be negatively affected by soil saturation and compaction which contributes to anaerobic conditions. The use of effluent may also increase the thickness of the thatch layer, create algae, or even develop a black layer. These problems increase the possibility of anaerobic conditions (Kozlowski 1984). The lack of oxygen can be toxic and eventually kill the bentgrass roots (Baird et al. 1996).

With these potential problems in mind, a study was initiated to investigate several aspects of effluent use on bentgrass. Three research objectives/questions were addressed. The first was 'what is the chemical composition of effluent?'. Effluent composition can be influenced by the general water quality of a geographical area as well as the commercial or public entities contributing to waste and degree of treatment. Up to this time, there has not been an examination of the plant nutrients in effluent generated in different areas. The second question was 'is there potential for direct salt damage to bentgrass?'. Because of the droughts in summer, the climate can take on characteristics of arid areas in the Southwest, which might lead to adverse salt effects. The third question was 'how susceptible is bentgrass to anaerobic soil conditions?'. Low oxygen levels may result from excessive water loading, especially in situations when drainage in the soil profile is restricted.

MATERIALS AND METHODS

Effluent Analysis

Samples were taken every 4-6 weeks over an 18 month period from three golf courses in North Carolina currently applying effluent to turfgrasses. These courses were Carolina National (Bolivia, NC), Governor's Club (Chapel Hill, NC), and St. James Plantation (Southport, NC). At each golf course, samples were collected from effluent and freshwater ponds that are used for irrigation. Samples also were collected from the North Cary Water Reclamation Facility to use as a standard for high quality effluent. The effluent was analyzed for nutrient anions and cations using ion chromatography: nitrate, total nitrogen, phosphate, potassium, carbon, sodium, chloride, magnesium, sulfate, and ammonium. Conductivity and pH were also measured.

Salinity Experiments

Experiments in hydroponics culture were designed to test salt tolerance of bentgrass at six different conductivity levels: 0, 1.5, 3, 6, 12, and 18 dS/m. Experiments also compared salt tolerance at two temperatures, 24 ◦C and 30 ◦C. The experiments examined creeping bentgrass (*Agrostis palustris* Huds.) cvs. A-1, G2, L-93, and Crenshaw.

Hydroponic treatment solutions were composed of 0.6 mM KNO_3, 0.5 mM KH_2PO_4, 2 mM $CaSO_4$, 1 mM $MgSO_4$, and 71.6 µM Fe as 10% chelated iron, 0.61 µM H_3BO_3, 0.12 µM $MnCl_2$, 0.11 µM $ZnSO_4$, 0.13 µM $CuSO_4$, and 0.003 µM Na_2MoO_4. A pH of 6.5 +/- 0.1 was maintained in six 12 L continuous flow hydroponics units with a flow rate of 12 L/min. Solution temperature was maintained with an automated temperature control system at 24 ◦C and 30 ◦C. The temperatures were constant with no change during the night and day. The hydroponics units were housed in an enclosed environmentally controlled walk-in growth chamber. Aerial temperature was 28 ◦C during day and 22 ◦C at night. The lighting was provided by 1000-watt metal halide lamps and 100 watt incandescent bulbs (Philips, Maddox Supply, Durham, NC) at 18 inches above canopy (shoots), which provided approximately 1200 µmol/m^2/sec of photosynthetically active irradiance.

On top of each hydroponic unit was placed a lid containing 16 circular openings. A polyethylene cup with a fine nylon mesh was placed into each opening so that the bottom touched the solution surface. Each cup contained 0.04-0.05 g of seed. For the first five days of germination, the seeds were exposed to solutions containing only 800 μM $CaSO_4$. At the time of radical emergence, additional nutrients were added. Plants were then allowed to grow for five days before establishing KCl salt treatments of 0, 1.5, 3, 6, 12, 18 dS/m. KCl, which is commonly used in hydroponics experiments when salt is needed, was chosen because the addition of large amounts of KCl does not cause toxic or confounding effects in this system. Plants were exposed to the salt solutions and temperatures for 35 days. The solutions were monitored approximately every four days and ionic constituents of samples were quantified using ion chromatography (Dionex Corp. Sunnyville, CA). Nitrate analyses were conducted with AS4G and AS4A anion separating columns placed in front of an electrical conductivity cell through a mobile phase solution of sodium bicarbonate/sodium carbonate at a flow rate of 2 mL/min. Data were recorded and integrated using Peaknet 5.1 software (Dionex Corp. Sunnyville, CA). After analysis, a NO_3^- aliquot from a 1 M KNO_3 stock was added to each chamber to return the NO_3^- concentration to the original 600 μM concentration. Nitrate uptake was estimated from the samples collected.

At the end of the exposure period, cups containing populations of bentgrass were harvested and the roots were separated from shoots just below the crown. Fresh weights for roots and shoots were measured, then the tissue was dried at 60 ◦C for 48 hours, and the dry weights recorded. Treatment effects measured include NO_3 uptake over time, shoot and root mass at the end of treatment period, and total N and C content for the root and shoot at termination of the experiment. For total N and C analysis, dried plant material was pulverized using a Geno 2000 ball grinder (Spex Certiprep, Metuchen, NJ), 2-4 mg of powdered tissue was weighed using a Metler M5 microbalance and placed in 8x5 mm tin capsules and organized in microplates. Capsules were analyzed for total N and C using a Thermo Finnigan Flash EA1112 elemental analyzer (Waltham, MA). The data were calculated and summarized using Microsoft Excel and Sigma Plot.

Anaerobic Stress Experiments

Bentgrass seedlings

Single A-1 creeping bentgrass seedlings were established from seed, using the same method as for the salt stress experiments. The hydroponics units were housed in an enclosed environmentally controlled walk-in growth chamber. Aerial temperature was 28 ◦C during day and 22 ◦C at night. The lighting was provided by 1000-watt metal halide lamps and 100 watt incandescent bulbs (Philips, Maddox Supply, Durham, NC) at 18 inches above canopy (shoots), which provided approximately 1200 μmol/m^2/sec of photosynthetically active irradiance. Seedlings were allowed to acclimate in the continuous flow hydroponics systems for approximately two weeks, at which time six single seedlings were selected from the uniform population and roots and shoots were separated, and weighed. Twelve other seedlings were selected randomly for anaerobic experimentation, and their root length measured from the crown to the root tip.

Seedlings were exposed to oxygenated or anaerobic culture solutions in 350 ml tubes for 10 days. The treatment apparatus consisted of six control tubes and six treatment tubes, each fitted with an electronic level-control system. All tubes were aerated for three days to acclimate the plants and encourage new root growth. After three days, half the tubes were converted to N_2 gas where all available dissolved oxygen (DO) dissipated to zero as confirmed by a DO meter. Plants were allowed to grow 10 days, at the end of which, the length of the root mass was measured and shoots and roots were harvested, separated, and weighed.

