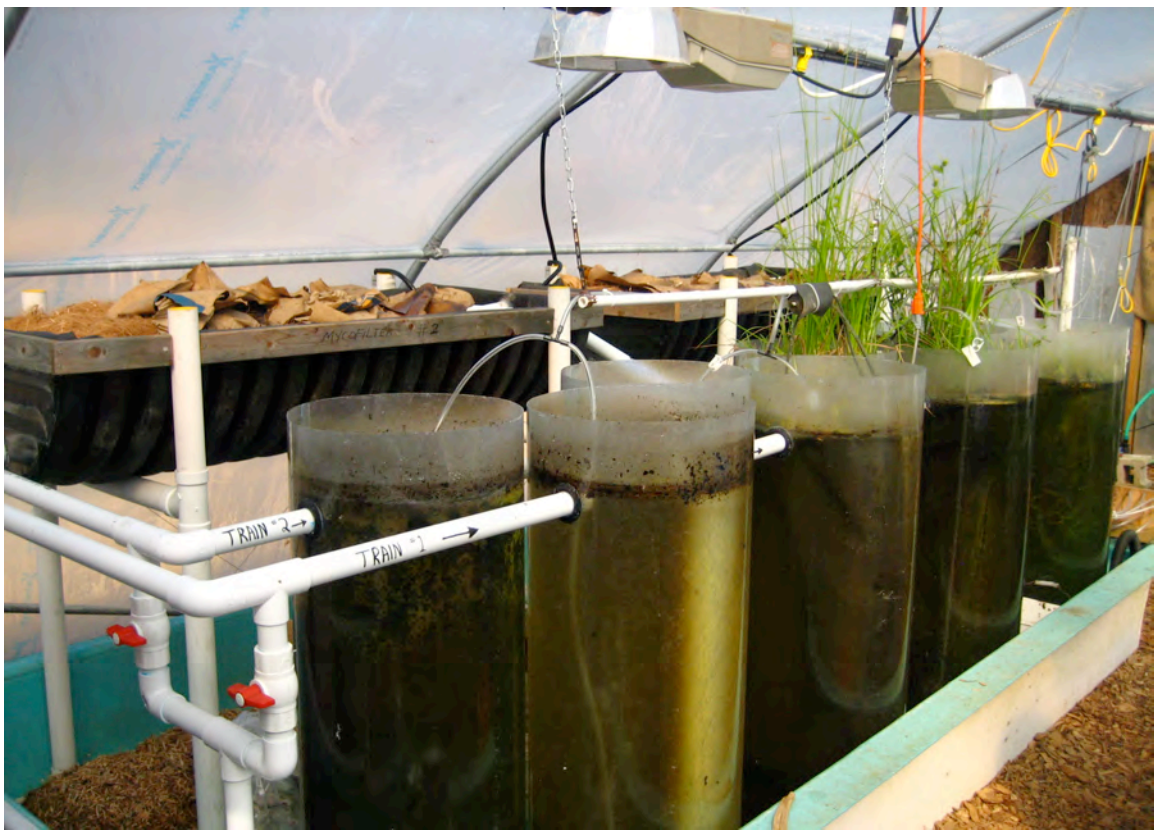
JOHN TODD ECOLOGICAL DESIGN, INC.

Solutions for Water Planning and Treatment

Fisherville Eco-Machine Pilot Final Report



April 27, 2007

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Executive Summary

The theoretical basis behind this project is the development of a complex ecology designed and naturally evolved to maximize dynamic and diverse biological surface areas and waste water/sediment contact in order to utilize and convert contaminated water and sediments to an energy source for biomass production. The specific goal of this project is to develop an eco-machine that will rapidly degrade # 6 oil, also known as Bunker C oil. Bunker C oil is a toxic and tar-like residual material from the manufacture of petroleum products. Bunker C is often used as a fuel in ships and in electrical power plants. When it spills out into the environment it can create environmental damage and persist for years in sediments and soils.

The Greenhouse Pilot:

The purpose of the pilot Eco Machine had three components:
1: To inform all the elements of the design to develop an effective Eco Machine for the treatment of the contaminant of concern.
2: To produce measurable empirical data proving the efficacy of our approach.
3: To facilitate human interaction with a natural systems technology to produce directed results.

The Design:

We designed and constructed a greenhouse based pilot system that housed a pair of Eco Machines that were operated in parallel. In order to maximize surface areas and ecological elements each system had four ecologically different cell types that include:
1: solar based cells which support algal turf communities;
2: higher plant based cells with marsh plants growing on rafts on the surface;
3: an open water fish dominated cell;
4: a fungi dominated “trickling filter” cell designed to support the rapid growth of fungi mycelia.
The first three cell types were housed inside clear-sided tanks to optimize solar penetration into the ecosystems. The fourth cell types were covered to prevent light penetration of the fungi dominated system. The pilot systems were operated in a continuous recycle mode.

Inoculation of Organisms and Addition of Contaminants

In December of 2006 the system was inoculated with organisms from local ponds and salt marshes, as well as from the Fisherville canal. A microbial chemostat was added in one of the Eco Machines to provide additional bacterial biomass to the system on an ongoing basis. A diversity of life forms established themselves.

Dominant were microbes, attached algae communities, zooplankton, snails and higher plants in addition to a few fish.

In mid December ten pounds of sediments were taken from the canal, split equally between each of the trains, and added to the fungal cells. Over the next three months 573 gallons of canal water were added to the systems and again divided between the two Eco Machines. Total hydraulic capacity of each Eco Machine was approximately 200 gallons.

Chemistry

The pre-treatment canal water and sediments were analyzed chemically. Measures included COD, TSS, alkalinity, ammonia, TKN, and conductivity. The petroleum measurements included TOC (Total Organic Carbon) and TPH (Total Petroleum Hydrocarbon) of the sediments and TPH in both the sediments and the canal water.

Preliminary Results

TOC in the sediments were 130,000 mg/kg and TPH were 110,000 mg/kg. Initial canal water samples had a COD of 1,500 mg/l, TSS of 1700 mg/l and TPH of 7.8 mg/l.

The first round of water tests on March 5th showed that the Total Petroleum Hydrocarbons in the water had dropped to Non Detect levels. The COD had dropped from 1500 mg/l to 50 mg/l in Train #1 and 51 mg/l in Train #2. As dilution alone lowered the COD to around 375 mg/l, the 50 mg/l represents an approximate 87 % reduction of COD in the system. TSS had dropped from 1,700mg/l to Non Detect levels in both treatment trains.

By April 2, despite additional contaminated canal water having been added, (at this stage a volume equivalent to 85.8 % of the total hydraulic capacity of the whole system) COD had dropped to 21 mg/l and 18 mg/l respectively. The Total Petroleum Hydrocarbons in the water measured 0.5 mg/l and 0.6 mg/l. The April data represented a 92 % removal TPH in the water.

The sediments themselves were significantly reduced in volume. The volume of sediment material in Train #1 was reduced 89% and in Train #2 was reduced 57%. Also the “character” of the remaining material had changed. Unexpectedly, the snails began to eat the Bunker C sediments attached to the sides of the translucent tanks. A composite sample of sediments from the various aquatic cells on April 2nd showed a TPH of 66,000 mg/kg in Train #1, a 40 % reduction, and 49,000 mg/kg in Train #2 for a 56 % reduction in TPH concentration of the remaining sediments. These numbers, when combined with the reduction in Bunker C sediment volume, indicate that the overall concept of the Fisherville Eco Machine is valid and that the system is working.

Technological Optimization of the Pilot

What is needed now are more measurements of water and sediment quality to further quantify the reduction of Bunker C hydrocarbons in the system. Also the system needs to be optimized with new biological inoculations from the wild and an increase in mineral diversity within the system. Although we have learned that a diverse, ecologically-based living technology has the capability to degrade and render harmless Bunker C oil, we need further quantification of chemical parameters and technological optimization.

