PLANT SPECIES AS A SIGNIFICANT FACTOR IN WASTEWATER TREATMENT IN CONSTRUCTED WETLANDS

A Senior Thesis
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Abstract

Plant Species as a Significant Factor in Wastewater Treatment in Constructed Wetlands Tracey W. Varvel, (Dr. Richard W. Weaver) University Undergraduate Fellow, 1997-1998, Texas A&M University, Department of Soil and Crop Sciences

Constructed wetlands are one of the newest wastewater treatment technologies. They should reduce the Biochemical Oxygen Demand (BOD) and utilize a large amount of the influent. The BOD determines how much oxygen is used by microorganisms while oxidizing organic matter. If BOD is high, the effluent is high in organic material, which clogs the soil of the drainfield. Reductions in the BOD can increase the life of a drainfield. The water usage of wetlands is important to drainfields. Reducing the amount of effluent through water uptake can result in smaller drainfields. This study was conducted using Arrowhead (Sagittaria lancifolia), Umbrella Palm (Cyperus alternifolius), Dwarf Umbrella Palm (Cyperus isoclaudus), and Cattail (Typha latifolia) in microcosms fed rural septic influent. The water parameters studied were water usage, ammonium-nitrogen, phosphorus, coliforms, suspended solids, BOD, pH, and turbidity. The BOD for all plants was reduced below the standard levels but none were significantly different. The Umbrella Palm utilized an average of 30 % of the wastewater it received over a two-day period and its water usage was significantly different from the others. The Umbrella Palm reduced the BOD and influent volume, making it the best plant choice for use in constructed wetlands.

Plant Species as a Significant Factor in Wastewater Treatment in Constructed Wetlands

Tracey W. Varvel

Constructed wetlands are one of the newest methods of municipal wastewater treatment and are the focal point of many current studies. They are designed to mimic natural wetlands, leaving the majority of wastewater cleanup to wetlands plant life. The use of plant life to remove or reduce environmental contaminants is known as phytoremediation (Anderson, 1996). The environmental contaminants present in wastewater include nitrogen (N), phosphorus (P), Biochemical Oxygen Demand (BOD) levels, suspended solids, and pathogens. These constructed wetlands have many advantages such as; energy efficiency, simplicity, low cost, advanced treatment levels, reliability, and versatility. They also provide aesthetic value in the plants chosen for the wetland. Many regulators and environmentalists have recognized constructed wetlands as a favorable alternative to contemporary wastewater treatments. For example, the Tennessee Valley Authority developed Constructed Wetlands Technology and began educating state environmental agencies and health organizations in 1986. The result of their efforts in recent years is increased use of constructed wetlands(IS-1).

<u>Constructed Wetlands</u>

Constructed wetlands are an innovative wastewater treatment technology based on older natural processes. These processes occur in natural wetlands and have existed longer than any other treatment technology, Mother Nature's method of dealing with impurities. The last half of the eighties and the beginning of the nineties decades have seen a rise in the use and study of constructed wetlands. However, according to Freeman

(1993) a sound design principle for the systems has yet to be developed. He blames this on the lack of meaningful data and understanding of the physical and biological processes at work in the system. Recently more research has been conducted on these systems and long-term operational data exists for full-scaled engineered wetland systems. The research conducted in pilot facilities has provided an adequate understanding of the physical, biological, and chemical processes occurring in the constructed wetlands (Kadlec and Knight, 1996).

Constructed wetlands vary in size from small ones that serve one household to extremely large ones that serve entire cities. The Orlando Easterly Wetlands in Orlando, Florida is one of the nation's largest treatment facilities of its kind. It consists of 17 cells planted with three types of plant communities, one with bulrush and cattails, one with a variety of submergent and emergent herbaceous species, and the third with hardwood species. It was discovered at this time that in the large wetland systems, gravel clogs the system as opposed to small wetlands in which they work well (Gillette, 1996). Two types of constructed wetlands systems exist, subsurface flow systems (SFS) and free-water surface systems (FWS). A SFS wetland is essentially a lined gravel control bed supporting various wetland plants and the flow of wastewater is below the surface of the gravel control. A FWS wetland is also lined and supporting wetland plants but the flow of wastewater occurs along the top of a shallow bed. Less required land and more cold tolerance are advantages of SFS wetlands over the FWS wetlands. The SFS wetlands also have no visible flow, fewer pest problems, and fewer odor problems. On the other hand, advantages of the FWS wetlands include a lower installment cost, simpler hydraulics and the ability to incorporate more natural wetland aspects (Freeman, 1993).