Bentgrass sod

Bentgrass sensitivity also was tested using sod acquired from the Sandhills Turf (Candor, NC). Bentgrass plugs (1 in. diameter) were cut, transferred to plastic cups, and allowed to grow in the hydroponics system for 30 days under continuous aeration. The growth and treatment conditions were otherwise similar to those used with bentgrass produced from seed. As before, the cups were transferred to the 350 mL tubes containing a complete nutrient solution and subjected to either aerobic or anaerobic treatments.

Soybean

A preliminary experiment using soybean (*Glycine max* L.) cv. 'Young' was conducted to determine anaerobic affects on a sensitive species and ensure the experimental system was working properly. Soybean seeds were germinated for three days in germination paper rolls soaked in 0.1 mM $CaSO_4$ solution. To expedite germination, seeds were placed in an incubator with 95% relative humidity at 28 ◦C. Soybean plants were placed in the continuous-flow hydroponics with a complete nutrient solution for one day to acclimate. From a uniform population, 12 seedlings were randomly selected and harvested, separating roots from shoots for initial fresh weight data. Another 12 seedlings were randomly selected and root length measured from posterior end of the hypocotyl to the end of the root tip; with the seedlings then transferred to the aerobic or anaerobic tubes. Following three days of exposure, root lengths doubled in the control and all plants were harvested, separated as before, and weight and root length were measured.

Microscopy

Bentgrass plants were removed from the treatment apparatus and placed on a tray, submerged in water. The roots were then dissected, with one cm sections cut from the top of the root and the root tip. Each was embedded in agarose and sliced in longitudinal sections using a vibratome (1000 Plus, The Vibratome Company, St. Louis, MO). The cross sections were placed on a glass slide under a microscope and images taken using a digital camera.

RESULTS AND DISCUSSION

Effluent Analysis

The analyses of effluent from different locations indicated that nutrient components in the effluent varied markedly at particular locations during the year and the variability was even greater among the locations (refer to tables in appendix). There were several trends of consequence. Perhaps the most important observation was that nitrate and total N were both extremely variable (Figure 2). Nitrate was the main N component in the effluent, and the highest levels were consistently recorded at Governor's Club. At this location, the highest nitrate levels were observed between December 2006 and April 2007. Nitrate levels were relatively consistent at Cary and remained fairly low throughout the year. Carolina National sampling site also remained fairly low, with the exception of one sampling date in September 2006. The nitrate concentration in effluent contrasted with nitrate levels in freshwater ponds at each site, which remained low during the 17 months of sampling. It should be noted that predicting N amounts to include in a fertilization plan has generally been difficult because of variations in N concentrations in effluent water (Pettygrove et al. 1985).

Nitrogen typically enters the turf system as fertilizer and leaves the system through leaching, clipping removal, as a gas, or under some conditions, runoff. Nitrate leaching is potentially the most significant environmental impact. Most studies show a low potential for NO_3 leaching from healthy turf, which is most likely due to the high efficiency of N absorption by turfgrass (Bowman et al. 2006). Several reports have found that both the uptake and metabolism of N are restricted by root zone salinity, which increases the potential for nitrate leaching (Bowman et al. 2006). Studies by Bowman et al. (2006) suggest that moderate root-zone salinity, like in soils associated with effluent use, should not increase the potential for NO_3 leaching. However, the effects of salinity on N uptake might be different in the field, where soil water content and salinity fluctuate in response to periods of rain or drought (Bowman et al. 2006).

Phosphorus is the second most essential element for plant growth and a potential pollutant. The P levels in effluent from the different sites ranged widely between very low from the Cary treatment plant, to over 10 mg/L at the Governor's Club. The P levels tended to be higher than those for N. Phosphorus values have been generally observed to fall between 6 and 15 mg/L in treated water from a variety of treatment plants (Pettygrove et al. 1985), which is consistent with our data. While these levels would not have an immediate fertilization impact when applied to turf, P can gradually build up in the soil over time and subsequently reduce the need for P fertilizers in the future.

Conductivity was measured to determine the amount of soluble salts in the solution water. Although there was some variability, the conductivity values were relatively low throughout the year at all locations (Figure 3). Levels stayed around 0.5 dS/m, except for the conductivity of samples from the Governor's Club which ranged up to 0.8 dS/m, and levels at Carolina National were consistently lower (0.25 to 0.35 dS/m).

In addition to salinity, pH is known to play an indirect role in turfgrass growth. Even at low salinity levels, injury to turf may occur due to a high or low pH, which can cause other toxicities or deficiencies (Harivandi 1992). Turfgrasses vary in their appropriate pH levels, but a range of 5.5 to 7.0 is usually considered optimal. The normal pH range of municipal wastewater is 6.5-8.5 (Pettygrove et al. 1985).

The pH levels found in this research ranged from about 7.0 at the lowest, to as high as 10.0 in some cases (Figure 4). If a sufficient amount of high pH effluent water was applied, this could cause a multitude of nutritional problems, including many micronutrient deficiencies. The micronutrient cations (Zn, Cu, Mn, Fe) all become unavailable for plant uptake at high pH, and insoluble $CaPO_4$ forms to reduce Ca and P availability. In addition, the precipitation reactions increase the likelihood of malfunction of sprinkler heads. It has been shown previously that irrigation with high pH effluent may lead to increases in soil pH (Schipper et al 1996). Qian and Mecham (2005) reported that soil pH was approximately 0.3 units higher in golf course fairway sites irrigated with effluent as compared to the control sites.

Salinity

The results indicated that root growth of creeping bentgrass was negatively impacted by salinity at about 6 dS/m (Figure 5). At higher salinity, root growth declined sharply and the effect was similar with all bentgrass types tested. The response was similar at moderate and high temperatures.

In previous research, Harivandi et al. (1992) identified different turfgrass sensitivities to salinity. They found that in soils with salt levels of 3 to 6 dS/m growth of some turfgrasses is restricted, at 6 to 10 dS/m the growth of many turfgrasses is restricted, and above 10 to 15 dS/m only very salt-tolerant grasses will grow. Based on our results, the framework of Harivandi et al., and on the levels of effluent water salinity measured at the four sites in North Carolina, it seems unlikely that salinity alone would cause a problem for the golf courses sampled. Effluent salinity was always $\ll 1.0$. This interpretation must include the caution, however, that in periods of extended drought when evapotranspiration would be high, salt problems may develop. As in the Southwest, golf courses need periodic leaching cycles to remove salts from the root zone and minimize the likelihood of salt issues.