The Pilot and Future Designs

The pilot has provided preliminary evidence that an engineered ecosystem can effectively treat oil contaminated canal sediments and poor river water quality. Information from the pilot will inform and direct the design of a full-scale system for the treatment of canal sediments. The data from the pilot will allow us to calculate retention times, hydraulic capacity and treatment capacity of future full-scale systems.

The pilot will also inform and direct the design of canal restorer Eco Machines for the purification of contaminated river and canal waters. Data from the pilot will help determine the size and substrate surface areas of the restorers.

Technology Transfer

One of the objectives of this project is that the technologies being developed can have wide-scale application throughout the watershed. This included a scaleable approach so that sediment decontamination Eco-Technologies can be built at appropriate scales in the river based on sediment volumes in need of treatment.

The same would apply to the upgrading of river and canal water quality as well. For example it will be possible to design restorers throughout the watershed specifically adapted to the nature and scale of contaminants most needing treatment.

Eco-Machine Design and Operation

Physical Infrastructure

The physical infrastructure of the system influences the ecological conditions within the system. Diversity in the physical characteristics of the system creates gradients of environmental characteristics such as light, oxygen, turbulence, and the size and relative availability of different physical environments. These different physical environments translate into a diversity of habitat types, which support a wide range of ecological communities. Diverse ecologies are resistant and resilient to disturbances, including the addition of highly polluted water and sediment. These communities in turn change the environment they live in, including water and sediment chemistry, which is of particular importance. Another strategy used in the physical design was to maximize surface area for the establishment of attached ecological communities that are crucial to the system's function. Transition zones within and between cell types (representing different ecologies) allowed the different ecological communities to interact, adding to the diversity and, therefore, the adaptive potential of the system. Several components of the Fisherville Eco Machine Pilot (FEP) influenced the structure, composition, function, and processes within the system, including: clear tanks, screens, floating plant racks, and mycofiltration troughs.

Figure 1 Clear tank walls maximize solar penetration within the aquatic cells.

Figure 2 Screens submerged vertically provide a matrix of surface area for algae turf communities

Clear tanks enhance solar penetration into the cells, increasing the photosynthetic base of the system. The large surface-area-to-volume ratio with access to light serves to create an environment dominated by algal turf communities. Screens submerged vertically within cell #2 provide a matrix of surface area for algae, microbes, and other species to colonize. These communities have proven to be important to the functioning of the system through algae and snail interactions discussed below. Increased insolation also raises water temperatures related to chemical reaction rates and expedites the degradation of compounds through UV radiation and other photoreactions. Further, the clear tanks allow the system operators to more closely monitor the internal processes of the tanks, which is valuable for observing the development and changes in system ecologies that are indicators of system performance.

The layout of the cells influences the amount of solar gain available to each cell. While all the cells used in the FEP were the same dimensions, with the exception of the mycofilters, the potential to create temperature, light, and depth gradients through the selection and orientation of different size cells poses further opportunities to influence the complexity and function of the system to benefit the degradation process.

Floating plant racks play many essential functions in the system. Plant racks suspend higher plants at the water-air interface. The emergent vegetation creates habitat niches for insects and other organisms, and the biomass of the plants (and anything eating them) acts as a sink for nutrients derived from the break-down of pollutants. The plant roots form complex structures in the water with huge amounts of surface area. These self-organizing and self-repairing root matrices host communities of microbes that metabolize pollutants or externally degrade them with enzymes. The excretions from these microbial communities and the simple compounds created by the degradation of complex compounds by these communities are then absorbed by the root hairs and sequestered as plant biomass.

Figure 3 Floating racks support higher plants at the air-water interface.

The mycofiltration troughs were constructed with opaque materials to create a low-light environment. These cells are also terrestrial and create a different type of habitat designed to facilitate the growth of fungi. The physical characteristics of the substrate in these cells, along with a different water delivery system, created a ‘trickle-filter’ pathway for water to pass through substrate colonized with diverse fungi and microorganisms, while maintaining high oxygen levels in the absence of light.

Figure 4 Mycofilter troughs create a “trickle-filter” pathway for exposing contaminants to mycelium.

Ramp Up of Biology and Concentration

The ramp up period allowed the inoculated biology to organize and establish itself before being exposed to pollutants. The concentration of pollutants was then gradually increased to give the ecological communities time to acclimate to the waste stream and to select for species tolerant of the pollutants in the canal water and sediments. The FEP continues to evolve and adapt to the chemically rich conditions created by increasing concentrations of polluted canal water. At the time of this report, the system is approaching, but has not yet reached biological capacity. The initial inoculation has provided a strong base of genetic information to the system. Additional inoculations are necessary to introduce species of varying seasonality and to increase mineral and biological diversity within the system.

Figure 5 The current system is continually undergoing internal cycles as it progresses towards capacity.

As the biological capacity and full concentration of polluted water are reached, the ability to assess the design and operation variables influencing long term system functioning increases, providing key information for optimizing efficiency and minimizing costs of further efforts. The initial positive results of the FEP demonstrate a need to continue to experiment with chemical, biological and ecological pathways as well as technology strategies employable in the degradation of bunker C oil and restoration of polluted environments.

Ecological Development

The biological components of the system have organized themselves into ecological communities complete with food chains and symbiotic relationships. These communities are interacting with the water and sediments individually as organisms, species and, more importantly, as groups of associated organisms and species. When broken down to the simplest level, we may identify a particular species "x" that is best at breaking down TPH. One might be inclined to monoculture this species in an attempt to maximize TPH reduction. However, when interspecies interactions are considered, we may find that this brilliant species “x” only achieves high population growth in the presence of species “y” that does not break down TPH at all, and that by managing for a species that does not break down TPH as a part of a community achieves better TPH reduction, higher adaptability, and more resistance to disturbance. Within the ecological diversity of the FEP, multiple key players and key associations that merit more testing have been identified and outlined below.

Algae – Snails

Cycles of algae blooms followed by pouch snail (*Physa gyrina*) population booms have been observed. As algae attached to tank walls and grew, TPH rich sediments suspended in the water became trapped in the waving mass and adsorbed to the tank walls and attached algal colonies. Algae populations exploded, covering the majority of the surface area available. After several weeks of growth, pouch snails appeared and began to scrub the tank walls, eating algae and sediments alike. The EPA has classified the pouch snail as a bioindicator generally associated with high nutrient levels and poor water quality (http://www.epa.gov/bioindicators/html/pouchsnails.html) The snail populations initially boomed with the readily available food source, and then declined after the attached algae communities were consumed. At the time of this writing, the cycle is starting over again, though slower than the initial iteration. This may be due to the temporary discontinuation of contaminated water input and/or improvement in water quality within the system. From the relatively large amount algal and snail biomass observed, we hypothesize that this relationship is playing a major role in the reduction of TSS and TPH in the system. This observation opens a line of research options with potential.

Figure 6 Snails grazing algae laden with sediments, creating visible pathways along tank walls

Fungi

Pleurotus ostreatus and *Trametes versicolor*, fungi species with noted ability to degrade petroleum hydrocarbons (Stamets 2005), were inoculated within the substrate. As the mycelial mass expands and colonizes more substrate, the amount of mycelium surface area exposed to contaminated water and sediments increases. Most white rot fungi secrete extracellular enzymes that can catalyze oxidation processes (Srebotnik and Hammel 2000). Cerniglia (1997) notes that several mechanisms for enzymes secretion are used by *P. ostreatus* to oxidize PAH. When the mycofilters were excavated and sediments weighed, only .549

pounds were recovered from Train #1 and 2.174 pounds from Train #2 of the 5 pounds input to each, equaling 89% and 57% reductions, respectively. The characteristics of the sediments also changed, becoming more pliable and less sticky.

Microbe Chemostat

A microbial chemostat containing microbes engineered by Hydros Inc. was placed in-line of Train #1. This chemostat supplied a steady stream of bacteria and other microorganisms to the system over the course of several months. Observations of the system revealed that the train containing the microbial chemostat had more root mass and a higher percentage of TPH reduction than the one without supplemental microbes. However, variables, including higher insolation existed in Train #1.