The advantages of the SFS wetland exhibit how it is suitable for residential areas whereas the FWS wetland is not. It is more useful in large systems where the cost saving are more evident and the upkeep of the system is easier (Sauter and Leonard, 1995).

Plant species for Constructed Wetland Use

The most important aspect of a constructed wetlands system is the plant life. They provide a large surface area for microorganisms, reduce the nitrogen content, and facilitate evapotranspiration of water (Hofmann, 1997). The plants are also important in the reduction of BOD levels, and the removal of P. Currently, the most abundantly used plant species are *Phragmites australis* (common reed, *Sagittaria* (arrowheads), *Typha* (cattails), and *Scirpus* (bulrush). It is common practice to plant a variety of wetland plants because the best plant species have yet to be determined (Kadlec and Knight, 1996). According to Gregory Sauter and Kathleen Leonard (1995), plant life should be chosen from indigenous species of *Typhaceae* (cattails), *Cyperacaea* (sedge), *Gramineae* (grass), *Scirpus* (bulrush), and *Phragmites* (reeds). They also suggest that plant species that tend to choke out other species should be avoided.

Research and study of constructed wetlands has centered mainly on the quality of the effluent and not the particular plant or plants that work the best. In recent years more studies have been performed. For instance in Sand Mountain, Alabama and Putman County, Georgia, various plant species used in the constructed wetlands for dairy wastewater were evaluated. However, these studies were more concerned with the suitability of plant life for wetlands systems than they were with the plant or plants that maximized the treatment process. Much of the data on plant species pertains to agricultural operations. For instance, varieties of wetland plant species were evaluated in constructed wetlands systems for dairy operations in Sand Mountain, Alabama and Putnam, Georgia. These studies concentrated on the survival of various species (Surrency, 1993).

Constructed wetlands use is not limited to the United States, in the United Kingdom facilities are using constructed reed beds for tertiary treatment. These reed beds have attained significant decrease in the BOD levels and in the suspended solids (Green, 1994). Research in Germany has also been conducted using reeds. Studies have shown that the reeds improve the oxygen supply of the constructed wetland through their roots. The roots not only leak oxygen, but also are responsible for the formation of air pockets through the growth of their root system. The reeds have also shown a high demand for water and facilitate its loss through evapotranspiration. The reed bed show significant reductions in the N content when compared to the control beds (Hofmann, 1997).

Water Quality Parameters

Constructed wetlands are created with the primary purpose of water quality improvement, specifically decreases in the concentrations of suspended solids, BOD, N, P, and pathogens (Brix, 1993). Total-N and total-P reductions of 75% and 74% respectively occurred in a study of the use of constructed wetlands treating dairy farm wastewater (Tanner, 1995). Based on the National Pollutant Discharge Elimination System, BOD_5 levels after treatment must be at or below 30 mg/L (Leonard, 1995). In a study in the United Kingdom, reed beds used as tertiary treatment were monitored using the before mentioned water quality parameters. The BOD_5 levels were reduced from around 25 mg/L to less than 5 mg/L and total suspended solids from around 50 mg/L to less than 10 mg/L (Green, 1994). A study in Richmond, New South Wales, Australia, consisted of seven pilot scale treatment wetlands; three of which were gravel control and cattails, gravel control and bulrush, and gravel control only. The systems were monitored for two years, and during that time the gravel control treatment showed an average percent reduction of 92 for BOD_5, 93 for TSS, 21 for phosphorus, 46 for ammonium-nitrogen, and 99.8 for fecal coliforms. Cattails showed percent reductions of 91 for BOD_5, 93 for TSS, 19 for phosphorus, 47 for ammonium-nitrogen, and 99 for fecal coliforms. Bulrush showed percent reductions of 89 for BOD_5, 91 for TSS, 12 for phosphorus, 45 for ammonium-nitrogen, 99 for fecal coliforms (Kadlec and Knight, 1995).

The objective of the present study is to determine which plant species are most effective at phytoremediation of wastewater from rural septic tanks. The effectiveness of the plant is determined by comparing the improvements it made to the water quality parameters with other plants.