Anaerobic Studies

There has been limited research examining whether anaerobic conditions have a strong negative affect on turfgrass. When using effluent, frequent irrigation to disperse effluent is common and can reduce soil oxygen availability. Jiang and Wang (2006) found that (based on depth in soil profile) waterlogging decreased soil redox potential, and reduced root dry weight and visual quality in cultivars A-4, G-6, Pennlinks, and

Penncross after 7 days. The only creeping bentgrass cultivar that remained visually acceptable throughout the course of the experiment was L-93. At the conclusion of the experiment, formation of aerenchyma was found in L-93 and Penncross cultivars. Waterlogging prompted the growth of adventitious roots in all cultivars. This experiment demonstrated that even partial waterlogging (that not seen on the surface) could have an effect on turf growth.

In our experiments, there was not a negative effect of anaerobic conditions on root fresh weight in bentgrass sod, and only a slight reduction in root fresh weight when bentgrass seedlings were grown from seed (Figure 6). There was, however, a consistent negative effect on rooting depth for bentgrass, with reductions of 40% in bentgrass sod and 60% in bentgrass seedlings. The differing degrees of inhibition likely reflect sod being stronger and more established than the individual bentgrass seedlings, making it less sensitive to adverse conditions. Parallel experiments were conducted with the check plant soybean, which is generally viewed as being sensitive to low oxygen conditions in the root zone. That certainly was true in our experiments, as root fresh weight and rooting depth were decreased by 80% when compared to the control (Figure 6). Clearly, in our experimental system, bentgrass was much more tolerant to low oxygen than soybean.

The explanation for the decrease in root depth with only a slight decrease in root fresh weight was that adventitious roots were formed in the upper part of the root system. This was similar to earlier observations with bentgrass (Jiang and Wang 2006). Adventitious roots grow from the stem and can access oxygen because they are located near the surface of the solution.

Microscopic examination of root cross sections in our experiments also revealed the presence of aerenchymous tissue in bentgrass under anaerobic conditions (Figure 7). Aerenchyma is formed via the breakdown of the cortex, and providing an internal pathway for the transport of oxygen and carbon dioxide throughout the plant (Armstrong 1972). It has been recognized that mechanisms of tolerance to anaerobic conditions may be associated with plant traits that improve oxygen uptake, such as changes in anatomy and morphology (Setter and Waters 2003). While formation of aerenchyma may aid in root survival under anaerobic conditions, a connection between amount of aerenchyma and saturation tolerance is seldom seen (Setter et al. 1999).

CONCLUSIONS

The use of effluent for irrigation is essential to lessen damage to surface waters and the strain on freshwater supplies. Applying effluent to turfgrass, especially on golf courses, is an effective alternative water source for irrigation and limits pollution of rivers and streams.

The analyses of effluent from different sources showed that nutrient levels were highly variable at a particular location and between locations. This makes it difficult for superintendents to build effluent into a comprehensive fertility plan. Based on several experiments in hydroponics, it seems that bentgrass would not be damaged by salt effects resulting directly from effluent additions. It is conceivable that damage could occur if salt accumulates during intense drought. Also, reduced N uptake (possible from high salinity), might then contribute to greater NO_3 leaching when rain or heavy irrigation cause the soil water to move beyond the root zone (Bowman et al. 2006). Our results also suggest that there is little likelihood that bentgrass would be severely damaged directly by anaerobic conditions if effluent is added to putting greens. The shift in morphological development to more shallow rooting, however, would predispose bentgrass to high temperature stress and desiccation during hot summer months.

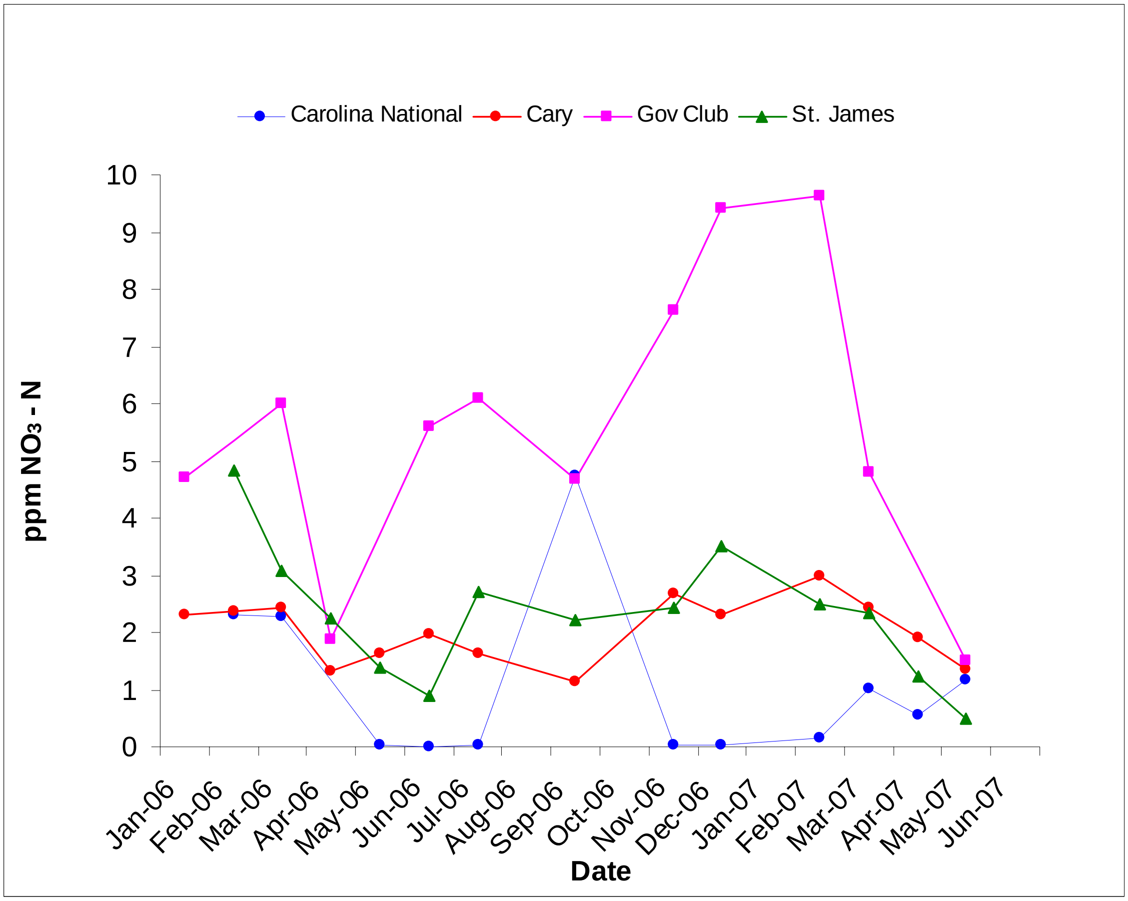

Figure 2. Nitrate levels from all four effluent locations between January 2006 and May 2007.

