U.S. Army Corps of Engineers (USACE), Fort Worth District
Pre-Application Meeting Request

Box 1 Basic Project Information Project Name: Trinity River Bioremediation Pilot Plan		Date: November 19, 2019
City Dallas	County Dallas	State Texas
Total Size of Property in Acres 1	Latitude 32.856376 GPS 32°51'22.2"N	Longitude -96.892312 GPS 96°53'32.1"W
Box 2 Property Owner Name City of Dallas (Arnelle Woods is the Assistant City Manager)		Email arnelle.woods@dallascityhall.com
Mailing Address 1500 Marilla St. (physical address is: 2301 North Stemmons Freeway)		Phone 214-670-3111
Box 3 Applicant Name Virginia Kilgore		Email virginia@waterisalive.org
Mailing Address 710 Ida Vista Ct. Duncanville Texas 75116		Phone 971-212-8337
Box 4 Agent Name		Email
Mailing Address		Phone

Box 5 Information Required to Accompany Request - check as much information as is available:

Project Description: Provide a brief summary of the proposed project including development plans, size in acres, potential impacts to Waters of the U.S., existing land use/cover, etc.: **X**

Project Purpose: In Situ bioremediation of contamination and e coli in the Trinity River

x Accurate Location Maps (from County map, USGS Quad Sheet, Aerial Photos, etc.)
x Map of the Project Site
Conceptual Site Plans for the Overall Development
X Approximate acreage of wetland impact: 2 acres.
x Approximate linear feet of stream impact: 1584 feet is .3 miles, and with both banks, 3168 linear feet.
Impact Type: (e.g., Forested Wetland, Emergent Wetland, Intermittent Stream, etc.)
Pre-Application Meeting Agenda

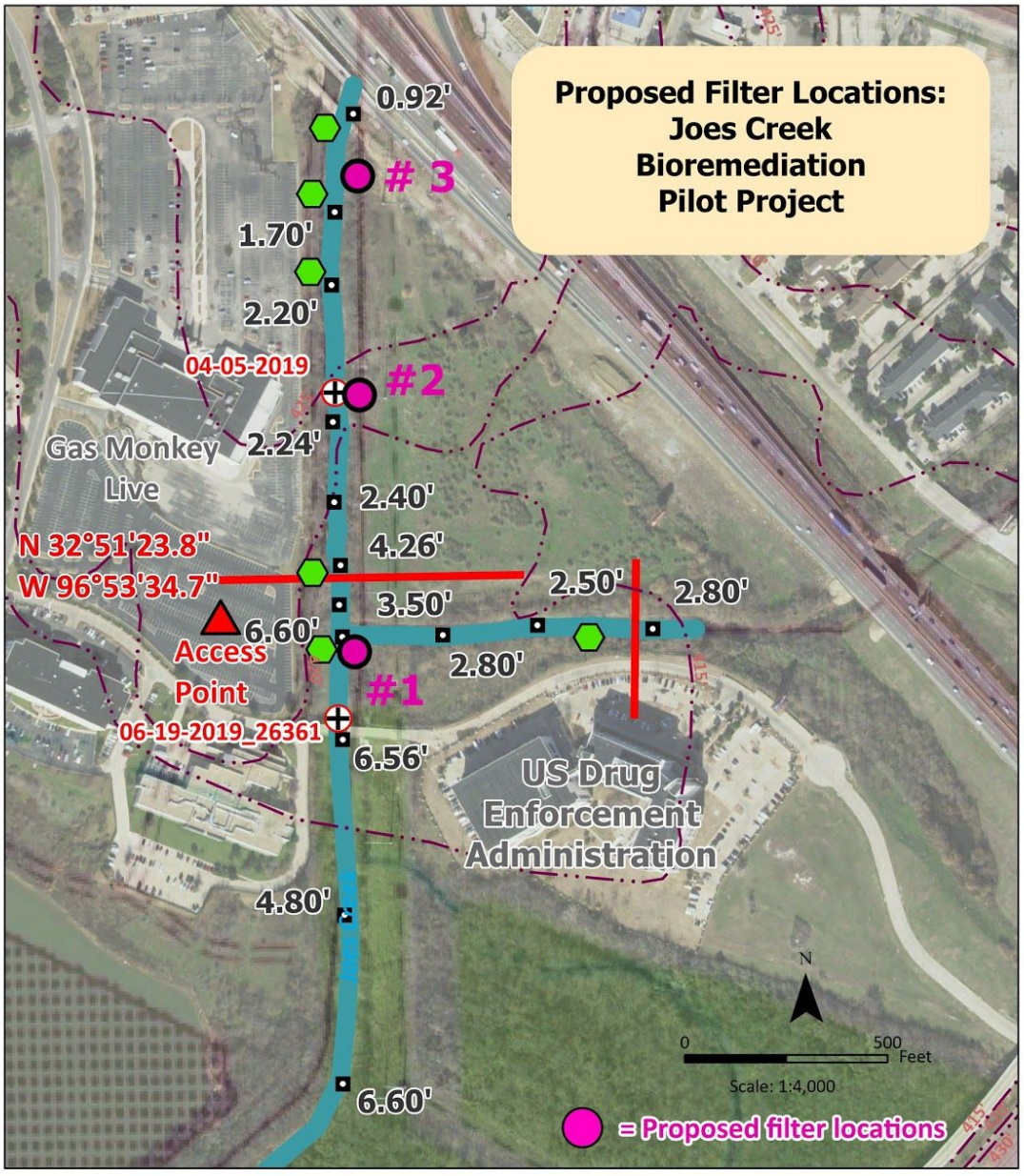
Box 6 Optional Additional Information: Any information you can provide about the proposal, project site, and/or surrounding area will facilitate a more effective pre-application meeting. Additional information may include, but is not limited to:

Delineation of the Waters of the U.S. on the Property or a Jurisdictional Determination from the USACE
Threatened or Endangered Species Information, and/or Any Coordination With USFWS
Historic Properties Cultural Resources Information, and/or Any Coordination With the SHPO
X Conceptual Mitigation Information
X Floodplain Information
X Color Photograph
X Aerial Photograph
X Other Authorizations Obtained or Required: Comments from TCEQ and TPWD
X Other: Video of Joe's Creek with proposed permit locations

The applicant will be responsible for taking meeting notes and submitting them to the USACE for review.

Copies of this request may be obtained at: http://www.swf.usace.army.mil/Missions/Regulatory.aspx

Please mail this form to:
Regulatory Division (CESWF-DE-R)
Fort Worth District U.S. Army Corps of Engineers 819 Taylor Street, Room 3A37 P.O. Box 17300



Trinity River Bioremediation Pilot Plan Project Description

Using 3 methods of bioremediation adapted by Water Is Alive, we propose to work with Groundwork Dallas, area schools and other potential stakeholders to bioremediate present e coli threats while addressing other contamination issues in Joe's Creek, a tributary to the Elm Fork of the Trinity River.

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Joe's Creek Bioremediation Pilot Plan Summary

Water Is Alive and partner organizations are applying to The Army Corps of Engineers for a pilot project permit to place biodegradable microorganism inoculated filters along 50-100 feet stretches in Joe's Creek. These small biodegradable filters may be anchored to existing debris along the creek. Water Is Alive is also suggesting various forms of natural anchors to place the filters in the deeper parts of the creek where the water level is sufficient to keep the filters moist year round.

Water Is Alive proposes producing several kinds of filters. Myceliated filters and filters activated with food grade bacteria, yeasts and fungi. Water Is Alive has been teaching students how to grow these filters since 2017. Prior to filter placement, the myceliated bio filters can produce gourmet mushrooms. 100 small myceliated filters, placed along the middle of the creek and both banks, should be sufficient to notice a change in the contamination levels in Joe's Creek. Current contamination levels, focused on the elevated levels of e coli, are monitored by the City of Dallas Water & Utilities Department and the Texas Stream Team, with the volunteers and staff from Groundwork Dallas.