MATERIALS AND METHODS

The experiment was conducted using wetland microcosms, miniature wetlands. Microcosms were constructed in a greenhouse using five-liter cylindrical containers filled with washed gravel control. The containers were equipped with a drainage hole to retain a level of septic effluent similar to that in a constructed wetland and a 0.25-inch tygon tubing with clamp to aid in sample collection. Septic effluent from both units of a two-bedroom duplex was added to the microcosms and replaced every two days to simulate a similar retention time in the wetland.

Experimental Variables and Control

The experimental variables in this project were four different plant species; Arrowhead (*Sagittaria lancifolia*), Umbrella Palm (*Cyperus alternifolius*), Dwarf Umbrella Palm (*Cyperus isocladus*), and Cattail (*Typha latifolia*). Two of each plant species were used to obtain dependable results. The plants were bought full grown and then potted in the five-liter cylindrical containers containing washed gravel control. They were fed plant food and water until they became established in the pots, and then were switched to septic effluent. Data collection began after a period of three weeks, allowing the plants to become accustomed to the effluent. The controls in this experiment were two pots filled only with washed gravel control and given septic effluent at the same times as the plants.

Data Collection

Water usage data were collected by adding a known volume of distilled water until level with the container's drainage hole. The amount of distilled water added represented the amount that the plants had removed through evapotranspiration. It also served the purpose of bringing the sample up to volume. This more accurately shows how much the contaminants were reduced from their original concentrations. After the water usage data were collected, each plant's effluent was completely drained into separate clean buckets. They were stirred and a one to five hundred milliliter sample, depending on the number and type of testing, of each was collected in appropriately labeled sampling bottles. The containers were again filled to the drainage hole with the influent and a one to five hundred milliliter sample of the influent was taken as well. The collection bottles were then transported to the laboratory were they would be tested upon arrival or stored in a refrigerator at 4oC until testing could begin. On Mondays water usage data were collected and the samples from the plants were analyzed for turbidity, temperature, pH, BOD, total and volatile suspended solids, and fecal and total coliforms. On Tuesdays, the septic influent was changed in the containers and a sample to the influent was analyzed for ammonium-nitrogen (NH_4^+-N) and P. On Fridays water usage data was collected and the septic influent was replaced. A sample of the influent was analyzed for turbidity, temperature, pH, biological oxygen demand, total and volatile suspended solids, and fecal and total coliforms. Samples from the plants were analyzed for NH_4^+-N and P.

Testing Procedures

The turbidity test requires a small portion of the sample to be placed in a special cuvette and measured using a turbidity meter (LaMotte Model 2008). It is important that before performing the test the sample is not agitated because this will impair the results. The temperature and pH of the samples are measured using a pH meter (Sentron 3001 pH). The 5-Day BOD Test as described in the Standard Methods for Examination of Water and WasteWater (1995) was used to analyze the samples for biological oxygen demand. Total suspended solids and volatile suspended solids of the samples were determined by using the methods "2540-D Total Suspended Solids Dried at 103-105° C" and "2540-E Volatile Suspended Solids Ignited at 500°C," respectively (APHA-AWWA-WEF, 1995). Total coliforms are determined using the Standard Total Coliform Fermentation Technique and carrying it only through the presumptive stage (APHA-AWWA-WEF, 1995). In this method, the samples are serially diluted in peptone water (appendix 2). Then one milliliter of each is added to an LTB tube (appendix 2) and incubated in a water bath at 35°C for 24 hours. After 24 hours, if growth has occurred it is considered positive for coliforms and one milliliter from the positive tube is added to an EC tube (appendix 2). If no growth has occurred, the tube is incubated for another 24 hours. Tubes that have a positive presumptive test are tested using the Fecal Coliform Procedure 1 which is similar to the total coliform method (APHA-AWWA-WEF, 1995). One milliliter of a positive LTB tube is added to an EC tube and incubated in a water bath at 44.5 °C for 24 hours. If the tube is positive after this period, then the tube is considered positive for fecal coliforms. The NH_4^+-N content of the samples was determined using the Indophenol Blue Method adapted from method 4500-NH3 D in Standard Methods for Examination of Water and Wastewater(see appendix 1). The samples were examined for P content using the Molybdate Method adapted from method 4500-P E in Standard Methods for Examination of Water and Wastewater (see appendix 1).