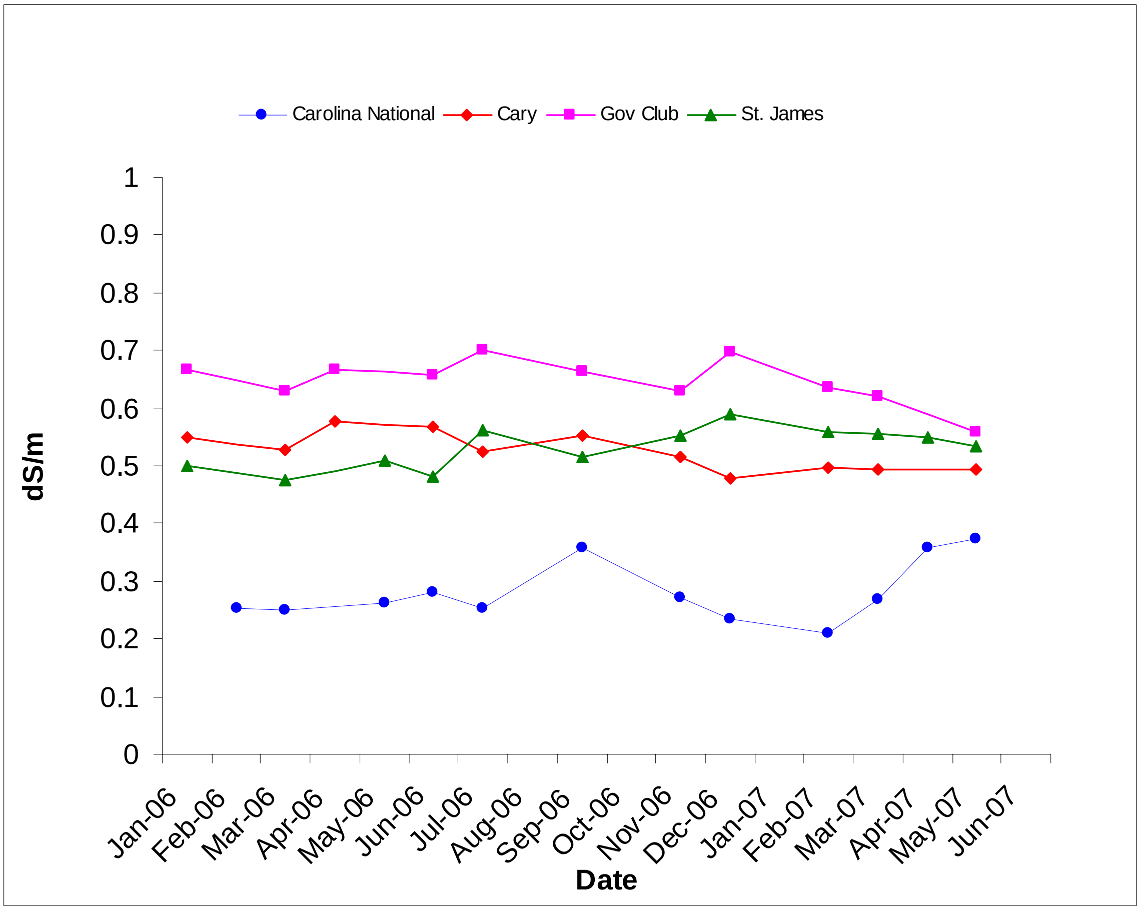

Figure 3. Conductivity levels for all four effluent locations from January 2006 to May 2007.

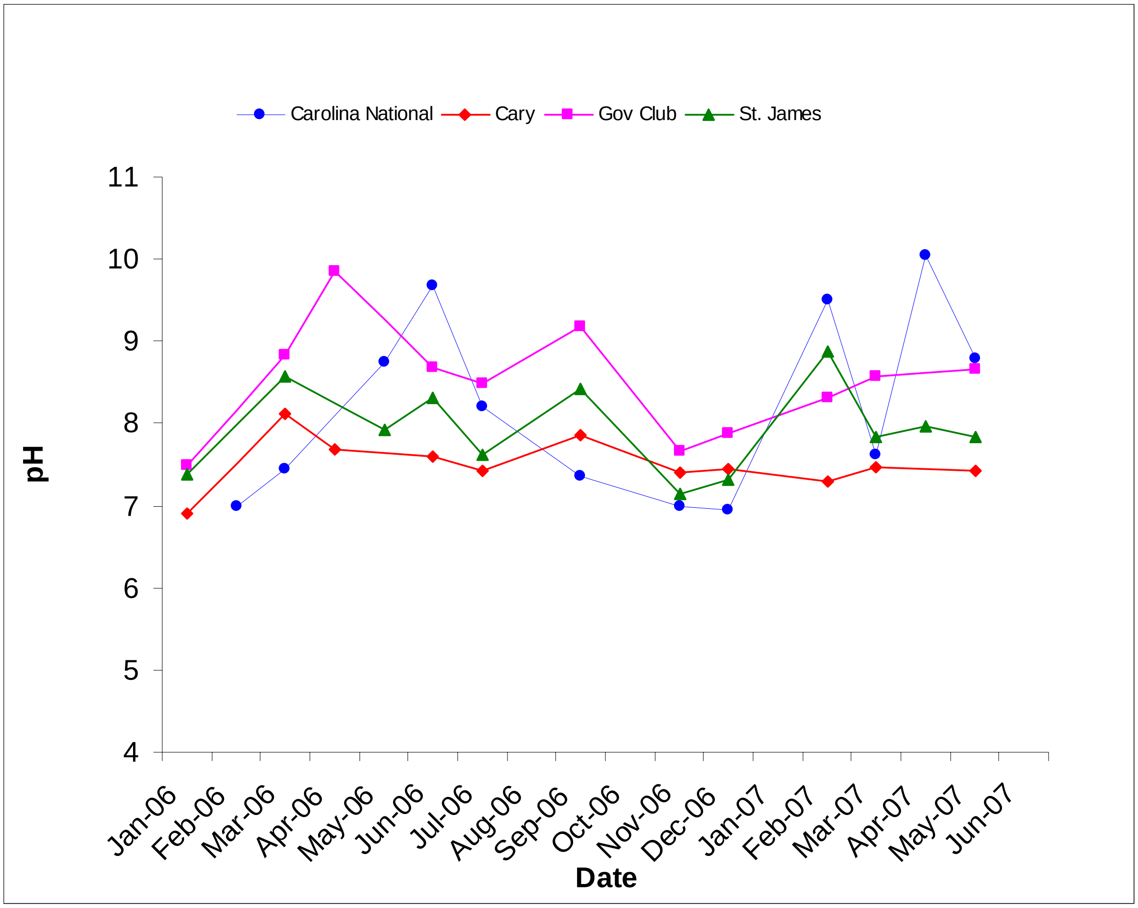

Figure 4. pH values from all four effluent locations from January 2006 to May 2007.