Concerns regarding the introduction of non-native species of food grade mushrooms as well as non genetically modified microorganisms are addressed in this permit summary, with comments from the TECQ and TPWD.

Our goals are to:

- Improve the water quality in Joe's Creek.
- Raise awareness regarding contamination in the Trinity River.
- Help restore and improve the natural ecology and bioremediation capabilities of the Trinity River Basin.
- Equip students, businesses and individuals with the materials, methods and recipes needed to grow biofilters that will mitigate and metabolize contamination in water and soil.

The survey and accompanying video of Joe's Creek

The video included in the application starts in the "West Fork" of Joe's Creek near the underpass of I-35E. The above map, "Orthomosaic View", details the depths of the creek at 17 locations on both forks of Joe's Creek and follows the joined creeks to the mouth of the creek on the Trinity River. The survey was conducted on July 28, 2019. The water level was down approximately six feet compared to when Groundwork Dallas conducted water monitoring on June 12 of 2019. The difference in water levels was noted during the July 28th survey by measuring the water level between the creek and the sewer main line. In June the water was passing several inches under the sewer main line and on July 28 there was 6 feet of clearance between the creek and the sewer main. Proposed location #1 of the Joe's Creek Bioremediation Pilot Project, is just down-stream of the sewer main line.

Current use of the land

The specific GPS location is near Gas Monkey Live (32°51'22.2"N 96°53'32.1"W). The creek immediately east of the Gas Monkey Live parking lot is north of the conjunction of the East and "West" forks of Joe's Creek. The fork we are applying to bioremediate is runoff water from storm drains and natural drainage from neighborhoods and industrial areas in Northwest Dallas in the Royal Lane/Webb Chapel vicinity. For the purposes of this permit application, the portion of the creek for which we are seeking a permit will be called the "West Fork" of Joe's Creek. Along the stretch of the creek next to the Gas Monkey Live parking lot, approximately .3 miles long, Water Is Alive is proposing 3 locations to place biofilters. Joe's Creek is a very full creek after rains, yet in more arid times of the year, the creek under I-35 is a small trickle of water. The total area covered along the creek could be up to .5 acres, including the surface area of the water between the filters.

The banks of the creek are wooded areas. On the west and east banks of the "West Fork" of Joe's Creek, trees stands approximately 60 feet wide line the creek bed. Inside the 3 proposed locations of the creek are 8 storm drains, and crossing the creek is one sewer main line. A man-hole is present on the west bank of the creek close to Proposed Location #1.

Residential and industrial paths of the two forks of Joe's Creek

The "West Fork" of Joe's Creek

The specific location where the West Fork of Joe's Creek appears to originate from on Google Maps is Northeast of Webb Chapel Road in the Northaven Road area. A Google

Maps satellite image of the Joe's Creek headwaters in the form of a storm drain for surface runoff can be seen from Webb Chapel Road at 32°54'03.4"N 96°52'20.9"W.

Continuing down the West fork of Joe's Creek, the creek can be seen passing under Brockbank Drive at: 32°53'32.0"N 96°52'45.8"W. And again, passing under Harry Hines Boulevard at 32°53'04.0"N 96°53'12.7"W. After passing under Allegany Lane at 32°53'20.9"N 96°52'53.6"W, the creek enters a heavily industrialized area. Joe's Creek combines with a branch of the creek running from another heavily industrialized area at: 32.8674988,-96.8916858
A map of this conjunction may be viewed at the following google maps link:
Conjunction of two West Fork's of Joe's Creek

The West Fork of Joe's Creek continues to run under Loop 12 and Stemmons Highway (I-35 E). A homeless community is located near the underpass of I-35, in the woods near Joe's Creek. This is the northernmost point on the West Fork of Joe's Creek at which we measured a depth of .92 feet on July 28, 2019.

The East Fork of Joe's Creek
20 meters downstream of the sewer main line, Joe's Creek meets the East Fork of Joe's Creek. This branch of Joe's Creek appears to originate near Rosser Park off of Deep Valley Drive. Those GPS coordinates are 32°54'42.6"N 96°50'38.8"W. After winding through five miles of residential neighborhoods, the East Fork of Joe's Creek enters the industrial area at Brockbank Drive**.**
The East Fork of Joe's Creek only runs two miles through an industrialized area, crossing under Harry Hines, Loop 12, I-35 and enters the Gas Monkey Live area joining the West Fork of Joe's Creek adjacent to the inlet from the parking lot close to Proposed Location #1.

Bioremediation Methods
Method 1) Biofilters made of biodegradable myceliated woodchips: Semi submerged masses of sawdust and woodchips that have been allowed to harden due to the consumption of the wood by the mycelium. When these small to medium sized myceliated blocks, are solidified with mycelium, we will anchor arrays of these filters onto the banks and into the middle of Joe's Creek.

Method 2) Effective Microorganisms: A product based on 31 species of non-genetically modified food grade microorganisms. These are primarily photosynthetic and lactic acid bacteria, yeast, actinomycetes, and fermenting fungi. The medium Effective Organisms is grown in is filtered water and organic molasses. The Effective Microorganisms reproduce in an anaerobic fermentation process.

The Trinity River Bioremediation Plan Time Line
The pilot project will run at 3-4 week intervals and would start in May of 2020 and run through November of 2020. The two main kinds of filters will be anchored in the creek at 3-4 week intervals from mid spring through the fall. For the purpose of the pilot project, Method 1 using myceliated wood chips, and Method 2 using Effective Microorganisms will be used in alternating intervals, as to be able to empirically observe the effectivity of the different approaches.

The Texas temperate winters are usually warm enough to allow for slow bacterial growth and mycelium development. Depending on the level of participation from area schools and other interested groups, we could consider running the pilot project through the winter months.

The biofilters can be grown in the winter and early spring of 2020, and the placement of the filters in May, as to benefit from the cooler temperatures and the rains. These conditions are more easily achievable in the winter by controlling a microclimate where the mycelium is growing, thus keeping the filters from freezing and keeping the filters humid.

Method 1) Myceliated Biofilters

Using strains of the Oyster mushroom, the *Pleurotus ostreatus* and the Garden Giant, or Stropharia rugosoannulata as well as native species, we will grow 100% biodegradable filters. Grown in cardboard boxes, or another easily obtainable source of casts, the biofilters will be placed along 50-100 foot section(s) of both banks of Joe's Creek. We hope to produce many myceliated filters and anchor them in the creek with natural fiber ropes and large rocks or by fastening the biofilters to debris already present.

The placement of biofilters along the banks and in the creek composed of myceliated (mushroom) substrates such as: myceliated straw bales, wicker baskets containing myceliated corn husks and straw and gunny sacks full of myceliated wood chips. Small to medium grade sawdust will help fully myceliate the entire surface of the substrates. Water Is Alive and interested parties will experiment with other innovative methods to naturally filter the creek water as it is flowing along the filters. As the water flows through the porous and biodegradable biofilters, bacteria and contamination are absorbed and metabolized by the mycelium. To protect the integrity of the filters different biodegradable materials will be tested during the pilot project. The bottom of the myceliated woodchip mass and the plant roots will be submerged in the water. The mycelium will continue to grow as more wood chips are added.

The approximate size of the filters is 17 inches x 11 inches, and the floating filters are the size of paint buckets. The cardboard boxes and bucket molds will be removed prior to placement in the creek. For the deeper parts of the creek, a floating islands of myceliated filters will be developed. This filter will also serve to host plants specific to the remediation of the contaminants found in water. This floating bioremediation project is based on phytoremediating plants interspersed between the linked bucket-sized mycelited biofilters. The mycelium and plants can be sensitive to cold weather and are subject to damage from extreme cold or heat or dryness. Pictures and demonstrations of a myceliated filter will be presented at the pre-permit meeting.

The myceliated biofilters are designed to biodegrade and be carried downstream to continue the inoculation and bioremediation process in the event of rain and high waters. Even in dry conditions, the biofilters will continue to biodegrade and bioremediate the creek bed. In dry and cold conditions the filters are less active.