Data analysis

Turbidity, pH, and temperature data required no conversions or calibration curves. The data from these tests were subjected to Anova Single Factor data analysis on Microsoft Excel 97. The data collected from the BOD test was the initial dissolved oxygen (IDO) and the final dissolved oxygen (FDO). It is converted into the BOD level by the following equation:

$$BOD=[(FDO-IDO)_{sample}-(FDO-IDO)_{seed}]300/\text{amount of sample used.}$$

The BOD levels are then subjected to the Anova Single Factor data analysis on Microsoft Excel 97. Total and volatile suspended solids were computed in the following manner (weights in milligrams, volumes in liters):

Total suspended solids = (wt. of dry sample-wt. of filter)/vol. of sample added

Volatile suspended solids = (wt. of fired sample-wt. of dry sample)/vol. of sample added

These amounts were then analyzed using Microsoft Excel 97's Anova Single Factor analysis. Total and fecal coliforms were estimated by using the Most Probable Numbers (MPN) technique. The log of these numbers was then taken because bacterial growth is exponential. The log number is used in the Anova Single Factor analysis mentioned previously. N and P both require a calibration curve using the concentrations for their respective standards. This curve was used to estimate the concentrations for the samples. The concentrations were also analyzed using Anova Single Factor. The least significant differences (LSD) between the data were determined to decide if the results were statistically different from one another.

RESULTS

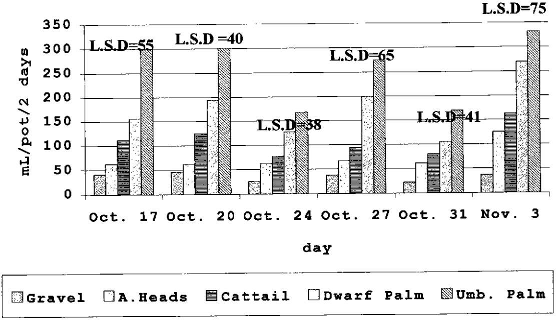

Figure 1. Water usage data comparing the amount of wastewater from a rural septic tank utilized by plant species and gravel controls in a greenhouse over a retention period of two days.

The umbrella palm utilized the greatest amount of water per pot per day. It was significantly different from the other plant species and gravel control on October 17, 20, 24, 31 and November 3. When only the plant data were analyzed, umbrella palm was significantly different on October 12, 20, and 31. The other plants were not generally significantly different from one another in either analysis.

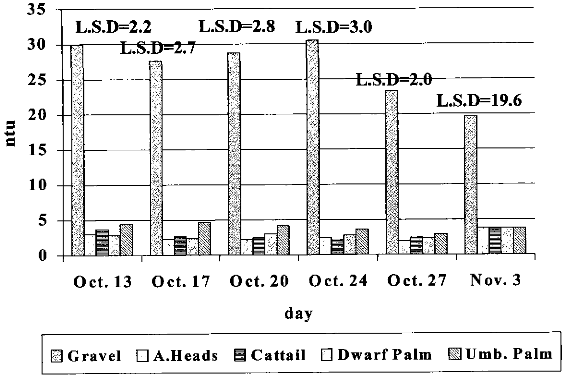

Figure 2. A comparison of the effects of gravel controls and plant species on the reduction of turbidity levels of rural septic water over a retention period of two days. The influent levels of wastewater entering the microcosms for October 13, 20, 27, and November 3 were 49.6ntu, 53.4 ntu, 46.0 ntu, and 57.3 ntu respectively.

The gravel control was significantly different from all the plant species, but the plant species were not consistently different from one another. The gravel control only improved the turbidity of the effluent by an average of 52 %. The arrowheads, cattails, and dwarf umbrella palms reduced it by 94 % and the umbrella palms by 92 %.

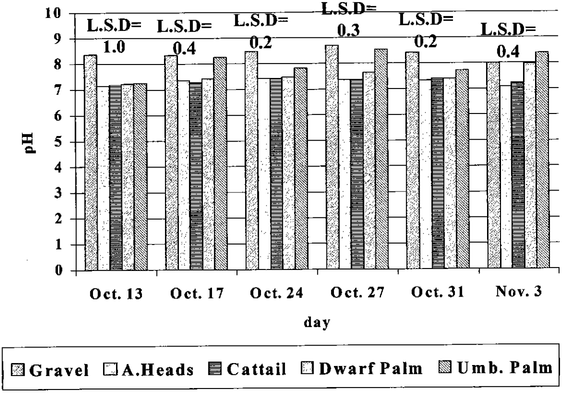

Figure 3. A comparison of the influence of plant species and gravel control on the pH of rural septic wastewater over a two-day retention period. The initial pH of the rural wastewater was 7.61, 7.87, and 7.49 for October 17, 27, and November 3 respectively.