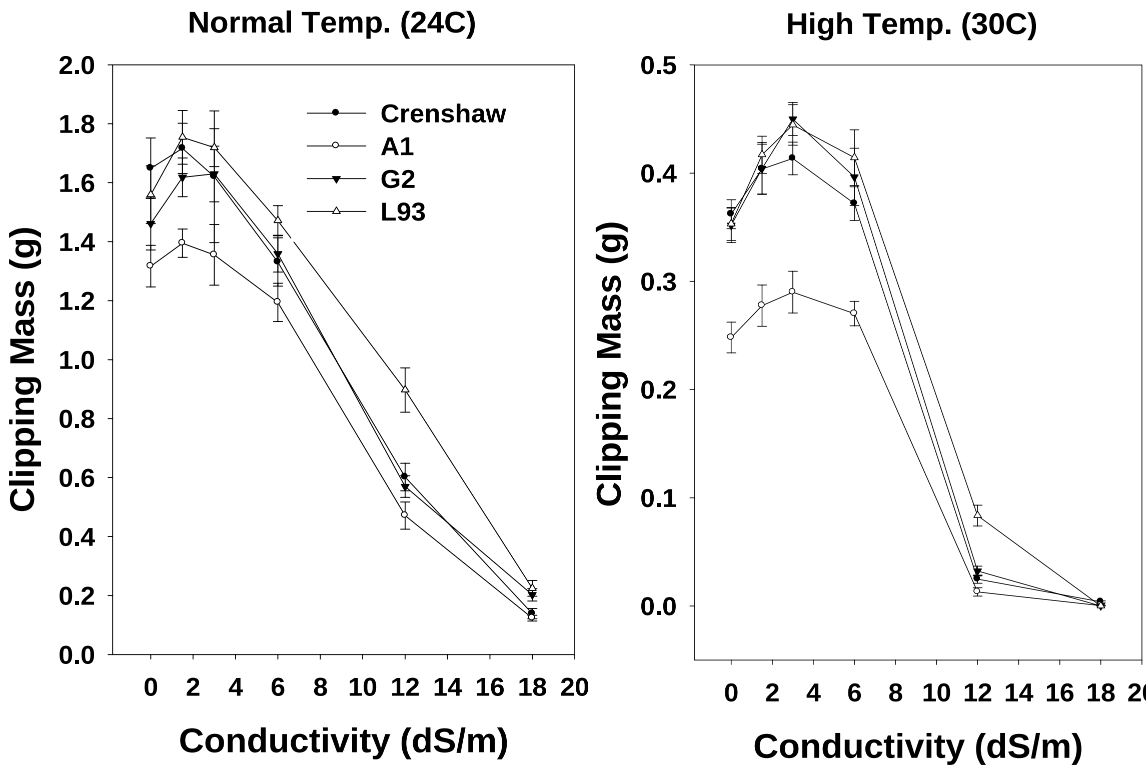

Figure 5. Graphs from salinity experiment show growth rates over the full range of conductivity levels, both in optimal and high temperature situations.

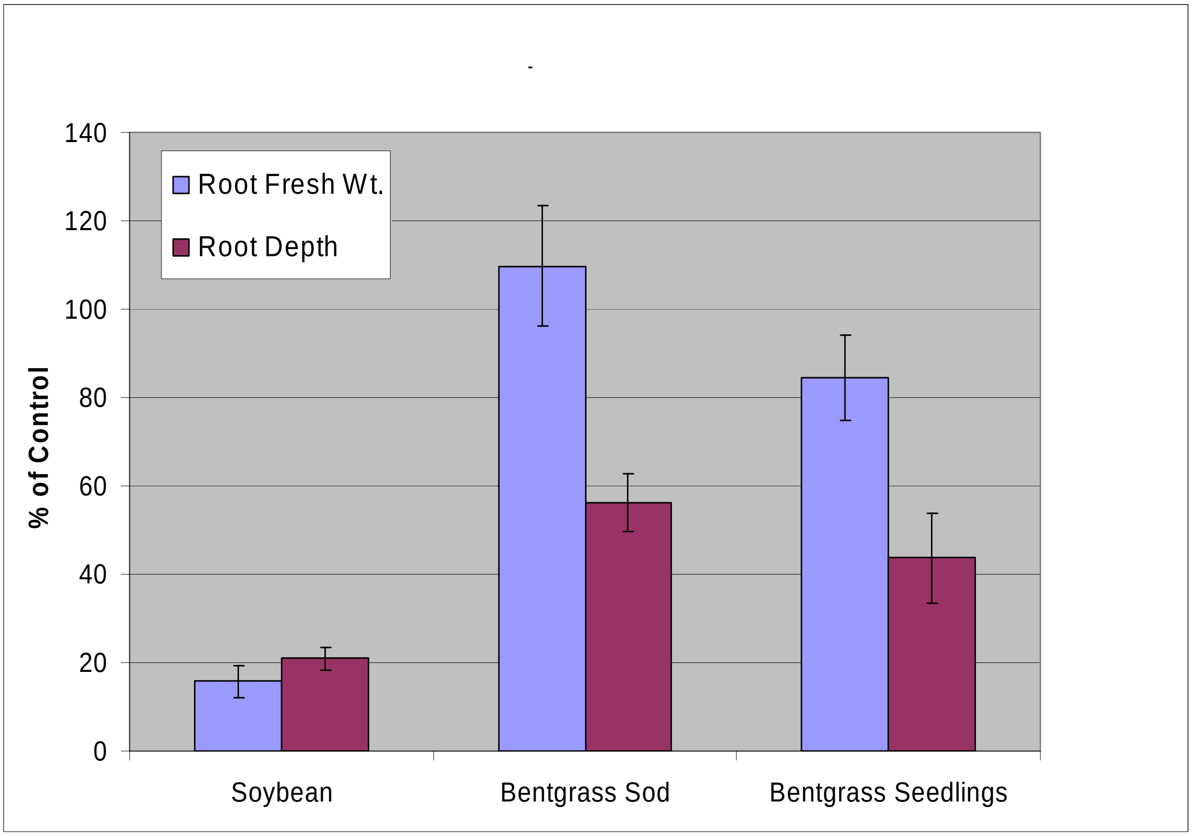

Figure 6. Results from the anaerobic experiment. The data for percent of control for plant mass and root elongation were plotted as percent of control.