Method 2: Effective Microorganisms

Effective Microorganism filters are essentially probiotics for the Trinity. This kind of filter is based upon fermenting fruits and is primarily designed as a passive timed-release of

Effective Microorganisms. The fruits are sources of enzymes. These enzymes are made by living organisms and they help bacteria break down nutrients and rebuild the nutrients into new compounds. Microorganisms such as bacteria gain their energy by transferring energy from an electron donor (sugars in the fruit) to an electron acceptor (oxygen, nitrate, sulfate, carbon dioxide and some pollutants) (17). These sugars are a source of energy (electron donors) the introduced bacteria (Effective Microorganisms) can eat and grow from. Strains of *Lactobacillus* as well as other bacteria in the Effective Microorganisms have been proven in clinical studies of e coli infections in human and animal intestinal tracts to adhere to e coli and inhibit growth and reproduction (2,11). This Trinity River Bioremediation Pilot Plan Method 2 will essentially attempt to treat the tributaries of the river with scientifically proven remedies that can reduce the presence of e coli and suspected and monitored chemicals that are a result of storm runoff waters and industrial pollution. This proposed form of bioremediation has the potential to prove the use of fermented compost processing in the in-situ clean up of contamination.

Some Effective Microorganism biofilters will be located under water, in earth and water permeable metal or ceramic baskets, secured in the creek bed or as integral component inside anchored myceliated biofilters. These biofilters will be anchored to prevent tampering from animals in the area. Combinations of biofilters may be developed by students and researchers in conjunction with the accompanying Water Is Alive curriculum, designed to stimulate the science of bioremediation. The fruit and wood chip filters are designed to biodegrade as the microorganisms and fruit are decomposing. Parameters for student biofilter development will be based on the permit.

Fruits high in fructose will be acquired from markets, wholesalers and grocers and can also be dried fruits that are too old, to ugly, not ripe, etcetera, to be sold for consumption (5). These fruits are inoculated with Effective Microorganisms in an anaerobic setting (airtight buckets) for a period of 2 weeks. After the two weeks the inoculated fruits are anchored and submerged in the creek for a period of 2-4 weeks. Preference will be given to biodegradable containers, that can withstand turtles and animals attempting to eat the sweet contents in the filters.

Biofilms are naturally occurring bacterial layers that adhere to the surface of organic matter, especially in water. To inhibit biofilms from developing around the inoculated fruit, citrus fruit injected with colloidal silver will be included as an ingredient with the inoculated fruit prior to submersion in the creek. While the colloidal silver is also an effective and inexpensive method to treat gram-negative and gram-positive strains (8,10,11) of e coli in open water and effluent, for these purposes, the colloidal silver is used to prevent biofilm growth and promote the dispersion of the Effective Microorganisms into Joe's Creek.

Environmental monitoring before, during and after the first method are required to judge the effectivity of the rate of metabolism of the e coli by the *Lactobacillus* bacteria and other microorganisms present in the Effective Microorganisms. The e coli present in the water from human or avian sources bind naturally with heavy metal contamination. The Effective Microorganisms help in the conversion of heavy metals to less harmful elements to support healthy water and a healthy ecosystem. Effective Microorganisms also aids in the further metabolism of the e coli.

Effective Microorganisms were developed in the 1970's and 1980's and were originally created with 81 non-genetically modified food-grade microorganisms (1). More recent DNA analysis of Effective Microorganisms has shown the numbers of the diverse microorganisms have decreased. The genetic study did not identify which microorganisms remain in the recipe. This is due to the stronger microorganisms consuming the others. All licensed producers of EM are under obligation not to tamper with the recipe. During the study, depending on the level of interest and participation, Water Is Alive may consider looking at more recently specifically studied and strictly food grade non-genetically engineered microorganisms and potentially applying these as timed released treatments to the Trinity River Bioremediation Pilot Plan. This method could be better at evaluating which specific strains of bacteria and mycelium are most effective in restoring the ecosystems of the Trinity River. Strains of the specific microorganisms studied to remediate contamination problems in open water are commonly available from microbe banks or from the environment.

Typically, 'EM mudballs', where clay is inoculated with Effective Microorganisms and then dried for a week or longer, are added to water bodies to reduce contamination. Effective Microorganisms may also be added to open water as a regular addition as in the release of tons of liquid Effective Microorganisms (EM). The 'EM mudballs' have been applied to large and small bodies of water and the results are inconclusive. The water quality of many rivers around the world has reportedly improved as a result of the addition of Effective Microorganisms (13). Effective Microorganisms have been scientifically proven to naturally remediate certain types of contamination in water and soil (15,16). Water Is Alive proposes utilizing a source of fructose (fruit) together with the EM as to promote the continued organic processes of metabolization and reproduction of the microorganisms while submerged in Joe's Creek.

Phytoremediation, the third line of defense.
Plants and trees that remediate contamination are called phytoremediators. These plants may be grown indoors by nurseries, interested individuals, schools and stakeholders until placement in the floating biofilters. The phytoremediators listed here can be invasive species, once prevalent in the Trinity River Basin.

Two of the native species listed here are cattails and iris's. There are 4 native species of Iris to Texas. In the event these plants establish themselves in the Trinity River, they will continue to aid in the metabolism of e coli as well as other contaminants.

A concerted effort will be made to construct the floating islands only with indigenous species of plants. There are other native reeds and plants that are native to Texas. A small list of native plants that could be included on the island filters are: "*Pontederia cordata* (pickerelweed), *Thalia dealbata* (alligator flag), and *Canna glauca* (golden canna) as they remove excess nitrogen and phosphorus from standing water. Their beautiful flowers enhance the aesthetic value of wet ponds. *Sagittaria* (arrowhead) binds cadmium and zinc to its roots, accumulates heavy metals and may even remediate diesel. *Eleocharis* (spikerush) and *Equisetum* (horsetail) are lead hyperaccumulators. *Schoenoplectus* (bulrush) uptakes zinc and *Justicia* (water willow) uptakes iron. These plants break down or degrade pollutants by acting as filters to improve the quality of water entering our streams and lakes." (19)

"Salix nigra (black willow) often volunteers in riparian zones. Salix phytoremediates copper, zinc and lead. Willows also facilitate denitrification (a microbially facilitated process where nitrate is reduced to nitrogen) and provide a more hospitable soil condition for native plant species which are not able to tolerate high soil nitrate levels. *Helianthus annuus,* the annual sunflower, was used in Chernobyl, Ukraine to remove radioactive contaminants from groundwater." (19)

Depending on the levels of contamination present in the water and soil, some of the phytoremediator plants could need to be removed and disposed of, due to absorption of the heavy metals and contaminants, such as willow trees growing in heavily contaminated soil. Focusing on native Texas plants for the floating pilot biofilter, may help restore the population of naturally occurring contamination metabolizers along the Trinity River Basin when the plants go to seed. The plants will be established in a bulk substrate of myceliated wood chips and sawdust with interspersed biodegradable tubes filled with soil to help the native plants establish themselves in the floating filter. The bulk of the mass will naturally float and can be fastened to naturally occuring floating debris in Joe's Creek.

The introduction of non-native species to The Trinity River
The primary concern is the use of non-native species. Various species of mushrooms including Pleurotus and Stropharia will initially be applied. Indigenous species in the Pleurotus and Stropharia exist in Texas and will be collected and attempts to train these more localized species to metabolize typical contaminants found in the Trinity River will be made.

Joe's Creek is full of trash. This situation has improved due to the clean-up effort organized by Groundwork Dallas. Water Is Alive believes the proposed food-grade microorganisms are less of a threat to our health and that of the ecosystem than the plastic, styrofoam, metal, paper, biological and chemical contamination found in the Trinity River and her tributaries today. These organisms offer a reasonable chance to improve the water quality, be cost efficient, aid in the use of other 'waste' materials in the bioremediation of the river and will not be harmful for school children, interns, volunteers, and staff to work with when placing and growing the filters. There has been no evidence found that these organisms will harm local wildlife. In the event of an invasive species spread, we could have an outbreak of pollution-eating gourmet mushrooms along the river banks.