Plants

Plants have multiple nutrient cycling pathways that influence microclimatic conditions with mechanisms and effects that vary by species. Plant roots secrete enzymes that promote microbial diversity and activity in a specific way depending on the plant and conditions. (Nedunuri et al 2000) Having these roots in an aqueous solution may have a similar enhancing effect on enzyme production as noted by Lenz and Holker for fungi. Matching plant adaptive strategies and chemical pathways to the challenge of degrading complex hydrocarbon chains poses great opportunities for further research.

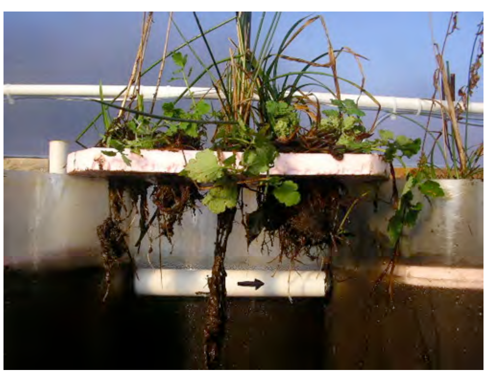


Figure 7 Root mass increased by several orders of magnitude as the system developed. The picture on the left was taken at the time of inoculation, while the picture on the right was taken after four months of operation.

Plants are key to the photosynthetic base of the system and further diversify the system with habitat for terrestrial and flying insects, which play a yet-to-be understood-role in overall balance and health of system. Plants also provide opportunities for value-added yields from the system through specific species selection.

Data Analysis

Water Quality Data

Prior to treatment in the FEP, raw canal water was tested for several water quality parameters with particular interest paid to total petroleum hydrocarbon (TPH) as a measurement of bunker C oil. Alkalinity, ammonia, chemical oxygen demand (COD), total suspended solids (TSS), total Kjehldahl nitrogen (TKN), conductance, and total phosphorous were also measured (Table 1**)**. TPH concentration in the raw input water was measured at 7.8 mg/L. COD and TSS tested at 1,500 mg/L and 1,700 mg/L respectively. TKN was at a concentration of 17 mg/L, and total phosphorous was measured at 15 mg/L.

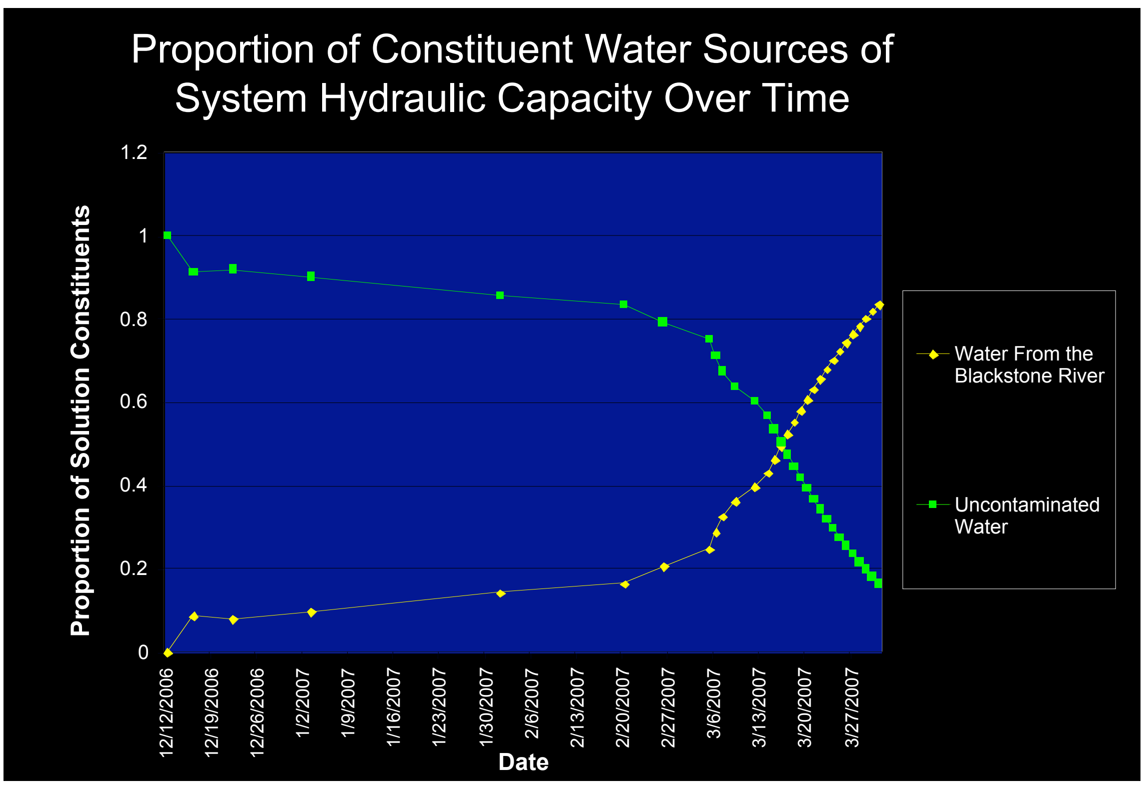
Table 1: Water quality measurements from the Blackstone River Canal in Fisherville, MA (1/12/2006).

Water Quality Measurement	**Raw Canal Water Prior to Treatment**
TPH (mg/L)	7.8
DO (mg/L)	9
Alkalinity (mg/L as CaCo)	14
Ammonia as Nitrogen (mg/L)	2.1
COD (mg/L)	1,500
TKN (mg/L)	17
TSS (mg/L)	1,700
Conductance (umohs/cm)	390
Total Phosphorous (mg/L)	15
Waste Strength	**100%**

Between 12/12/2006 and 3/5/2007, 56.5 gallons (213.86 L) of raw canal water were added to each train in the FEP system. This slowly brought the concentration of the waste stream up to 24.82% of the total system hydraulic capacity during the ecological ramp-up period, referred to as test period 1 hereafter. From 3/5/2007 until the end of the pilot period on 4/2/2007, an additional 230 gallons of raw canal water (870.64 L) were added to the system in daily doses of 10 gallons (37.85 L). In this period, referred to as test period 2 hereafter, the concentration of polluted water rose from 24.8% to 85.8% of the total system hydraulic capacity. The concentration of polluted water as a proportion of hydraulic capacity over the course of the pilot study is shown in **figure 8.** For a log of actual additions along with concentration calculations, including average evaporative loss, see **Appendix .**

At the end of test period 1, samples taken from cell 4 in both trains were tested for the same water quality parameters measured for the raw influent water (**Tables 2 and 4**). The test results were then compared to the contaminant levels expected from dilution at the time of sampling to arrive at reduction percentages. The THP levels were non-detect (ND) for both trains at a reporting limit (RL) of 0.5 mg/L representing a reduction of at least 74.15%. Ammonia levels were also not detectable at a RL of 0.2 mg/L in Train #1, representing a reduction of 61.6% or greater. Ammonia levels in Train #2 were measured at 0.3 mg/L, a 42.4% reduction. COD tests revealed an 86% reduction in both trains leaving 50 and 51 mg/L COD in Trains #1 and #2 respectfully. TSS was at ND levels in both trains at a RL of 5 mg/L representing at least a 98.8% reduction. Total phosphorous was at ND levels in Train #1 and measured at 0.7 mg/L in Train #2 representing a reduction of at least 86.5% in Train #1 and 81.2% in Train #2.

Figure 8: Proportion of contaminated water to uncontaminated water constituting the total hydraulic capacity of the FEP system as a measure of pollutant concentration.



At the end of test period 2 samples taken from cell 4 in both Trains were tested for the same water quality parameters measured for the raw influent water (**Tables 3 and 5**). The test results were then compared to the contaminant levels expected from dilution at the time of sampling to arrive at reduction percentages. The THP level in Train #1 was 0.5 mg/L representing a reduction of 92.53%. The TPH level in Train #2 was measured at 0.6 mg/L representing a reduction of

91.03%. Ammonia levels in both trains were not detectable at a RL of 0.2 mg/L, representing a reduction of 88.9% or greater. Tests revealed that over 98% of COD was removed in both trains, leaving a mere 21 and 18 mg/L in Trains #1 and #2 respectfully. TSS levels were also ND in both trains at a RL of 5 mg/L, representing a reduction of 99.7% or greater. Total phosphorous was at ND levels in both trains for a reduction of at least 96.1%.