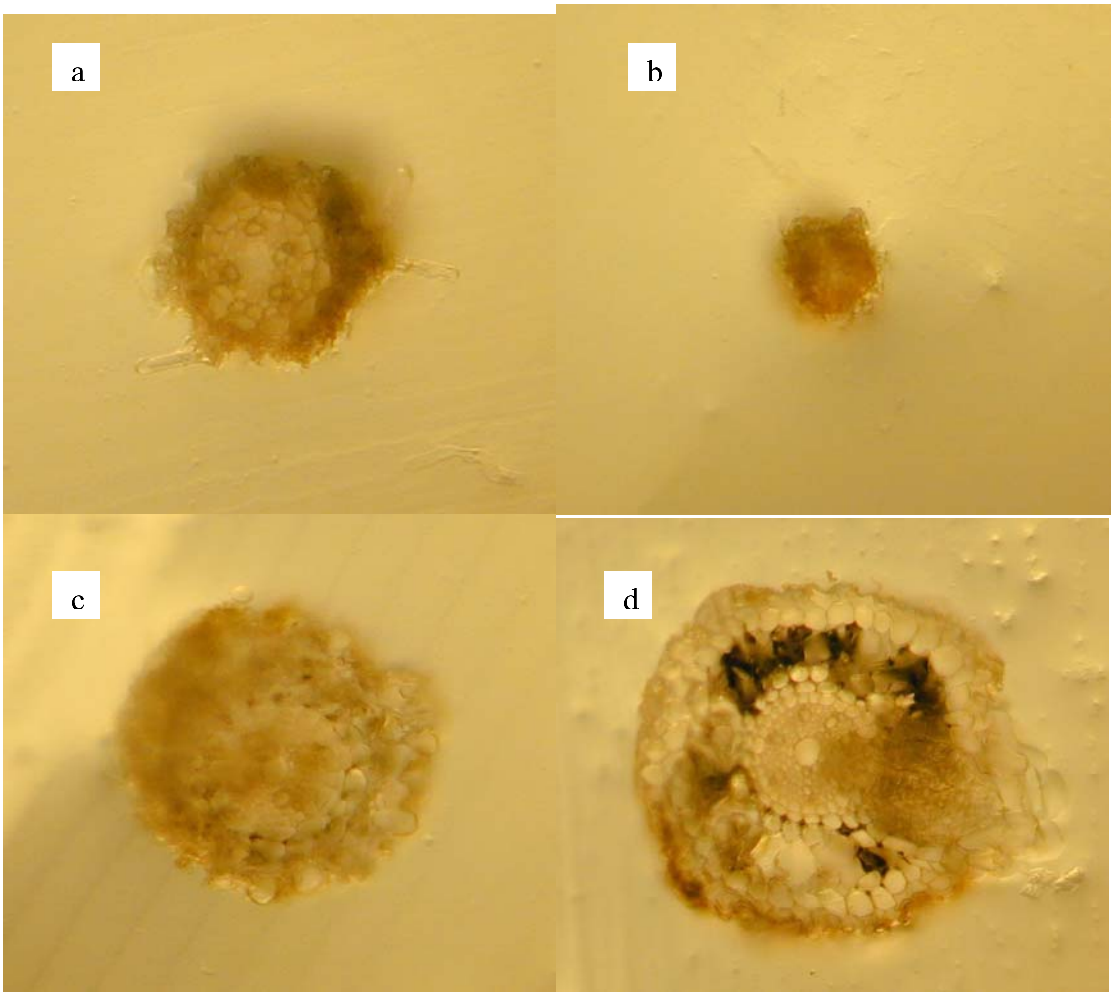

Figure 7. Images of possible aerenchyma formation. Sections were taken 1 cm from the top of the root and 1 cm from the root tip: a. aerobic root (top), b. anaerobic root (top), c. aerobic root (tip), d. anaerobic root (tip)

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APPENDIX

Appendix Table 1. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for municipal effluent from the North Cary Water Reclamation Facility - Cary, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
			ppm										
Jan-06	0.55	6.9	3.4	45.0	78.8	2.3	0.0	2.8	50.5	4.3	15.2	13.5	13.9
Mar-06	0.53	8.1	0.7	35.4	65.6	2.4	0.0	3.0	49.9	4.2	16.9	14.2	13.6
Apr-06	0.58	7.7	0.7	46.7	69.0	1.3	0.0	1.4	18.9	4.0	16.1	13.5	8.9
Jun-06	0.57	7.6	2.4	54.2	66.7	2.0	0.0	4.8	80.3	4.2	18.0	12.9	7.0
Jul-06	0.53	7.4	0.7	50.5	62.2	1.6	0.2	6.2	71.4	4.0	17.0	12.6	7.2
Sep-06	0.55	7.9	0.3	53.3	60.2	1.1	0.0	4.8	77.0	3.8	16.5	12.8	10.8
Nov-06	0.52	7.4	0.5	49.4	58.9	2.7	0.3	4.1	69.3	3.9	15.6	11.6	7.9
Dec-06	0.48	7.5	0.2	48.7	54.7	2.3	0.2	2.5	65.0	3.5	14.2	10.8	9.7
Feb-07	0.50	7.3	0.2	48.1	58.2	3.0	0.5	3.4	70.0	3.7	14.8	11.4	12.9
Mar-07	0.49	7.5	1.8	44.9	57.8	2.4	0.2	2.7	67.1	3.9	14.5	10.3	12.8
May-07	0.49	7.4	0.4	40.3	53.0	1.6	0.0	1.6	75.3	3.6	16.9	13.1	7.9
Average	0.53	7.5	1.0	46.9	62.3	2.1	0.1	3.4	63.1	3.9	16.0	12.4	10.2
Std. Dev.	0.03	0.3	1.0	5.5	7.4	0.6	0.2	1.5	17.6	0.3	1.2	1.2	2.7
CV	6.26	4.1	100.7	11.7	11.9	28.4	125.1	43.1	27.9	6.5	7.6	9.9	26.2