Already present mycelium and plant species can be supported by the deposit of substrates and methods to encourage indigenous mycelial and plant growth. Mycelium requires a substrate to grow on, moisture and air.

Implementation:
Part of the mission of Water Is Alive is to advance the understanding and application of bioremediation, or the in-situ clean-up and restoration of depleted and contaminated ground and water. As Method 1 and 2 of the Trinity River Bioremediation Plan are proven to be successful, we would like to continue producing biofilters and improving water quality in the Trinity River and her tributaries.

The filters can be deposited in areas of acute discharge to assist with the biodegradation and metabolization of toxicity, such as the "Dallas water and environmental officials investigating murky, pungent discharge into Trinity River",as reported by the Dallas Morning News on July 30 of 2019.

Filters can also be placed in the river and along the banks all year long to assist in the neutralizing of contaminants due to storm water runoff and other sources.

In the event wood chips accumulate as a result of a filter breach or the intentional deposit along the banks of the creek, these wood chips will protect moisture levels in the soil so already present mycelium species can further develop. The presence of wood chips will provide an additional substrate for the indigenous mycelium species to grow on. Deposited wood chips may be inoculated with food-grade microorganisms and mycelium. As the waters surge, the myceliated wood chips will be carried downstream to help metabolize contamination and naturally process e coli.

Signage
Signs will be placed along the banks indicating a test is in progress and to explain the presence and function of the myceliated biofilters. A link to various websites will be listed for interested parties.

Public Outreach
Social media, printed media, on-site signs, web sites, a mobile application and local associations, environmental groups and corporate volunteer support will be sought to foster participation and awareness of a growing bioremediation clean up of the Dallas waterways flowing into the Trinity River. Extreme creativity and involving students are key components to a successful campaign.

Analyses
A baseline bacterial and chemical analysis on a broad spectrum of chemical, metal and bacteriological contaminants in the water is needed. Core samples to observe the initial depth of contamination in the creek bed is wished. This is to document the depths in the sediment at which the various contaminants are located. A toxicological study to evaluate the effect of contaminants on small insects is desired as to identify potential baseline values and hazards of contamination in the sediment.

Potential obstacles The pilot project expects to be confronted with natural predicaments such as fast running water, floating trees and other debris and wildlife wanting to consume the biodegradable and edible filters. Cages made from bamboo or another biodegradable material may be necessary, as we continue to develop better methods to house the bioremediating filters.

Education through hands-on environmental clean-up and food production. Method 1, mycelium, is not only a method proven to metabolize contamination, it is also a food source and can be grown in humidity controlled trailers and out-buildings all over the metroplex. After the myceliated filters have produced gourmet mushrooms for consumption, often producing as much weight in edible mushrooms as the weight of the

substrate they are growing on, they are ready for placement in the water as myceliated filters. Method 2 can be taught “as how to clean up pollution with trash” (composted organic materials) on a small to a large scale.

Method 1 and 2 have been taught by Water Is Alive at a Dallas County Community College District school, and soon more of the district is interested in participating. Composting and the construction of biofilters are skills children and adults can learn. Composting is essential for a sustainable future because the enzymes in organic matter are a nonrenewable resource and from the enzymes, natural bacteria in the environment builds DNA, the basic building blocks of all life.

Involving industry and schools in the DFW area, especially the TMDL I-Plan stakeholders and municipalities will be the key to an affordable and widespread clean-up of contaminants in the Trinity River. This can be a cohesive factor in communities and an opportunity for professionals, educators and students to learn about our individual roles in protecting water quality, storm water issues and the disposal of chemicals and medicines at home. We can feel better about ourselves by contributing to the clean-up of our polluted planet because we care enough to compost and be active in the construction and care of biofilters. The shape of the biofilters depends on the shape of the box or cage or fabric the wood chips are myceliated in. These forms can be grown to function as building materials, furniture and there are many uses of myceliated substrates that have not been discovered yet. These methods will help educate and inspire citizens of the Trinity River Basin to participate in improving water and soil quality as to protect our health.

Partners and Stakeholders
Water Is Alive. A Texas non-profit corporation seeking to establish a multifaceted and ongoing community based bioremediation project to clean the waters of the Trinity River Basin and other waters of the world.

Groundwork Dallas is an environmental non-profit whose mission is to regenerate, sustain, and improve the Dallas Elm Fork Greenbelt and Great Trinity Forest by developing community-based partnerships that educate and empower people, businesses, and organizations to promote environmental stewardship. This mission is carried on by monitoring the water quality in this location along Joe's Creek. Groundwork Dallas has also organized volunteers to clean up the large household debris in the creek. They are currently active in encouraging local business to pick up litter along the creek.

Dallas Zoo, also considering a permit request to participate in the Trinity River Bioremediation Pilot Project for the creek running through the zoo. Benjamin Jones, the Senior Director of Conservation is also a member of the Board of Directors of Groundwork Dallas.

Potential stakeholders:
City of Dallas. Chris Morris is from the Storm Water Management Water Quality Team and coordinates water quality monitoring and water sampling for the Texas Stream Team.

Dallas Water Utilities: Kevin Hurley and Nusrat Munrir. They could help with chemical analysis of the site. The Joe's Creek location is currently only monitored for bacteria and other values, but not for chemical contamination.

Potential funding sources are: Rotary National fish and wildlife, Urban
5 Star Waters Grant (due in January), Meadows Foundation (Texas Stream Team), Cynthia and John Mitchel Foundation, Harold Simmons Park, and The Trinity Parks Conservancy.

Consultant Howard Sprouse, Founder of The Remediators Incorporated is known for his contribution to the remediation industry as an early commercializer of mycoremediation. A former research consultant to the Pacific Northwest National Laboratory and well known speaker at Washington based universities on environmental cleanup and restoration, Howard is involved in a variety of environmentally based projects in the Pacific Northwest and Alaska. Howard has proven mycoremediation and phytoremediation methods with projects for the U.S. Navy and EPA Region 9.

Other potential stakeholders: TMDL I-Plan stakeholders, parks and developments along the Trinity River, area universities, Texas A&M University, The Dallas County Community College District, area Independent School Districts, local businesses and corporations, environmental groups, and private individuals and foundations working toward social and environmental change.

Appendix

1) **"Effective Microorganisms (EM) Technology for Water Quality Restoration and Potential for Sustainable Water Resources and Management"**

2) **Bioremediation and Tolerance of Humans to Heavy Metals through Microbial Processes: a Potential Role for Probiotics?**

"Halttunen et al. (39) showed that *Lactobacillus* and *Bifidobacterium* species can bind lead and cadmium in solution. They observed a rapid binding phenomenon across all studied species, with the largest amounts of both lead and cadmium bound within 5 min to 1 h (39, 106). Most importantly, the metal remained strongly sequestered by the cell and did not disassociate, even 48 h after testing.

The rapid absorption of the metals from solution indicates cell surface binding. Ibrahim et al. (45) also compared the abilities of *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii* to bind and absorb lead and cadmium in solution. They reported a rapid effect of the bacteria to bind maximal amounts of metal after only 1 h of exposure; this was influenced by pH, as in *B. subtilis* and *E. coli* (52)."

"Lactobacilli and potentially other bacterial types used in the food industry or as probiotics are ideal organisms to use as an adjunct tool to prevent/reduce heavy-metal toxicity and prevent absorption of metals into the human body. Lactobacilli have a strong track record of safe application in the food industry and as probiotics, and they have the ability to bind and sequester metals. The use of lactobacilli as a tool to reduce the burden of metal exposure is advantageous, as it can be applied almost immediately; there is no requirement for expensive technology or infrastructure setup, as fermentation capability is either already available or easily set up."