The gravel control and umbrella palms generally increased the pH of the wastewater. The arrowheads, cattails, and dwarf umbrella palms generally lowered the pH by a marginal amount.

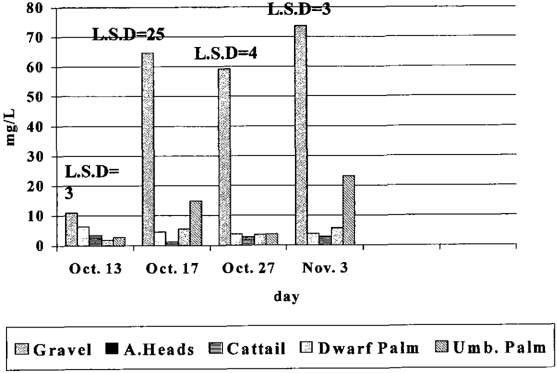

Figure 4. Biochemical Oxygen Demand (BOD) data comparing plant species and gravel controls' reduction of rural septic wastewater, over a period of two days, from levels of 160.2 mg/L, 211.2 mg/L on October 13 and November 3 respectively.

The biochemical oxygen demand was reduced by an average of 53% through the gravel control treatment. The arrowhead treatment reduced it by an average of 97 %, the cattail treatment by 99 %, the dwarf umbrella palm by 98 %, and the umbrella palm by 94%. None of the plant species were significantly different when compared only to one another.

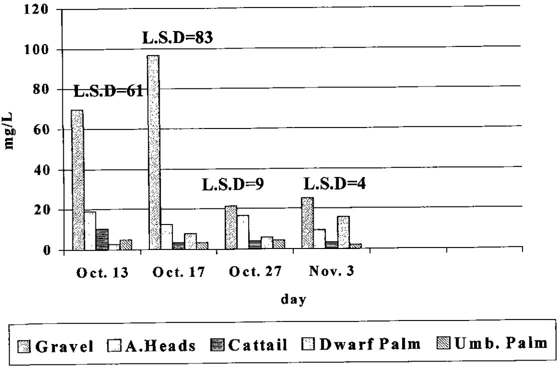

Figure 5. A comparison of the effects of plant species and gravel control treatments on total suspended solids in rural septic wastewater over a two-day retention period. Total suspended solids of the influent were 23 mg/L, 28 mg/L, and 38mg/L for October 13, 27, and November 3 respectively.

Gravel control and arrowhead treatments generally were not significantly different from one another and they produced the least percent reductions. Gravel control only reduced the total suspended solids by 45 % and the arrowheads by 58 %. The arrowheads were usually significantly different from the other plants. The cattails reduced the total suspended solids by and an average of 89 %, dwarf umbrella palms by 69 %, and umbrella palms by 90 %.

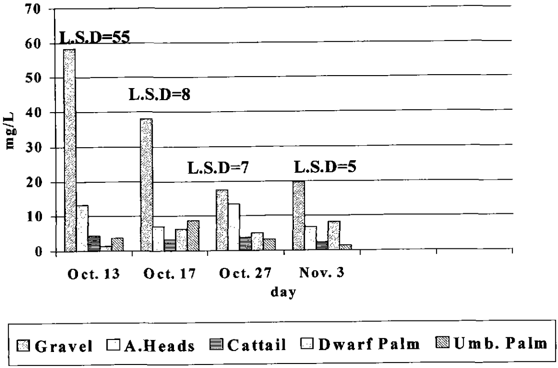

Figure 6. A comparison of volatile suspended solids after treatment with the plant species and with the gravel controls over a two-day period. For October 27 and November 3, volatile suspended solids of the influent were 85 mg/L and 84 mg/L respectively.

The gravel control treatment's reduction of the volatile suspended solids generally was significantly different from the plant species. It reduced the volatile suspended solids by an average of 33 %. The arrowheads reduced it by an average of 62 %, the cattails by 88 %, the dwarf umbrella palm by 77 % and the umbrella palm by 91 %. The volatile suspended solids of the influent comprised an average of 85 % of total suspended solids. For the gravel control, 80 % of the total suspended solids were volatile. 86 % of the total suspended solids for cattails was volatile, 77 % for arrowheads, 68 % for dwarf umbrella palms, and 74 % for umbrella palms.