Appendix Table 2. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for municipal effluent from Carolina National Golf Club - Bolivia, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
							ppm						
Feb-06	0.25	7.0	6.3	14.2	28.5	2.3	1.3	4.2	16.5	2.4	16.6	8.8	13.7
Mar-06	0.25	7.4	4.1	1.3	27.6	2.3	0.1	4.4	16.4	2.2	15.8	8.9	19.0
May-06	0.26	8.7	3.3	17.3	33.0	0.0	0.2	2.6	15.7	2.1	16.5	9.4	16.1
Jun-06	0.28	9.7	1.5	17.8	33.7	0.0	0.0	5.0	23.7	1.4	11.2	8.6	16.7
Jul-06	0.25	8.2	3.3	17.4	32.3	0.0	1.0	6.0	24.5	1.2	13.6	8.6	21.3
Sep-06	0.36	7.4	2.9	19.1	37.1	4.7	0.2	9.7	31.5	2.7	24.3	8.7	10.1
Nov-06	0.27	7.0	2.1	17.8	32.2	0.0	0.1	1.3	19.8	2.4	18.5	7.5	14.1
Dec-06	0.23	7.0	2.0	16.3	27.5	0.0	1.2	1.6	21.4	2.2	15.6	6.9	12.8
Feb-07	0.21	9.5	1.6	16.8	25.0	0.1	1.1	1.9	14.6	2.0	13.6	6.0	14.8
Mar-07	0.27	7.6	2.6	19.5	33.1	1.0	0.1	3.7	23.3	2.3	15.5	8.1	23.3
Apr-07	0.36	10.0	0.9	23.7	41.4	0.6	0.2	3.3	36.6	2.4	15.2	10.6	18.5
May-07	0.37	8.8	7.3	25.9	46.0	1.3	0.9	2.9	42.1	3.2	20.3	14.0	13.3
Average	0.28	8.2	3.1	17.2	33.1	1.0	0.5	3.9	23.8	2.2	16.4	8.8	16.1
Std. Dev.	0.05	1.1	1.9	6.0	6.0	1.4	0.5	2.3	8.7	0.5	3.4	2.0	3.8
CV	18.71	13.8	61.7	34.6	18.3	138.4	94.8	60.2	36.6	24.8	20.9	22.8	23.6

Appendix Table 3. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for a freshwater pond from Carolina National Golf Club - Bolivia, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
			ppm										
Feb-06	0.32	7.0	0.2	7.7	10.9	0.5	0.0	2.3	6.2	1.7	57.8	1.8	8.2
Mar-06	0.31	7.9	0.0	7.4	12.0	0.0	0.0	0.8	6.5	1.7	55.6	1.7	11.7
May-06	0.32	8.1	0.0	7.4	12.3	0.0	0.0	1.9	1.9	1.6	52.5	1.2	4.3
Jun-06	0.28	8.1	0.0	7.5	13.1	0.0	0.0	6.1	5.9	1.5	45.5	1.3	4.2
Jul-06	0.28	7.7	0.0	6.9	15.1	0.0	0.2	2.2	8.2	1.6	46.6	1.2	4.1
Sep-06	0.31	8.0	0.0	8.7	13.1	0.0	0.1	4.6	7.7	1.7	51.3	1.7	4.8
Nov-06	0.34	7.6	0.0	10.0	13.4	0.0	0.1	0.3	6.2	1.7	56.7	1.6	4.7
Dec-06	0.34	7.7	0.0	10.8	14.6	0.0	0.5	1.5	6.6	1.7	56.5	2.2	5.1
Feb-07	0.36	7.9	0.0	11.6	15.0	0.0	0.1	0.9	6.7	1.8	61.1	1.8	9.3
Mar-07	0.36	7.7	0.0	11.1	15.0	0.0	0.1	1.1	7.5	1.9	62.2	1.8	7.9
Apr-07	0.32	8.1	0.0	11.0	14.6	0.0	0.0	0.8	14.1	1.9	52.6	1.7	6.2
May-07	0.29	6.9	0.0	9.5	14.6	0.1	4.0	4.1	12.0	2.0	48.2	2.3	9.3
Average	0.32	7.7	0.0	9.1	13.6	0.1	0.4	2.2	7.5	1.7	53.9	1.7	6.7
Std. Dev.	0.03	0.4	0.1	1.7	1.4	0.1	1.1	1.8	3.1	0.1	5.4	0.3	2.5
CV	8.51	5.3	346.4	19.0	10.2	205.1	275.7	80.8	41.2	7.9	10.0	20.1	38.3

Appendix Table 4. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for effluent ponds from Governor's Club Golf Course - Chapel Hill, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
			ppm										
Jan-06	0.67	7.5	10.4	38.8	71.7	4.7	3.1	7.9	65.5	4.4	12.8	14.5	24.8
Mar-06	0.63	8.8	10.8	42.2	57.4	6.0	1.4	8.0	63.7	4.4	11.4	13.9	32.5
Apr-06	0.67	9.9	7.1	41.5	64.9	1.9	1.5	6.3	112.4	3.7	8.8	13.5	18.2
Jun-06	0.66	8.7	8.1	47.0	66.0	5.6	0.4	9.8	112.7	4.1	11.3	13.6	15.8
Jul-06	0.70	8.5	8.8	44.1	64.9	6.1	5.0	13.5	120.8	4.1	10.8	15.5	18.7
Sep-06	0.66	9.2	11.8	39.5	56.9	4.7	0.2	9.3	106.7	4.5	12.0	13.4	21.4
Nov-06	0.63	7.7	11.6	39.9	55.3	7.6	0.5	8.7	100.6	4.5	12.6	12.7	14.9
Dec-06	0.70	7.9	12.7	43.1	54.0	9.4	2.1	11.5	101.3	4.3	12.5	12.9	18.4
Feb-07	0.64	8.3	12.4	43.6	54.8	9.6	1.2	11.4	102.2	4.3	12.3	13.4	16.3
Mar-07	0.62	8.6	11.3	39.2	50.6	4.8	1.2	6.5	94.5	4.0	10.2	11.8	16.4
May-07	0.56	8.7	8.4	30.7	41.3	1.7	1.3	4.4	101.4	4.2	11.1	13.6	15.1
Average	0.65	8.5	10.3	40.9	58.0	5.6	1.6	8.9	98.3	4.2	11.4	13.5	19.3
Std. Dev.	0.04	0.7	1.9	4.2	8.4	2.6	1.4	2.7	18.2	0.2	1.2	1.0	5.3
CV	6.12	8.0	18.5	10.3	14.6	45.5	85.5	30.0	18.5	5.7	10.4	7.1	27.3