Table 2: Water quality test 1: Train #1:Comparison of raw water from the Blackstone River Canal to treated water from the Fisherville remediation pilot eco-machine with recent reduction of pollutants (3/5/2007).

Water quality measurement	Raw Canal Water	Lab test results for sample taken from Train #1, cell 4 on 3/5/2007	Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Percent Reduction of Pollutants
TPH (mg/L)	7.8	ND (RL: 0.5)	1.93	>74.15
DO (mg/L)	9	10		
Alkalinity (mg/L as CaCo)	14	11		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	0.52	>61.6
COD (mg/L)	1500	50	372	86.56
TKN (mg/L)	17	ND (RL:0.5)	4.22	>88.14
TSS (mg/L)	1700	ND (RL:5.0)	421.6	>98.81
Conductance (umohs/cm)	390	300		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	3.72	>86.56
Waste Strength	**100%**		**24.8%**	

Table 3: Water quality test 2: Train #1:Comparison of raw water from the Blackstone River Canal to treated water from the Fisherville remediation pilot eco-machine with recent reduction of pollutants, 4/2/2007.

Water	Lab	Lab test	Waste stream strength	Percent

quality measurement	test results for raw canal water	results for sample taken from Train #1, cell 4 on 3/5/2007	in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Reduction of Pollutants
TPH (mg/L)	7.8	0.5 (RL:0.20)	6.7	92.53
DO (mg/L)	9	9.8		
Alkalinity (mg/L as CaCo)	14	3.8		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	1.81	>88.89
COD (mg/L)	1500	21	1287	98.37
TKN (mg/L)	17	0.5	14.59	96.57
TSS (mg/L)	1700	ND (RL:5.0)	1458.6	>99.66
Conductance (umohs/cm)	390	460		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	12.87	>96.11
Waste Strength	**100%**		**85.8%**	

Table 4: Water quality test 1: Train #2:Comparison of raw water from the Blackstone River Canal to treated water from the Fisherville remediation pilot eco-machine with recent reduction of pollutants, 3/5/2007.

Water quality measurement	Lab test results for raw canal water	Lab test results for sample taken from Train #2, cell 4 on 3/5/2007	Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Percent Reduction of Pollutants
TPH (mg/L)	7.8	ND (RL: 0.5)	1.93	>74.15
DO (mg/L)	9	10		
Alkalinity (mg/L as CaCo)	14	15		
Ammonia as Nitrogen (mg/L)	2.1	0.3	0.52	42.4

COD (mg/L)	1500	51	372	86.3
TKN (mg/L)	17	1.1	4.22	73.9
TSS (mg/L)	1700	ND (RL:5.0)	421.6	>98.8
Conductance (umohs/cm)	390	300		
Total Phosphorous (mg/L)	15	0.7	3.72	81.2
Waste Strength	**100%**		**24.8%**	

Table 5: Water quality test 2: Train #2:Comparison of raw water from the Blackstone River Canal to treated water from the Fisherville remediation pilot eco-machine with recent reduction of pollutants, 4/2/2007.

Water quality measurement	**Lab test results for raw canal water**	**Lab test results for sample taken from Train #2, cell 4 on 3/5/2007**	**Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity**	**Percent Reduction of Pollutants**
TPH (mg/L)	7.8	0.6 (RL:0.20)	6.7	91.03
DO (mg/L)	9	9.8		
Alkalinity (mg/L as CaCo)	14	3.8		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	1.8	>88.9
COD (mg/L)	1500	18	1287	98.6
TKN (mg/L)	17	ND (RL:0.5)	14.59	>96.6
TSS (mg/L)	1700	ND (RL:5.0)	1458.6	>99.7
Conductance (umohs/cm)	390	470		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	12.87	>96.1
Waste Strength	**100%**		**85.8%**	

Sediment Data

Sediments from the Blackstone River Canal were tested for TPH and TOC content; TOC in the sediments were 130,000 mg/kg and TPH were 110,000 mg/kg. Of the 5 lbs of sediment added to each train, significant mass reductions were observed. At the end of test period 2 the sediments in Train #2 weighed 2.174 lbs, a reduction of 56.52%. Sediment reduction observed in Train #1 was 89.02% leaving only 0.549 lbs of the original 5 lbs. In addition to the major mass reductions measured, the texture and viscosity of the sediments had changed considerably from that of the original sediments; the treated sediments had become softer, less sticky, and had lost some of the metallic luster characteristic of petroleum. The minimal amount of sediments that settled out in the aquatic cells was tested for TPH in both trains. A mixed sampling from all aquatic cells revealed a TPH concentration of 60,000 mg/Kg in Train #1 and 49,000 mg/Kg in Train #2; when compared to the raw canal sediments, this was a reduction of 45.5% and 56% respectively.

Conclusions and Further Recommendations

The Fisherville Eco Machine Pilot (FEP) project has produced measurable empirical data proving the efficacy of the living system approach to the treatment of oil contaminated canal sediments and poor river water quality. This pilot project has provided many insights that will guide the appropriate design of an Eco Machine or Restorer system to treat the contamination experienced in the Blackstone river basin.

The FEP project demonstrated significant reductions in both sediment mass and pollutant concentrations in the sediments and the water. During the pilot study, significant biomass was generated in an environment poor in nutrients other than the incoming polluted sediments and water. All of this validates our thesis that a complex ecology designed along in natural principles of evolution to maximize dynamic biological surface area and including waste water/ sediment contact is capable of utilizing and converting contaminated water and sediments to an energy sources for biomass production.

Over the course of this pilot study we have observed life colonize all areas of the FEP and thrive. We have observed thick algal mats cover virtually every square inch of the aquatic tank walls to be entirely consumed and metabolized by snails and then reemerge to cover the tanks and begin the cycle anew. We have witnessed the plants, tiny when installed in the racks, grow to heights of 3 feet and send out thick root masses to the bottoms of their 5-foot-tall enclosures. While we did not achieve complete colonization of the mushroom substrate, we did observe significant patches *Trametes versicolor* of wild mycelium beginning to take a strong foothold in the system. This suggests that they could have played a significant role in pollutant degradation and also shows that we have an opportunity to greatly improve the technology's efficiency above what is reported here through optimization of the mycofilters. Major growth in biomass in the nutrient limited system was observed, and a major reduction in the pollutants added to the system was measured. This strongly suggests that the pollutants were converted to an energy and carbon source for biomass production. Future investigation in carbon isotope tracing could show exactly what pathways the contaminants are taking to end up as biomass, but this would simply be an expensive way to irrefutably prove what we can deduct from observation.

We measured a 56% mass reduction of sediment with a TPH concentration of 110,000 mg/Kg in Train #2 and an 89% reduction in Train #1. The sediment had also changed in character, indicating that it had been degraded to some extent, though we had insufficient funds to conduct a chemical analysis of this remaining sediment. In addition, the sediments that settled out of the water column had significantly lower concentrations of TPH -a reduction of 45.5% and 55.5%- than the sediments that have settled out in Fisherville and coated the Blackstone River Canal channel.

Water quality tests measured even greater removal rates of pollutants. TPH reductions were measured at >74.2% in both trains for tests period 1 and at 92.5% and 91% for test period 2 in Trains #1 and #2 respectively. COD and TSS were also reduced by 99% in both trains for test period 2. These are large reductions in the contaminants of interest, and these results prove the efficacy of Eco-Machine technology to treat waterways thus polluted. We attribute much of these reductions to the algae-snail cycles, fungi, and to the interaction of the dynamic root surfaces of higher plants and associated microbial communities with the water, but further testing is required to quantify the mechanics of these relationships and to determine relative importance.