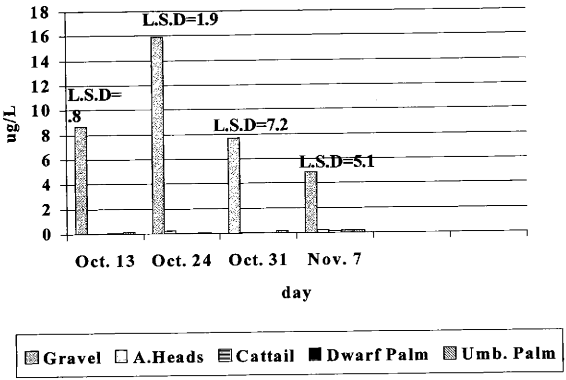

Figure 7. A comparison of the NH_4^+-N content after treatment with the plant species and with the gravel controls over a two-day period. The NH_4^+-N content of the rural septic wastewater was 33 ug/L, 29 ug/L, 23 ug/L and 30 ug/L for October 13, 24, 31, and November 7, respectively.

The gravel control treatment is significantly different from the plant species but the plant species are not significantly different from one another. The gravel control only reduced the NH_4^+-N content by 66 %. The arrowheads and umbrella palms reduced it by 99.6 % and the cattails and dwarf umbrella palms by 99.8%.

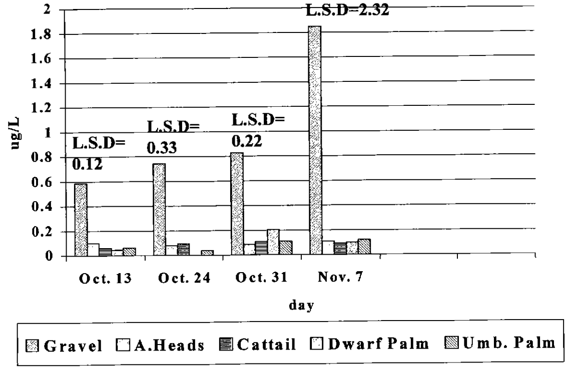

Figure 8. A comparison of the effects of plant species and gravel controls on the phosphorus content of rural septic wastewater after a two-day retention period. The phosphorus content of the rural septic wastewater was 0.67 ug/L, 0.95 ug/L, 0.92 ug/L, and 0.80 ug/L for October 13, 24, 31, and November 7.

The gravel control treatment was significantly different from all plant species and the arrowhead treatment was significantly different two out of five times. The other treatments were not significantly different. The gravel control reduced the phosphorus amount by an average of only 14 %. The arrowheads reduced it by an average of 88 %, the cattails reduced it by an average of 89 %, and the dwarf umbrella and umbrella palms reduced it by an average of 90 %.

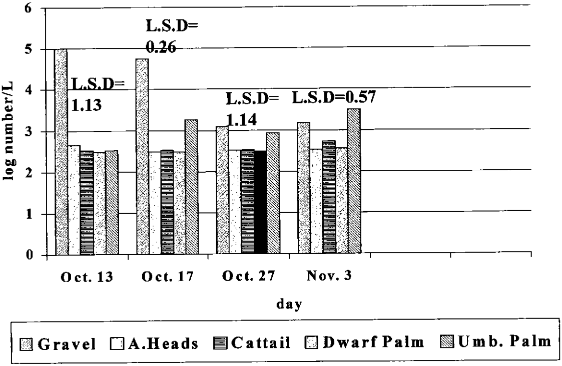

Figure 9. The effect of plant species and gravel controls on total coliforms in rural septic wastewater after a two-day retention period. For October 17, 27, and November 3, the total coliform content of the influent was 5.63 log number/L, 4.38 log number/L, and 4.63 log number/L respectively.

The gravel control treatment is significantly different in half of the comparisons. It reduced the total coliforms by an average of 94 %. The plants are generally not significantly different from one another. The arrowhead treatment reduced the total coliforms by an average of 99.7 %, the cattails by 99.8 %, the dwarf umbrella palm by 99.7 %, and the umbrella palm by 99.3 %.