3) **"Electron donors for biological sulfate reduction"**

4) **https://en.wikipedia.org/wiki/Electron_donor**

5) **List of high fructose content fruits**

6) **Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems**

7) **Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria**
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127560/

8) **Silver enhances antibiotic activity against gram-negative bacteria.**

9) **Colloidal silver complex as an alternative to sulphur dioxide in winemaking**
https://www.sciencedirect.com/science/article/pii/S095671351100243X

10) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria
https://www.sciencedirect.com/science/article/pii/S0021979704001638

11) Probiotics Reduce Enterohemorrhagic *Escherichia coli* O157:H7- and Enteropathogenic *E. coli* O127:H6-Induced Changes in Polarized T84 Epithelial Cell Monolayers by Reducing Bacterial Adhesion and Cytoskeletal Rearrangements
https://iai.asm.org/content/73/8/5183

"The adhesion of lactobacilli to receptors on surface epithelial cells could compete for binding sites with enteric pathogens. It is also possible that lactic acid-producing bacteria reduce both the viability and the virulence properties of *E. coli* O157:H7 (6) and other diarrheagenic *E. coli* (19). In this study, we have demonstrated that these probiotics affect the virulence of *E. coli* O157:H7 and *E. coli* E2348/69 by factors other than their ability to reduce the pH or produce lactic acid. The ability of these probiotic strains to attenuate the pathogen-induced drop in TER at neutral pH values strongly supports this contention. Several previous reports indicated that factors other than lactic acid produced by probiotics, including bacteriocins, proteinases, peroxides, and exopolysaccharides, could exert antibacterial effects (3, 32)."

"Lactobacilli inhibit adhesion of *E. coli* O157:H7 strain CL-56 and EPEC strain E2348/69 (O127:H7) to epithelial cells."

12) The water purification activity of the Nihonbashi River and Kanda River led to purification of the moat
https://emrojapan.com/case/detail/117

13) Effective Microorganisms® - An Earth Saving Revolution
https://www.teraganix.com/Effective-Microorganisms-History-and-Availability-s/194.htm

14) "The Technology Of Effective Microorganisms – Case Studies of Application"
http://www.futuretechtoday.com/em/sang.htm

15) "Effective microbial consortia to treat wastewater on site"
http://www.chemijournal.com/archives/2018/vol6issue3/PartBA/6-4-166-468.pdf

16) "Phytoremediation of Domestic Wastewater for Reducing Populations of *Escherichia Coli* and MS-2 Coliphage"
https://www.tandfonline.com/doi/abs/10.1080/09593330.2000.9618954

16a) Native to Texas: Cattails https://www.foragingtexas.com/2012/09/cattails.html
https://www.fs.fed.us/database/feis/plants/graminoid/typlat/all.html#MANAGEMENT%20CONSIDERATIONS

"VALUE FOR REHABILITATION OF DISTURBED SITES:
Broadleaf cattail's high wildlife value, potential for erosion control, and tolerance of heavy metals makes it desirable in reclamation or revegetation efforts [176]. Studies found broadleaf cattail grew on "industrially degraded habitats" with heavy metals and high acidity

in western Pennsylvania and in Ontario [150]. Broadleaf cattail also dominated slime ponds 3 years after phosphate mining was discontinued on Florida's central peninsula [17]."

16b) Iris species
https://www.wildflower.org/expert/show.php?id=1771
"There are 26 irises native to North America in our Native Plant Database. Of these, only 4 were shown to be found in Texas. Most of them seemed to favor the West Coast, or cool, wet areas. The four irises tough enough to grow in Texas are *Iris brevicaulis* (zigzag iris), *Iris fulva* (copper iris), *Iris hexagona* (Dixie iris), and *Iris virginica* (Virginia iris)."

17) Principles of Bioremediation
https://www.nap.edu/read/2131/chapter/4#21

18) "Plants and phytoremediation"
https://www.envirosurvey.com/plants-and-phytoremediation/h

19) "The Use of Stropharia rugosoannulata Mycelium for the Mycofiltration of Motor Oil in Water"
https://www.lcps.org/cms/lib/VA01000195/Centricity/Domain/27/1400%20Environmental%20Engineering.pdf

LCPS RSEF OFFICIAL ABSTRACT - Wonkyung Bae, Alanna Hill 2019
Surface runoff of motor oil into surrounding aquatic environments is a growing issue. While traditional methods such as booms and skimmers are often used in cases of large oil spills, little has been done to remediate cases of smaller-scale oil contamination. Mycofiltration is the use of fungal mycelium combined with a substrate, contained in a breathable covering to filter water. Mycofiltration has been most prominently used to remediate bacterially contaminated stormwater. Studies have proven the ability of the fungal species Pleurotus ostreatus to remediate oil in water and Stropharia rugosoannulata to remediate oil soil, but not water. This research will compare the efficacy of two mushroom species, Pleurotus ostreatus, and Stropharia rugosoannulata, to filter oil in water. Mycelium mycofilters were constructed of straw inoculated with S. rugosoannulata and P. ostreatus encased in linen and compared to non-mycelium inoculated filters. Filters were inoculated for two weeks then added to motor-oil contaminated water for four weeks. The efficacy of mycofilters to remediate the water quality was determined using a toxicology study and percent reduction in oil. Mycelium filters improved Danio rerio embryo hatchability rates when compared to non-mycelium filters. Filtered water from Pleurotus ostreatus and Stropharia rugosoannulata exhibited higher hatchability (6.67%), when compared to non-mycelium filters (0%) at 7 days post fertilization, indicating that both mycelium species improved oil-contaminated water quality. Remaining oil mass will be found using the hydrocarbon-binding polymer, EnviroBond. Mycofilters have the potential to be applied as an eco-friendly method of remediating oil in aquatic environments. Stamets, P., Beutel. M., Taylor, A., Flatt, A., Wolff, M., Brownson, K, (2013). Mycofiltration biotechnology for Pathogen management. Taylor, A., Wetzel, J., Mudrock, E., Cameron, J., King, K., Davis, J., & Mcintyre, J. (n.d.). Engineering Analysis of Plant and Fungal Contributions to Bioretention Performance Earth Resources Technologies, under Contract to NOAA, National Marine Fisheries Service. Water, 10. doi:10.3390/w10091226Thomas, S., Becker, P., Pinza, M.R.,,

J.Q. Word, 1998. “Mycoremediation of Aged Petroleum Hydrocarbon Contaminants in Soil.” NASA no. 19990031874.

20) Native Grassland Restoration in the Middle Trinity River Basin https://agrilifeextension.tamu.edu/library/ranching/native-grassland-restoration-in-the-middle-trinity-river-basin/

“Even though native grasslands (also known as tallgrass prairies and savannahs) are characteristic of the ecoregions present within the middle Trinity River basin, today true native grassland is believed to occupy only 1% of the historical land cover (Allen, 2007; Table 1)… Once wide-ranging, native grasslands are highly efficient in providing ecosystem services that benefit humans. These services include flood retention, erosion control, livestock forage, wildlife habitat, water filtration, and soil formation. Native grasslands are important to watershed protection due to the extensive root systems that increase the water holding capacity of the soil, reduce erosion by slowing runoff, and promote groundwater recharge by allowing water to infiltrate the soil more efficiently than many introduced grasses, such as bermudagrass (Cynodon dactylon; Thurow et al. 1986; Schuster, 2001; Teague et al. 2011).” Source: “Native Grassland Restoration in the Middle Trinity River Basin”

21)"MYCOFILTRATION BIOTECHNOLOGY FOR PATHOGEN MANAGEMENT" 2013 Fungi Perfecti, LLCPaul Stamets, Marc Beutel, PhD, Alex Taylor, Alicia Flatt, Morgan Wolff, Katie Brownson: https://assets.fungiperfecti.net/pdf/articles/Fungi_Perfecti_Phase_I_Report.pdf

22)"Filters that contain fungi with powerful antibiotic properties can help remove harmful bacteria from water.": https://www.yesmagazine.org/planet/portland-s-e-coli-scare-how-mushrooms-could-have-helped-prevent-it

23)"Pollan finally finds out that it was the bacteria produced by the wood itself that kept her original cheese E. coli-free.": https://www.slowfood.com/cheese-biodiversity-war-bacteria/

24) Application of EM•1® (Effective Microorganisms®) for Treatment of Diarrheic Disease in Piglets in Vietnam: https://www.teraganix.com/category-s/1189.htm