The FEP project has provided valuable information to direct and inform a full-scale system. The project has shown that the technology, using native organisms from all the kingdoms of life, works to remove contaminants of interest from water and sediments. With a second round of pilot studies the insights gained from this project can be used to optimize the technology and to quantify appropriate scale and treatment rate for a full scale system.

One of the main advantages of this technology is that it is inherently cellular in concept and in practical application. This means that it can easily be scaled up or down to adapt to changing conditions or needs with minimal costs. It also makes this technology ideal for large-scale watershed restoration because of the mobility a cellular design affords; Restorer / Eco-Machine hybrid technology can be broken down to small functional units that can be located up and down the watershed in situ to deliver treatment where it is most needed. These units can be linked to handle high loads where pollution is great, then split into independent, smaller units to clean less polluted areas simultaneously once the hotspots are neutralized. This keeps costs low and makes treatment highly responsive to continually changing conditions in the field. Furthermore, once the area is restored, these units can be transferred to other restoration efforts and continue to clean our polluted waters beyond the scope of any single project.

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Appendixes

Appendix 1 Lab Analysis of Raw Sediment Water 1/12/07

CERTIFICATE OF ANALYSIS Page: 1

Barnstable County Health Laboratory

Report Prepared For: Report Dated: 1/29/2007

Jonathan Todd
John Todd Ecological Design
P O Box 497
Woods Hole, MA 02543

Order No.: G0739307

Laboratory ID #: 0739307-01 Description: Water - Ground Water

Sample #: Sampling Location: M8L Greenhouse, Woods Hole, MA Collected: 1/12/2007

Collected by: Olin Christy Received: 1/12/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Tested
DISSOLVED OXYGEN	9.0	mg/L	1.0		SM 4500-0 G	1/12/2007
Alkalinity	14	mg/L as CaCO	2.0		EPA 310.1	1/12/2007
Ammonia as Nitrogen	2.1	mg/L	0.20		SM4500-NH3	1/18/2007
COD	1,500	mg/L	2.0		EPA 410.4	1/19/2007
TKN	17	mg/L	0.50		EPA 351.2	1/17/2007
Total Suspended Solids	1,700	mg/L	5.0		EPA 160.2	1/12/2007
TPH by GC\FID	7.8	mg/L	0.20		EPA 8015B	1/23/2007
Conductance	390	umohs/cm	2.0		EPA 120.1	1/12/2007

Approved By: (Lab Director)

1/31/2007

MCL = Maximum Contaminant Level
RL = Reporting Limit

Superior Court House, PO. Box 427, Barnstable, MA 02630 Ph: 508-375-6605

Appendix 2: Lab Analysis of Water Chemistry 3/05/07

CERTIFICATE OF ANALYSIS

Page: 1

Barnstable County Health Laboratory

Report Prepared For: Report Dated: 3/21/2007

Order No.: G0739687

Laboratory ID #: 0739687-01 Description: Water - Ground Water

Sample #: Sampling Location: Train 1 Collected: 3/5/2007

Collected by: Olin Christy Received: 3/5/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
DISSOLVED OXYGEN	10	mg/L	1.0		SM 4500-0 G	TFB	3/7/2007	
Alkalinity	11	mg/L as CaCO	2.0		EPA 310.1	LAP	3/5/2007	
Ammonia as Nitrogen	ND	mg/L	0.20		SM4500-NH3	DCB	3/8/2007	
COD	50	mg/L	2.0		EPA 410.4	LAP	3/9/2007	
Phosphorus - Total	ND	mg/L	0.50		EPA 365.4	LAP	3/9/2007	
TKN	ND	mg/L	0.50		EPA 351.2	LAP	3/14/2007	
Total Suspended Solids	ND	mg/L	5.0		EPA 160.2	LAP	3/6/2007	
Conductance	300	umohs/cm	2.0		EPA 120.1	DCB	3/5/2007	

Laboratory ID #: 0739687-02 Description: Water - Ground Water

Sample #: Sampling Location: Train 2 Collected: 3/5/2007

Collected by: Olin Christy Received: 3/5/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
DISSOLVED OXYGEN	10	mg/L	0.1		EPA 360.1	TFB	3/7/2007	
Alkalinity	15	mg/L as CaCO	2.0		EPA 310.1	LAP	3/5/2007	
Ammonia as Nitrogen	0.30	mg/L	0.20		SM4500-NH3	DCB	3/8/2007	
COD	51	mg/L	2.0		EPA 410.4	LAP	3/9/2007	
Phosphorus - Total	0.70	mg/L	0.50		EPA 365.4	LAP	3/9/2007	
TKN	1.1	mg/L	0.50		EPA 351.2	LAP	3/14/2007	
Total Suspended Solids	ND	mg/L	5.0		EPA 160.2	LAP	3/6/2007	
Conductance	300	umohs/cm	2.0		EPA 120.1	DCB	3/5/2007	

Approved By: Gongmin Lei (Lab Manager)

3/21/2007

ND = None Detected RL = Reporting Limit MCL = Maximum Contaminant Level

Superior Court House, PO. Box 427, Barnstable, MA 02630 Ph: 508-375-6605

Appendix 3: Lab Analysis of TPH of Water and Sediments 3/05/07

CERTIFICATE OF ANALYSIS

Page: 1

Barnstable County Health Laboratory

Report Prepared For: Report Dated: 3/21/2007

Jonathan Todd
John Todd Ecological Design
P O Box 497
Woods Hole, MA 02543

Order No.: G0739687

Laboratory ID #: 0739687-01 — Description: Water - Ground Water

Sample #: — Sampling Location: Train 1 — Collected: 3/5/2007

Collected by: Olin Christy — Received: 3/5/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	ND	mg/L	0.50		EPA 8015B	yn	3/9/2007	

Laboratory ID #: 0739687-02 — Description: Water - Ground Water

Sample #: — Sampling Location: Train 2 — Collected: 3/5/2007

Collected by: Olin Christy — Received: 3/5/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	ND	mg/L	0.50		D3328-78	yn	3/9/2007	

Laboratory ID #: 0739687-03 — Description: Water - Ground Water

Sample #: — Sampling Location: Soil — Collected: 3/5/2007

Collected by: Olin Christy — Received: 3/5/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TOC	130,000	mg/Kg	50		EPA 9060	yn	3/16/2007	
TPH by GC\FID	110,000	mg/Kg	13000		D3328-78	yn	3/9/2007	

Approved By: Gongmin Lei (Lab Director) 3/21/2007

ND = None Detected RL = Reporting Limit MCL = Maximum Contaminant Level

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Appendix 4: Lab Analysis of Water Chemistry 4/02/07

04/18/2007 WED 16:23 FAX 5083627103 Barnstable CTY HealthLab 002/004

CERTIFICATE OF ANALYSIS

Page: 1

Barnstable County Health Laboratory

Report Prepared For: Report Dated: 4/18/2007

Jonathan Todd
John Todd Ecological Design
P O Box 497
Woods Hole, MA 02543

Order No.: G0739952

Laboratory ID #: 0739952-01 Description: Water - Ground Water

Sample #: Sampling Location: Train 1 Collected: 4/2/2007

Collected by: O. Christy Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Tested
DISSOLVED OXYGEN	9.8	mg/L	0.1		EPA 360.1	4/4/2007
Alkalinity	3.8	mg/L as CaCO	2.0		EPA 310.1	4/2/2007
Ammonia as Nitrogen	ND	mg/L	0.20		SM4500-NH3	4/11/2007
COD	21	mg/L	2.0		EPA 410.4	4/5/2007
Phosphorus - Total	ND	mg/L	0.50		EPA 365.4	4/9/2007
TKN	0.50	mg/L	0.50		EPA 351.2	4/9/2007
Total Suspended Solids	ND	mg/L	5.0		EPA 160.2	4/3/2007
Conductance	460	umohs/cm	2.0		EPA 120.1	4/2/2007