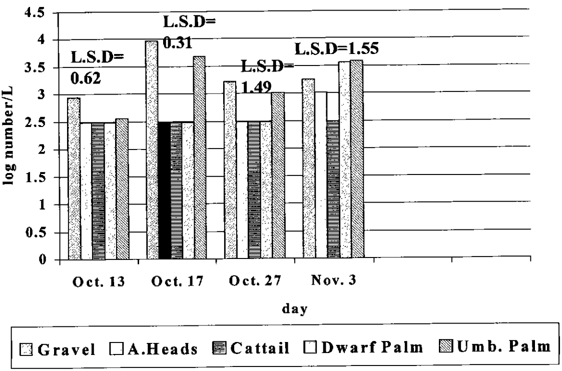

Figure 10. A comparison of the effects of plant species and gravel controls on fecal coliforms in rural septic wastewater over a two-day retention period. The concentrations of fecal coliforms in the rural septic wastewater were 4.63 log number/L, 4.38 log number/L, and 4.63 log number/L for October 17, 27, and November 3, respectively.

None of the treatments showed consistent significant difference in results. The gravel control treatment reduced the fecal coliforms by an average of 94%, arrowheads by 99 %, cattails by 99 %, dwarf umbrella palms by 98 %, and umbrella palms by 96 %. Of the total coliforms for the gravel control treatment, 97 % were fecal coliforms, 93 % were fecal for the cattails, 94 percent for the influent, and 100 % for the arrowheads, dwarf umbrella and umbrella palms.

DISCUSSION

The planted microcosms generally did not show any differences between plant species, as they were expected to, however overall they were significantly different from the gravel controls. As a whole, the plant species showed a decrease in the BOD levels, total suspended solids, volatile suspended solids, pH, and the concentrations of N, P and total and fecal coliforms. Generally, the gravel also reduced the water quality parameters.

The results did contain some basis for selection of plant species. For instance, the umbrella palm was the most effective species at evapotranspiration of water and was significantly different from the other plant species and the gravel controls (see fig. 1). It utilized an average of 254 mL of wastewater over a two-day period. Hofmann (1997) conducted studies on constructed wetlands containing only reeds (*Phagmites* sp.) and found that in beds with dense reed growth the annual evapotranspiration is1-2 meters per year in various European countries. Another difference is in the pH values for the gravel controls and umbrella palms. In both cases the pH levels increased after treatment, instead of lowering. This could be due to moderate algal growth in the pots. There was some visual evidence of algae in the microcosms. Kadlec and Knight (1996) discuss a Listowel constructed wetland, which periodically displayed high pH due to algal blooms in the influent. The concentrations of N and P after treatment in the gravel controls are also unexpected. The concentrations should not have been significantly reduced, but they were. In a concurrent study by Srini Nerella (1997), a graduate student at Texas A&M University, it was found that the high pH occurring in the microcosms, promoted ammonia volatilization. When the pH of the wastewater in the microcosms was kept at a normal to slightly acidic pH level, ammonia volatilization did not occur. Explanations for the reduction in P levels include precipitation on the gravel and P complexed by other compounds in the wastewater.

BOD is the measure of the oxygen used by microorganisms while oxidizing organic matter (Kadlec and Knight, 1996). Water quality parameters that affect BOD are volatile suspended solids. If large amounts of volatile suspended solids are present, then the BOD will probably be higher due to the large amount of organic matter that needs to be consumed. According to a study done by Gearheart and Higley (1993), the BOD was reduced by 41 to 65 % in a constructed wetland in Arcata, CA. The BOD was reduced by 94 to 99% in the planted microcosms. The gravel control showed a reduction of 53 %. When the gravel control's percent loss is averaged with the plant's percent loss the average is 74%. The common plants used in the Arcata wetland include common cattail, marsh pennywort, sago pond weed, alkali bulrush, lesser duckweed, hardstem bulrush, common spikerush and upland grass (Gearheart and Higley, 1993).

The total suspended solids are all solids in solution, inorganic and organic. The volatile suspended solids are only the organic solids. In the study, it is shown that the total suspended solids after treatments with gravel and arrowhead are not significantly different, but volatile suspended solids are significantly different. An explanation for this difference requires further study.

Nitrogen removal in the dairy wetlands shows a 34 % removal of NH_4^+-N in planted wetlands and a 7% removal in unplanted wetlands (Tanner, 1995). In this study, the NH_4^+-N in the planted microcosms was reduced by an average of 99.7% and in the control microcosms, it was reduced by 66%. These numbers may be affected by ammonia volatilization, causing them to be larger than they should. Further study of the fate of nitrogen in these systems is necessary to prove or disprove the effect of ammonia volatilization or other mechanisms of nitrogen removal.