Sample Data from Dallas Water and Utilities
AgrSubject: RE: Sample data
Date: 2019-06-19 04:44
From: "Pasley, Jonathan R" <jonathan.pasley@dallascityhall.com>
To: "virginia@waterisalive.org" <virginia@waterisalive.org>

Good Morning Ms. Kilgore,

Attached you will find the bacteria and field results for the 3 sampling events we conducted. Samples were collected to track results between dry and wet periods. The results are as follows:

April 5th 2019
Samples taken at Gas Monkey location
E. Coli: 2,851 MPN/100mL

April 8th 2019
Same location (post rain event the night before)
E. Coli: 15,531 MPN/100mL

April 22nd 2019
3 locations
2551 Lombardy (E. Coli: 256 MPN/100mL)
Gas Monkey (E. Coli: 134 MPN/100mL)
2395 Stemmons Trail (E. Coli: 327 MPN/100mL)

As of right now, this is all the information we have for this location.
Hopefully this gives you something to start with.
Regards,
JON PASLEY
_ Environmental Supervisor – WET Team_
CITY OF DALLAS | DALLASCITYNEWS.NET
Dallas Water Utilities

Sample data from Groundwork Dallas on behalf of the Texas Stream Team
2019-06-26 12:40
Hi Virginia,
I am attaching all the data sheets we have for Joe's creek for your reference. Below you will find the e.coli and total coliform data for easier reference:
Station ID: 81460 - Joe's Creek @Justice Way
08/28/2018
- E.coli Average: 158 cfu/100mL
- Total Coliform: 12,543 cfu/100mL

09/27/2018
- E.coli Average: 1,698 cfu/100mL
- Total Coliform: N/A

04/23/2019
- E.coli Average: 5,960 cfu/100mL
- Total Coliform: 46,775 cfu/100mL

05/17/2019
- E.coli Average: 421.5 cfu/100mL
- Total Coliform: 6,900.5 cfu/100mL

6/26/2019
- E.coli Average: 159.5 cfu/100Ml
- Total Coliform: 3,183 cfu/100mL

Hope this is helpful!

Liomari Diaz-Martinez, LEED Green Associate

Green Team Program Coordinator
Groundwork Dallas | GroundworkDallas.org
3001 Quebec, STE 201
Dallas, Texas 75247
C: 817.983.8271
O: 469.859.4705
Liomari@GroundworkDallas.org

13 data sample sheets are available from The Texas Stream Team on monitoring at the Gas Monkey Live location and will be included in the permit request.

Texas Parks and Wildlife Department Comments on concerns about non-native species
Below is the response from our TPWD permit coordinator Monica McGarity who is very knowledgeable about non-native species. From her summary, there is no regulation or permit that pertains to use the materials you suggested. Thus the decision is yours as to whether you use the non-native species or not. However since they are non-native species, it is suggested that more work be done to determine a native species that would work just as good for the biofilters.
Thanks.
Raphael Brock
Texas Parks and Wildlife Department
District Fisheries Biologist
6200 Hatchery Rd
Fort Worth, TX 76114
817-732-0761 - Office
817-233-5792 - Cell

-----Original Message-----
From: Monica McGarrity
Sent: Thursday, June 20, 2019 12:21 PM
To: Raphael Brock <Raphael.Brock@tpwd.texas.gov>; Brian VanZee <Brian.VanZee@tpwd.texas.gov>; Cynthia Fox <Cynthia.Fox@tpwd.texas.gov>
Cc: IF permits <IFpermits@tpwd.texas.gov>
Subject: RE: Trinity River Bioremediation Pilot Plan

Thanks for sending this to me--I'm very familiar with all of our TPWD regulations as well as federal AIS laws and am happy to help.

Fungi aren't regulated under TPWD exotic species regulations, as those apply only to fish/shellfish/aquatic plants. The only federal permits for non-native species that I'm aware of that could apply--as these aren't animals--would be USDA-APHIS PPQ permits for import or interstate transit of organisms. Their best course of action to determine whether such a permit would be needed would be to contact APHIS directly--they do have an online system where folks can search for FAQ answers and submit new questions.
https://www.usda.gov/ask-expert

Fungi also aren't be regulated under TPWD regulations pertaining to introduction into public waters as those also apply only to fish/shellfish/aquatic plants.

All of the invasive fungi I'm familiar with are pathogens--and of course those are the most high-profile non-native fungi. However, that's not to say that this isn't at all a concern. Fragments of mycelia could break off and escape and potentially invade natural habitats. Edible fungi have been introduced in some areas and escaped into others and, while I'm not aware of any documented impacts, it is hypothesized that introgression with native species like the Texas Pleuronotus could reduce their genetic variation and fitness.

Given that there is a native, closely related fungus species, experimenting with the use of that species FIRST seems to be the most ecologically responsible course of action. However, doing so isn't a requirement under our regulations.
Regards,

Monica E. McGarrity

Senior Scientist for Aquatic Invasive Species Management Inland Fisheries Division – Habitat Conservation Branch Texas Parks and Wildlife Department
4200 Smith School Rd., Austin, TX 78744
Cell: 512-552-3465

-----Original Message-----
From: Raphael Brock
Sent: Thursday, June 20, 2019 10:27 AM
To: Brian VanZee <Brian.VanZee@tpwd.texas.gov>; Cynthia Fox <Cynthia.Fox@tpwd.texas.gov>
Cc: Monica McGarrity <Monica.Mcgarrity@tpwd.texas.gov>
Subject: FW: Trinity River Bioremediation Pilot Plan

I received the information below from this group last week after a lengthy telephone conversation. I haven't reviewed or commented on the proposal yet but they were planning on using biofilters composed of organic material like wood chips and some species of mushrooms to lower E.coli levels of Joe's Creek which is a tributary of the Trinity River near Bachman Lake. She is basically wanting input in regards to making sure the materials she is using is appropriate and if she needs any permits for the materials she will be using from TPWD. The species of mushrooms she is using are non-native. She states this in the "Concerns" sections below (Concerns: The use of non-native species. Various species of mushrooms, Pleurotus and Stropharia, will initially be applied, these are not native to Texas. Indigenous species in the Pleurotus exist in Texas and will be collected and attempts to train these local species to metabolize typical contaminants found in the Trinity River will be made.)

I have included Monica in the email also as she has more knowledge of any permits she will need from us.

Raphael Brock
Texas Parks and Wildlife Department
District Fisheries Biologist
817-732-0761 - Office

817-233-5792 - Cell

Texas Commission on Environmental Quality Comments Thank you for reminding me about the data. I knew I was forgetting something. Unfortunately, TCEQ does not have any surface water quality monitoring data associated with Joes Creek. Most data we have is for the Elm Fork Trinity River Below Lewisville Lake (Segment 0822).

Please visit the TCEQ Surface Water Quality Viewer https://www.tceq.texas.gov/gis/segments-viewer for information.
Dania On 2019-06-20 03:25, Dania Grundmann wrote: Ms. Kilgore,