Laboratory ID #: 0739952-02 Description: Water - Ground Water

Sample #: Sampling Location: Train 2 Collected: 4/2/2007

Collected by: O. Christy Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Tested
DISSOLVED OXYGEN	9.8	mg/L	0.1		EPA 360.1	4/4/2007
Alkalinity	5.6	mg/L as CaCO	2.0		EPA 310.1	4/2/2007
Ammonia as Nitrogen	ND	mg/L	0.20		SM4500-NH3	4/11/2007
COD	18	mg/L	2.0		EPA 410.4	4/5/2007
Phosphorus - Total	ND	mg/L	0.50		EPA 365.4	4/9/2007
TKN	ND	mg/L	0.50		EPA 351.2	4/9/2007
Total Suspended Solids	ND	mg/L	5.0		EPA 160.2	4/3/2007
Conductance	470	umohs/cm	2.0		EPA 120.1	4/2/2007

Laboratory ID #: 0739952-05 Description: Water - Ground Water

Sample #: Sampling Location: Raw Canal Water Collected: 4/2/2007

Collected by: O. Christy Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Tested
Phosphorus - Total	15	mg/L	3.0		EPA 365.4	4/9/2007

ND = None Detected RL = Reporting Limit MCL = Maximum Contaminant Level

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Appendix 5: Lab Analysis of TPH of Water and Sediments 4/02/07

04/18/2007 WED 16:23 FAX 5083627103 Barnstable CTY HealthLab 004/004

CERTIFICATE OF ANALYSIS

Page 1

Barnstable County Health Laboratory

Report Prepared For: Report Dated: 4/18/2007

Jonathan Todd
John Todd Ecological Design
P O Box 497
Woods Hole, MA 02543

Order No.: G0739952

Laboratory ID #: 0739952-01 Description: Water - Ground Water
Sample #: Sampling Location: Train 1
Collected by: O. Christy
Collected: 4/2/2007
Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	0.50	mg/L	0.20		EPA 8015B	yn	4/5/2007	

Laboratory ID #: 0739952-02 Description: Water - Ground Water
Sample #: Sampling Location: Train 2
Collected by: O. Christy
Collected: 4/2/2007
Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	0.60	mg/L	0.20		EPA 8015B	yn	4/5/2007	

Laboratory ID #: 0739952-03 Description: Soil
Sample #: Sampling Location: Train 1
Collected by: O. Christy
Collected: 4/2/2007
Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	66,000	mg/Kg	14,000		EPA 8015B	yn	4/10/2007	

Laboratory ID #: 0739952-04 Description: Soil
Sample #: Sampling Location: Train 2
Collected by: O. Christy
Collected: 4/2/2007
Received: 4/2/2007

Test Parameters

ITEM	RESULT	UNITS	RL	MCL	Method #	Analyst	Tested	Note
TPH by GC\FID	49,000	mg/Kg	14,000		EPA 8015B	yn	4/10/2007	

Approved By: Gongmin Le
(Lab Director)
4/18/2007

ND = None Detected RL = Reporting Limit MCL = Maximum Contaminant Level

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Appendix 6 Additional System Data

Table 6: Log of water additions and removals with proportion of system capacity comprised of contaminated Blackstone River Canal water (cont.) and tap water (uncont.).

Note that prior to 3/5/2007 evaporative losses were topped-off with tap water

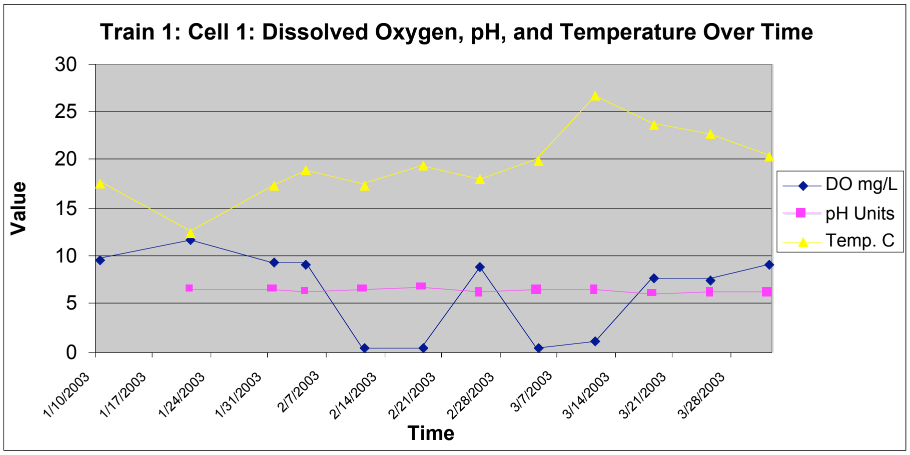
date	action	Proportion cont. water in system	Proportion uncont. Water in system
12/12/2006	initial filling of system	0	1
12/16/2006	removed 17.5 gal		
	added 17.5 gal cont.	0.0875	0.9125
12/22/2006	removed 17.5 gal		
	added 17.5 ecology	0.07984375	0.92015625
1/3/2007	removed 4 gal		
	added 4 gal cont.	0.098246875	0.901753125
2/1/2007	removed 10 gal		
	added 10 gal cont.	0.143334531	0.856665469
2/20/2007	removed 5 gal		
	added 5 gal cont.	0.164751168	0.835248832
2/26/2007	removed 10 gal		
	added 10 gal cont.	0.20651361	0.79348639
3/5/2007	evap. Of 2 gal.	0.208599606	0.791400394
	removed 8 gal		
	added 10 gal cont.	0.248169625	0.751830375
3/6/2007	evap. Of 2 gal.	0.250676389	0.749323611
	removed 8 gal		
	added 10 gal cont.	0.28814257	0.71185743

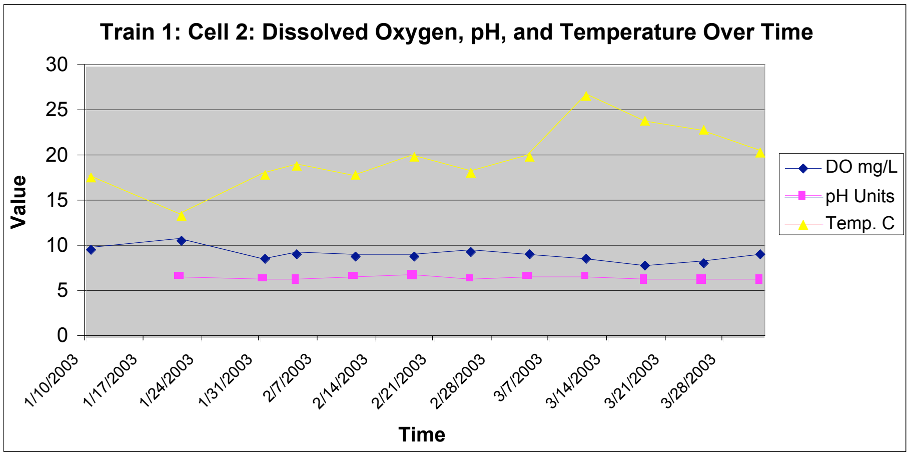
3/7/2007	evap. Of 2 gal.	0.291053101	0.708946899
	removed 8 gal		
	added 10 gal cont.	0.326500446	0.673499554
3/9/2007	evap. Of 2 gal.	0.32979843	0.67020157
	removed 8 gal		
	added 10 gal cont.	0.363308509	0.636691491
3/12/2007	evap. Of 2 gal.	0.366978291	0.633021709
	removed 8 gal		
	added 10 gal cont.	0.398629377	0.601370623
3/14/2007	evap. Of 2 gal.	0.402655936	0.597344064
	removed 8 gal		
	added 10 gal cont.	0.432523139	0.567476861
3/15/2007	evap. Of 2 gal.	0.43689206	0.56310794
	removed 8 gal		
	added 10 gal cont.	0.465047457	0.534952543
3/16/2007	evap. Of 2 gal.	0.469744906	0.530255094
	removed 8 gal		
	added 10 gal cont.	0.496257661	0.503742339
3/17/2007	evap. Of 2 gal.	0.501270364	0.498729636
	removed 8 gal		
	added 10 gal cont.	0.526206846	0.473793154
3/18/2007	evap. Of 2 gal.	0.531522067	0.468477933
	removed 8 gal		
	added 10 gal cont.	0.554945964	0.445054036