Total and fecal coliforms were reduced in the microcosms. It was believed that the gravel microcosm would decrease the number of coliforms more than the planted microcosms, due to the increased living surfaces on the plant roots. However, the plant microcosms reduced the coliform populations more than the gravel controls. It could be that the coliforms in the gravel pots did not receive as many nutrients as the ones in the planted pots or that they were aerobic and died because of oxygen deficiency. The plants leak exudates, leak oxygen, form air pockets around their roots (Hofmann, 1997).

In conclusion, the use of plants in constructed wetlands does help reduce the concentrations of various environmental contaminants present in septic wastewater. The most important of these contaminants is the BOD; optimal BOD levels are less than 30 mg/L. This parameter indicates the amount of organic matter present in the effluent. If the BOD is high, then a high amount of organic matter is present in the effluent. High levels of organic matter clog the soil as the water percolates in the drainfield. This can reduce the life of a drainfield and cause the size of drainfields to be increased. If the BOD is low, then the size of the drainfield can be reduced and its life extended. Another benefit of low BOD is cholorination. Low BOD allows for cholorination of the effluent to kill off the fecal coliforms (Weaver, 1998). The plants in the study reduced the BOD to levels below 30 mg/L. However, none of the plants was significantly better. The second most important parameter is the water usage. The size of a drainfield can be significantly reduced if a lower quantity of water needs to be dispersed (Weaver, 1998). In this study, the Umbrella Palm utilized a significantly higher amount of septic water than the other plant species. It reduced the amount of wastewater it received by an average of 30%. Based on this, a drainfield could be reduced in size by 30 % because of the reduction in the amount of effluent to be dispersed. The Umbrella Palm's performance in lowering the BOD and utilizing a significantly larger amount of septic water provides an adequate basis for selecting it as the optimal plant species. It is suggested that this plant species be used as the dominant plant in constructed wetlands.

APPENDIX 1

Indophenol Blue Method

1. Rinse all tubes with deionized water.
2. Number tubes according to scheme suggested.
3. Add 3.4 mL deionized water to the tubes.
4. Add 0.2 mL sample to appropriately labeled tubes. (INSTEAD of sample, use 0.2 mL deionized water for blanks.)
5. Add 0.2 mL EDTA reagent. Mix well and hold one minute.
6. Add 0.4 mL Phenol Nitroprusside Reagent.
7. Add 0.8 Buffered Hypochlorite Reagent and mix well.
8. Place tubes in a 40°C water bath for thirty minutes.
9. Record absorbance at 636 nm on a spectrophotometer.
10. Compare with standards. (Standards are prepared according to the following table)

Concentration (ug/L)	mL of Ammonium-nitrogen	mL deionized water
0	0.0	3.6
2	0.2	3.4
4	0.4	3.2
6	0.6	3.0

Then follow steps 5-9 above.

Molybdate Method

1. Rinse all tubes with deionized water.
2. Number tubes according to scheme suggested.
3. Add 4.0 mL deionized water to the tubes.
4. Add 0.2 mL sample to appropriately labeled tubes. (0.2 mL deionized water for blanks)
5. Add 0.8 mL of Reagent B (1.056 g Ascorbic acid in 200 mL of Ammonium Paramolybdate Reagent) and mix well.
6. Record absorbance at 680 nm on a spectrophotometer.
7. Compare with standards. (Standards are prepared according to the following table)

Concentration (ug/L)	mL Phosphorus standard	mL deionized water
0	0	4.2
0.5	0.2	4.0
1.0	0.4	3.8
1.5	0.6	3.6

Then follow steps 5-6 above.

APPENDIX 2

Media Preparation

Peptone water

Peptone water is prepared by adding 1.0 g LTB to 1 liter of distilled water and mixing well. 9.1mL of the media are dispensed into test tubes containing Durham tubes until it is depleted. These tubes are placed in racks and autoclaved. (Makes about one and a half racks).

LTB

This media is prepared by adding 71.2 g LTB to 2 liters of distilled water and mixing well. 9.1mL of the media are dispensed into test tubes containing Durham tubes until it is depleted. These tubes are placed in racks and autoclaved. (Makes about two and a half racks).

EC

This media is prepared by adding 37 g EC to 1 liter of distilled water and mixing well. 9.1mL of the media is dispensed into test tubes containing Durham tubes until it is depleted. These tubes are placed in racks and autoclaved. (Makes about one and a half racks).

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