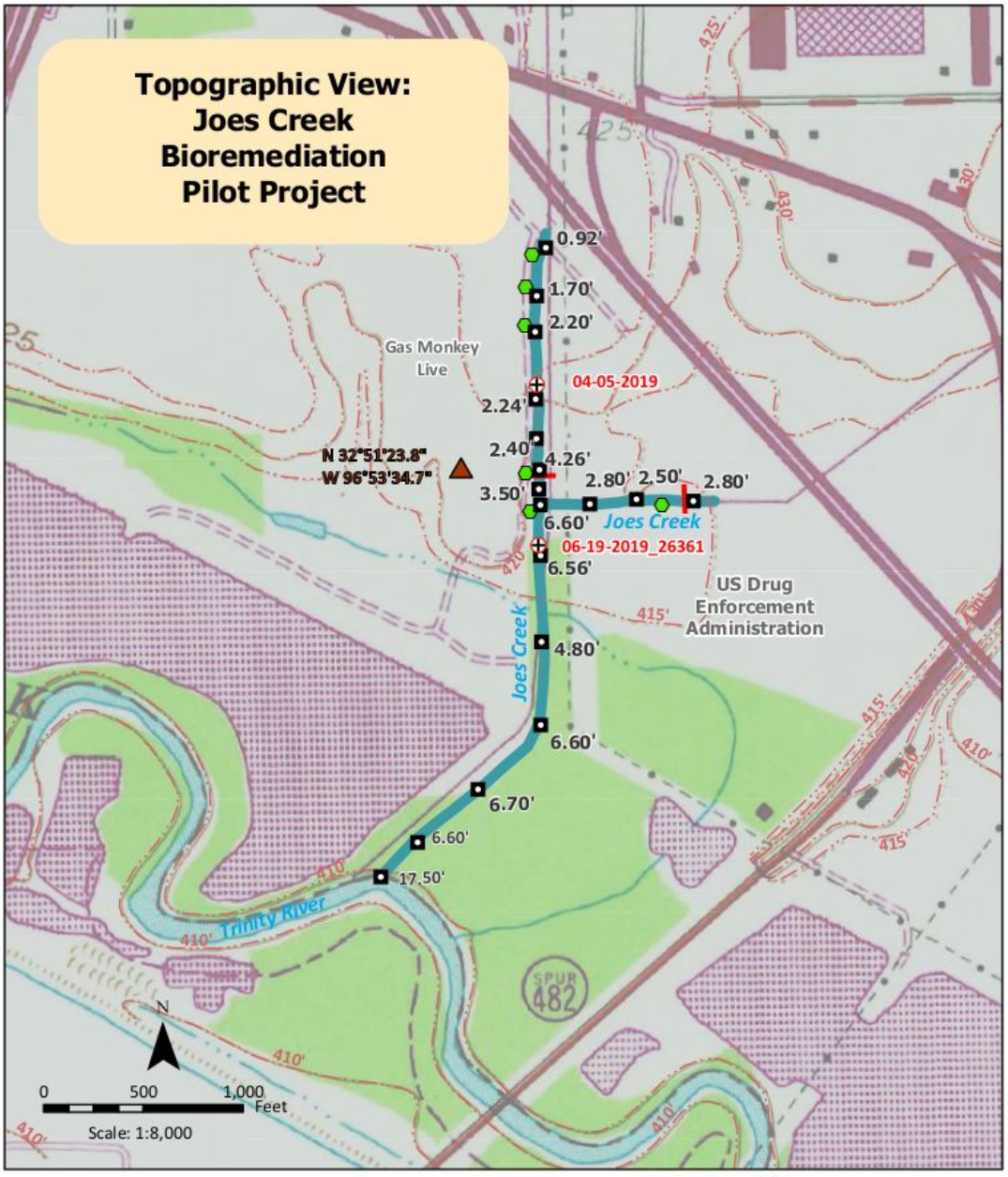
I enjoyed talking to you earlier this week about the bioremediation project on Joes Creek. Although I cannot provide too much assistance regarding your project we do have some ongoing projects that you may be of interest to you and our organization.

TCEQ works with NCTCOG to implement the bacteria TMDLs in the Greater Trinity River region. The web page for the group is located at https://www.nctcog.org/envir/natural-resources/tmdl

TCEQ also works with NCTCOG to hold semi-annual meetings to discuss approaches like WPPs or TMDLs to address bacteria issues in the Upper Trinity Basin. This web page is located at https://www.nctcog.org/envir/natural-resources/tmdl and a meeting is coming up in August. You will find meeting presentations and other information related to WPPs in the region that you may find helpful.

Best Regards,

Dania Grundmann | Project Manager, TMDL Program
Texas Commission on Environmental Quality Water Quality Planning Division



WATER IS ALIVE

This product is for informational purposes and may not have been prepared for or be suitable for legal, engineering, or surveying purposes. Portions of the information are derived from on on the ground survey conducted on July 28th, 2019 and represents only the approximate relative location of features.
Date Exported: 10/6/2019

Data Sources

Base map: USGS Irving, TX 1959 (1982) 1:24,000

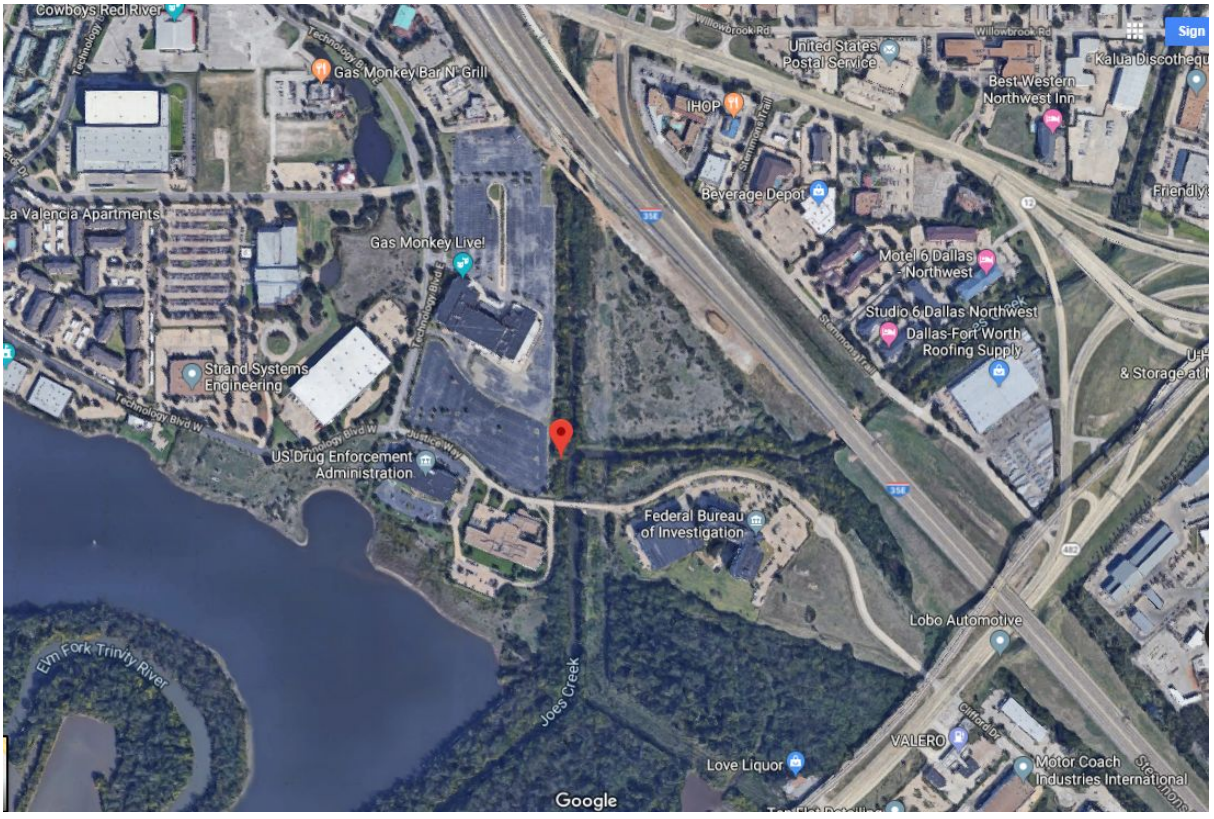
Contours: Strategic Mapping Program (StratMap). StratMap Hypso, 1958-02-01. Web. 2019-10-06.