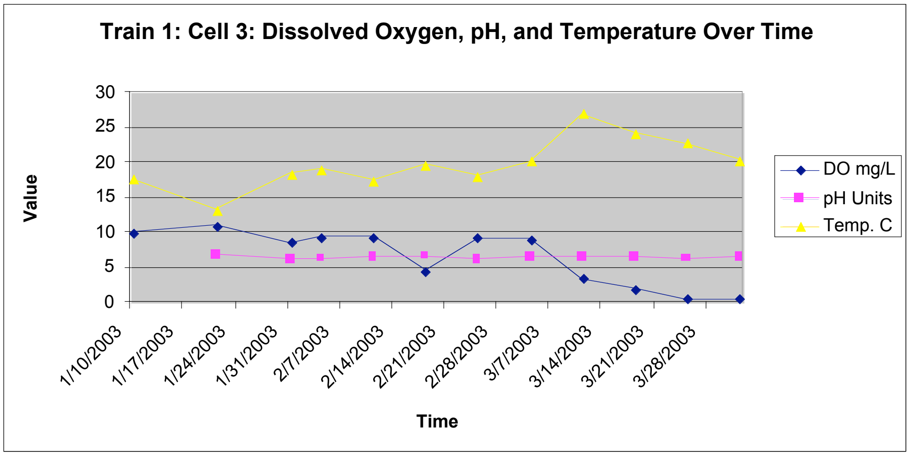
3/19/2007	evap. Of 2 gal.	0.560551478	0.439448522
	removed 8 gal		
	added 10 gal cont.	0.582523904	0.417476096
3/20/2007	evap. Of 2 gal.	0.588407984	0.411592016
	removed 8 gal		
	added 10 gal cont.	0.608987585	0.391012415
3/21/2007	evap. Of 2 gal.	0.615138975	0.384861025
	removed 8 gal		
	added 10 gal cont.	0.634382026	0.365617974
3/22/2007	evap. Of 2 gal.	0.640789925	0.359210075
	removed 8 gal		
	added 10 gal cont.	0.658750429	0.341249571
3/23/2007	evap. Of 2 gal.	0.665404474	0.334595526
	removed 8 gal		
	added 10 gal cont.	0.68213425	0.31786575
3/24/2007	evap. Of 2 gal.	0.689024495	0.310975505
	removed 8 gal		
	added 10 gal cont.	0.70457327	0.29542673
3/25/2007	evap. Of 2 gal.	0.711690172	0.288309828
	removed 8 gal		
	added 10 gal cont.	0.726105663	0.273894337
3/26/2007	evap. Of 2 gal.	0.733440064	0.266559936
	removed 8 gal		
	added 10 gal cont.	0.746768061	0.253231939
3/27/2007	evap. Of 2 gal.	0.754311173	0.245688827

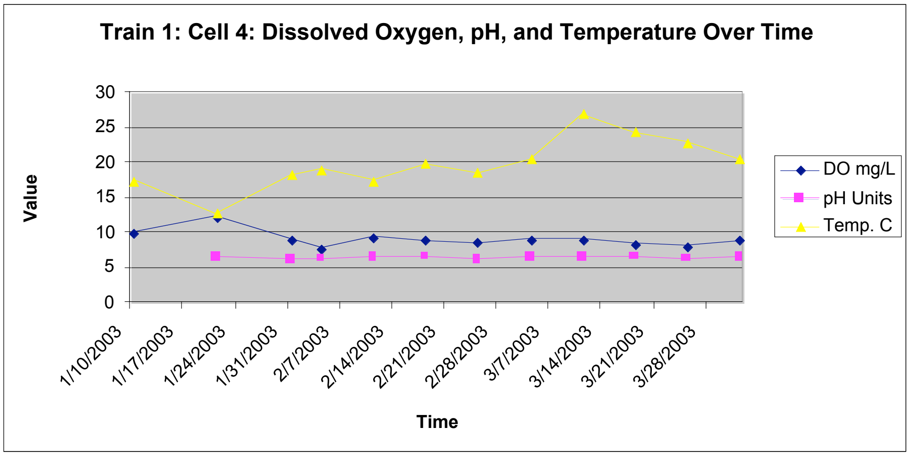
	removed 8 gal		
	added 10 gal cont.	0.766595614	0.233404386
3/28/2007	evap. Of 2 gal.	0.774339004	0.225660996
	removed 8 gal		
	added 10 gal cont.	0.785622054	0.214377946
3/29/2007	evap. Of 2 gal.	0.79355763	0.20644237
	removed 8 gal		
	added 10 gal cont.	0.803879749	0.196120251
3/30/2007	evap. Of 2 gal.	0.811999746	0.188000254
	removed 8 gal		
	added 10 gal cont.	0.821399759	0.178600241
3/31/2007	evap. Of 2 gal.	0.829696726	0.170303274
	removed 8 gal		
	added 10 gal cont.	0.83821189	0.16178811
4/1/2007	evap. Of 2 gal.	0.84821189	0.15178811
4/2/2007	evap. Of 2 gal.	0.85821189	0.14178811

Figure 9: Do, pH, and temp. for each aquatic cell and average across cells for train 1.


Train 1: Cell 1: Dissolved Oxygen, pH, and Temperature Over Time
Value
Time
DO mg/L
pH Units
Temp. C


Train 1: Cell 2: Dissolved Oxygen, pH, and Temperature Over Time
Value
Time
DO mg/L
pH Units
Temp. C


Train 1: Cell 3: Dissolved Oxygen, pH, and Temperature Over Time
Value
Time
DO mg/L
pH Units
Temp. C