Appendix Table 5. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for a freshwater irrigation pond from Governor's Club Golf Course - Chapel Hill, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
							ppm						
Mar-06	0.16	7.4	0.0	18.5	15.9	0.3	0.0	0.7	12.5	2.6	8.6	4.4	8.9
Apr-06	0.19	7.5	0.0	21.4	17.0	0.0	0.1	1.3	15.4	3.3	11.0	4.7	8.7
Jun-06	0.23	8.1	0.0	34.0	17.4	0.0	0.0	2.7	16.0	4.4	16.7	5.3	8.6
Jul-06	0.21	7.8	0.6	20.7	15.4	0.0	1.2	4.4	17.4	3.6	13.9	5.5	15.9
Sep-06	0.16	7.5	0.3	11.7	11.8	0.1	0.4	6.8	13.0	2.7	9.7	4.6	10.8
Nov-06	0.12	6.8	0.0	12.7	9.4	0.2	0.1	0.5	7.5	2.0	6.7	3.5	9.3
Dec-06	0.12	6.8	0.0	13.5	10.5	0.2	0.1	2.8	8.6	2.1	7.0	3.3	10.0
Feb-07	0.16	6.9	0.0	13.3	18.1	0.1	0.4	1.3	12.3	2.2	7.4	6.3	11.3
Mar-07	0.14	7.5	0.0	14.1	12.8	0.1	0.1	1.2	10.2	2.3	7.9	3.4	12.8
May-07	0.15	8.6	0.0	13.6	10.8	0.0	0.1	2.2	14.0	3.0	11.0	4.3	12.5
Average	0.16	7.5	0.1	17.4	13.9	0.1	0.2	2.4	12.7	2.8	10.0	4.5	10.9
Std. Dev.	0.04	0.6	0.2	6.8	3.2	0.1	0.4	1.9	3.2	0.8	3.3	1.0	2.3
CV	22.18	7.6	224.7	39.2	23.1	96.3	147.0	81.4	25.2	27.2	32.6	21.5	21.4

Appendix Table 6. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for effluent ponds from St. James Plantation - Southport, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
							ppm						
Jan-06	0.50	7.4	6.4	68.8	50.0	4.8	4.0	8.8	29.8	4.8	39.5	12.9	14.3
Mar-06	0.47	8.6	4.9	60.4	45.6	3.1	0.3	4.5	31.1	4.4	34.8	12.8	28.5
May-06	0.51	7.9	6.3	42.9	46.3	1.4	0.6	3.6	43.9	4.4	43.8	11.4	17.9
Jun-06	0.48	8.3	4.4	41.1	45.8	0.9	3.3	8.1	41.3	4.1	36.8	11.0	17.6
Jul-06	0.56	7.6	7.0	30.6	44.2	2.7	1.7	6.5	36.6	4.3	55.6	9.8	17.5
Sep-06	0.52	8.4	3.2	41.4	45.8	2.2	1.1	7.5	42.4	3.6	43.0	10.3	17.5
Nov-06	0.55	7.2	6.9	42.7	57.4	2.4	0.2	7.6	54.1	4.0	39.6	11.9	14.3
Dec-06	0.59	7.3	6.5	51.4	64.1	3.5	0.7	4.2	61.1	4.2	37.8	13.0	14.4
Feb-07	0.56	8.9	4.8	53.7	62.5	2.5	1.0	3.5	61.3	3.7	32.3	13.2	18.9
Mar-07	0.56	7.8	6.9	54.5	64.5	2.3	0.3	3.1	66.4	3.9	29.4	15.1	26.8
Apr-07	0.55	8.0	5.5	36.5	54.0	1.3	0.0	2.5	56.0	4.1	52.2	12.6	20.9
May-07	0.53	7.8	2.8	25.4	44.3	0.5	0.3	2.7	42.5	3.7	62.7	9.2	16.4
Average	0.53	7.9	5.4	45.8	52.0	2.3	1.1	5.2	47.2	4.1	42.3	11.9	18.7
Std. Dev.	0.04	0.5	1.4	12.4	8.1	1.2	1.3	2.3	12.3	0.4	9.9	1.7	4.6
CV	6.76	6.7	26.6	27.2	15.5	51.6	114.1	44.3	26.0	8.6	23.5	14.0	24.6

Appendix Table 7. Electrical conductivity, pH, anion, cation, total nitrogen, and total carbon values for a freshwater pond from St. James Plantation - Southport, NC.

Date	EC (dS/m)	pH	PO_4^{2-}	SO_4^{2-}	Cl^-	NO_3^--N	NH_4^+-N	Total N	Na^+	Mg^{2+}	Ca^{2+}	K^+	Total C
							ppm						
Jan-06	0.07	7.7	0.2	3.1	8.0	0.5	0.0	0.7	4.6	0.6	9.1	1.2	26.8
Mar-06	0.28	7.9	0.0	1.5	15.1	0.0	0.0	1.0	6.7	1.7	51.2	0.6	17.4
May-06	0.38	7.9	0.0	0.8	13.2	0.0	0.0	1.5	10.6	2.1	65.4	1.0	7.9
Jun-06	0.28	8.0	0.0	2.7	12.4	0.0	0.0	1.5	6.2	1.7	48.2	1.0	13.6
Jul-06	0.40	8.0	0.0	0.8	19.5	0.0	0.1	1.3	10.3	2.1	69.5	0.9	6.9
Sep-06	0.30	7.9	0.0	4.1	12.6	0.0	0.1	2.0	8.5	1.9	47.4	1.0	20.5
Nov-06	0.29	7.4	0.0	4.6	13.7	0.1	0.0	0.8	7.2	1.9	46.8	1.1	15.4
Dec-06	0.19	7.1	0.0	5.8	13.7	0.1	0.0	1.1	6.4	1.6	28.4	1.0	27.7
Feb-07	0.21	7.1	0.0	5.9	14.0	0.1	0.2	1.3	6.0	1.7	31.9	0.9	22.8
Mar-07	0.37	7.5	0.0	2.5	14.9	0.0	0.1	2.6	9.7	2.2	64.1	1.3	14.8
Apr-07	0.41	7.6	0.0	1.4	13.8	0.0	0.1	3.2	16.2	2.4	76.3	1.1	7.4
May-07	0.39	8.0	0.0	1.4	12.6	0.1	0.6	6.9	12.5	2.3	73.5	1.0	7.7
Average	0.30	7.7	0.0	2.9	13.6	0.1	0.1	2.0	8.8	1.8	51.0	1.0	15.8
Std. Dev.	0.10	0.3	0.1	1.8	2.6	0.1	0.2	1.7	3.3	0.5	20.3	0.2	7.5
CV	34.43	4.3	346.4	64.3	19.1	148.2	179.1	85.9	37.7	25.4	39.8	17.7	47.6