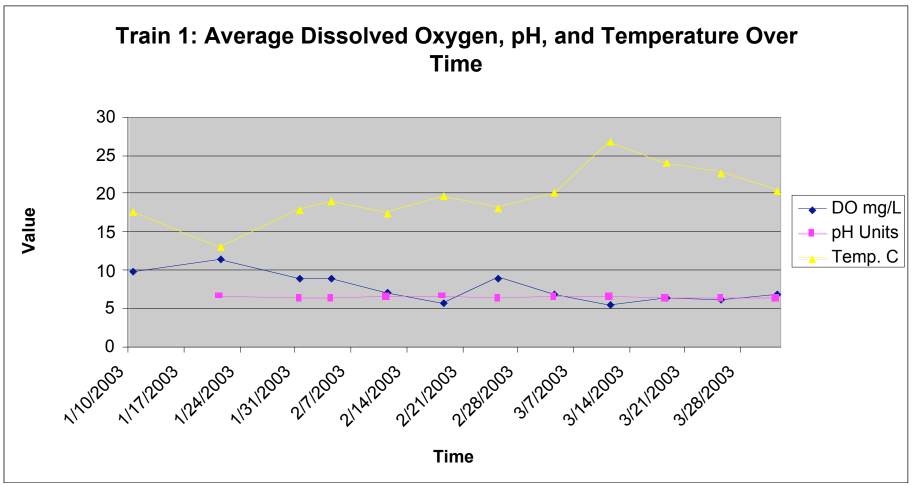
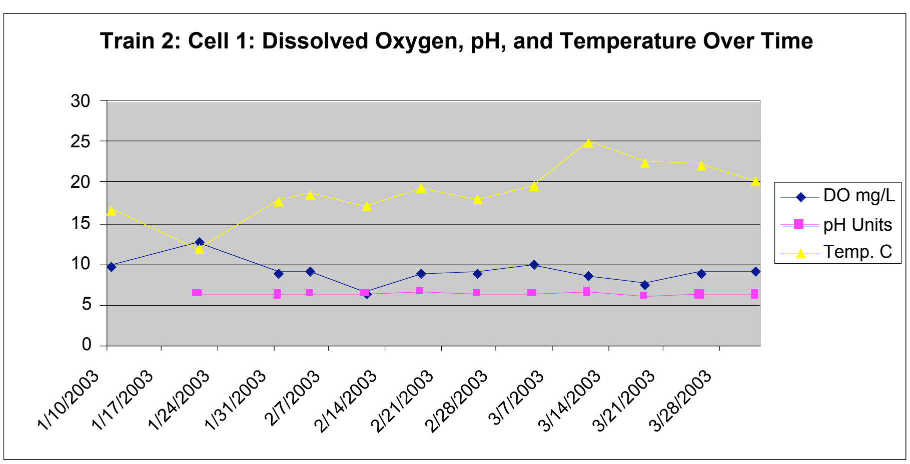
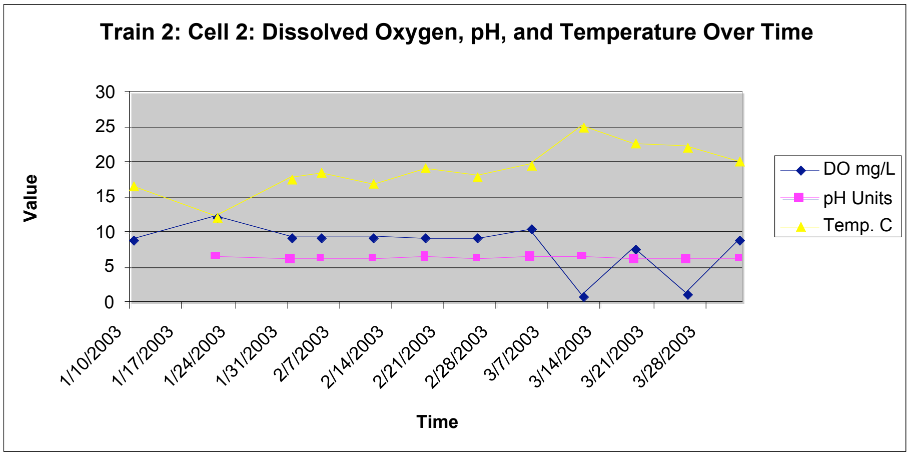
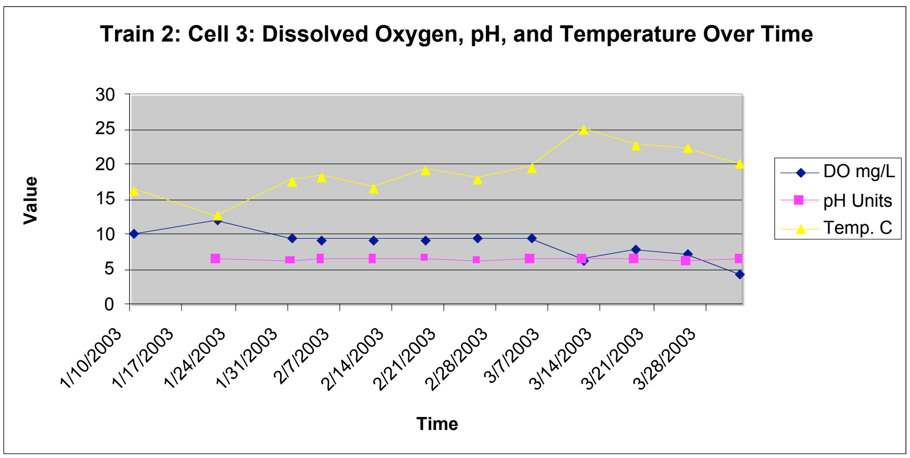
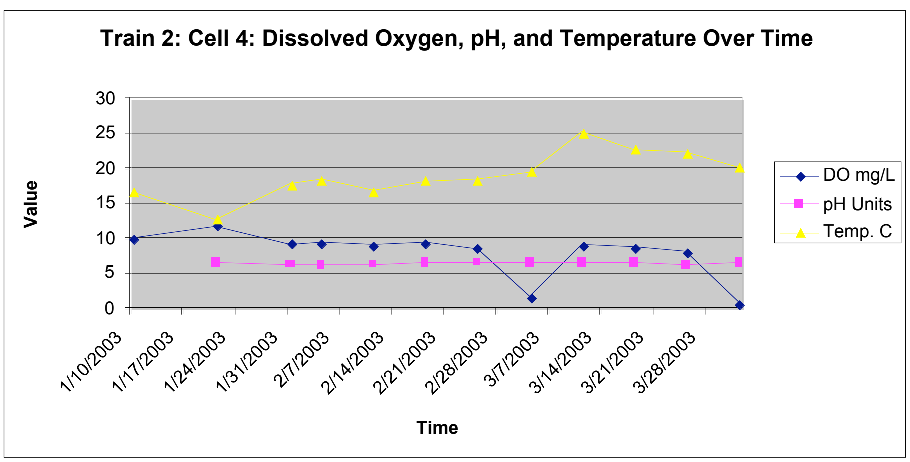


Figure 10: **Do, pH, and temp. for each aquatic cell and average across cells for train 2.**




Train 2: Cell 2: Dissolved Oxygen, pH, and Temperature Over Time
30
25
20
15
10
5
0
Value
1/10/2003
1/17/2003
1/24/2003
1/31/2003
2/7/2003
2/14/2003
2/21/2003
2/28/2003
3/7/2003
3/14/2003
3/21/2003
3/28/2003
Time
DO mg/L
pH Units
Temp. C


Train 2: Cell 3: Dissolved Oxygen, pH, and Temperature Over Time
30
25
20
15
10
5
0
Value
1/10/2003
1/17/2003
1/24/2003
1/31/2003
2/7/2003
2/14/2003
2/21/2003
2/28/2003
3/7/2003
3/14/2003
3/21/2003
3/28/2003
Time
DO mg/L
pH Units
Temp. C


Train 2: Cell 4: Dissolved Oxygen, pH, and Temperature Over Time
30
25
20
15
10
5
0
Value
1/10/2003
1/17/2003
1/24/2003
1/31/2003
2/7/2003
2/14/2003
2/21/2003
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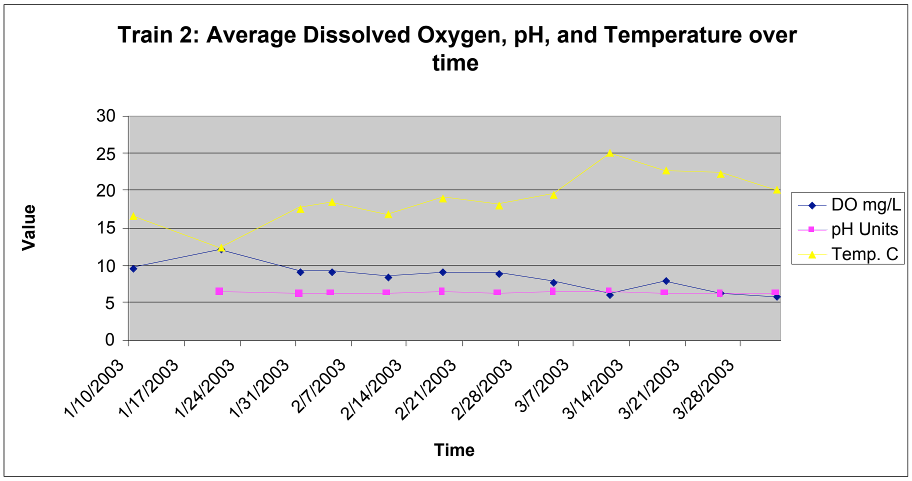
3/7/2003
3/14/2003
3/21/2003
3/28/2003
Time
DO mg/L
pH Units
Temp. C


Train 2: Average Dissolved Oxygen, pH, and Temperature over time
Value
30
25
20
15
10
5
0
1/10/2003
1/17/2003
1/24/2003
1/31/2003
2/7/2003
2/14/2003
2/21/2003
2/28/2003
3/7/2003
3/14/2003
3/21/2003
3/28/2003
Time
DO mg/L
pH Units
Temp. C