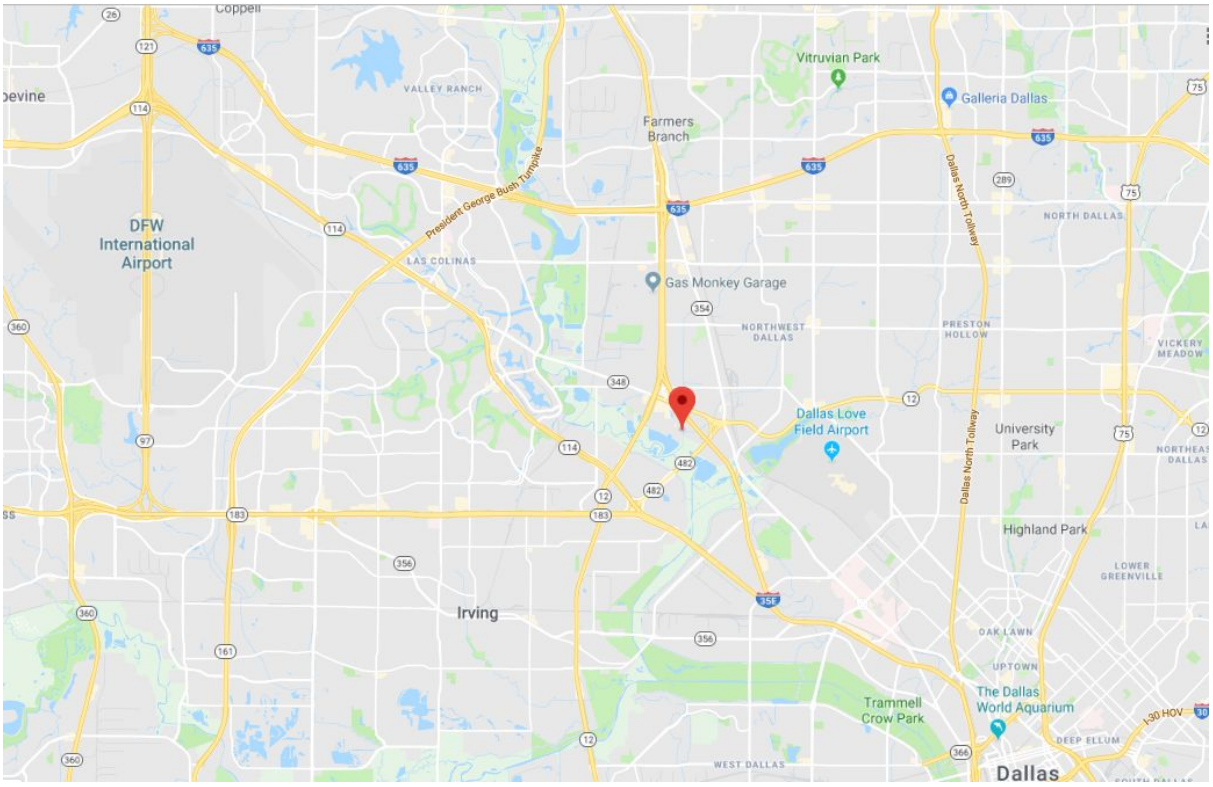
Survey Data: Garmin GPS (Horizontal)
Philadelphia Rod (Water Depths)
Date of Survey July 28th, 2019

Spatial Reference
PCS: NAD 1983 2011 StatePlane Texas North Central FIPS 4202 FtUS
Projection: Lambert Conformal Conic

- Storm Water Drains
- Water Quality Samples
- Water Depth Samples (6.70' - Water Depth)
- Sewer Pipeline Crossings
- Contours (5' Intervals)
- Joes Creek


Joes Creek
Justice Way


Cowboys Red River
Gas Monkey Bar N' Grill
United States Postal Service
Willowbrook Rd
Best Western Northwest Inn
IHOP
Beverage Depot
La Valencia Apartments
Gas Monkey Live!
Motel 6 Dallas Northwest
Studio 6 Dallas Northwest
Dallas-Fort Worth Roofing Supply
Strand Systems Engineering
Technology Blvd W
US Drug Enforcement Administration
Justice Way
Federal Bureau of Investigation
Lobo Automotive
Elm Fork Trinity River
Joes Creek
VALERO
Love Liquor
Motor Coach Industries International
Google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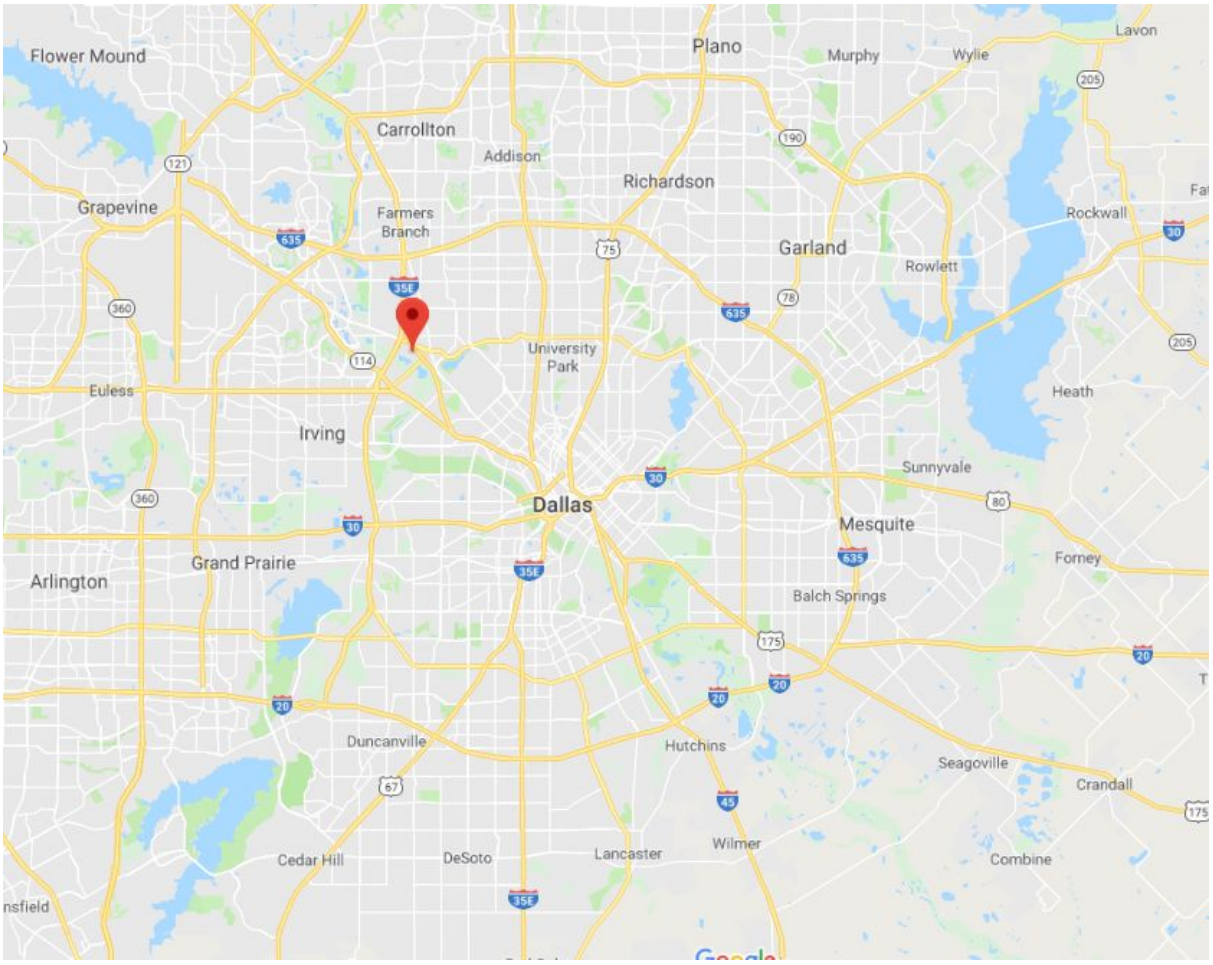
Vitruvian Park
Galleria Dallas
VALLEY RANCH
Farmers Branch
DFW International Airport
President George Bush Turnpike
Dallas North Tollway
NORTH DALLAS
LAS COLINAS
Gas Monkey Garage
NORTHWEST DALLAS
PRESTON HOLLOW
VICKERY MEADOW
Dallas Love Field Airport
University Park
Highland Park
LOWER GREENVILLE
Irving
OAK LAWN
UPTOWN
Trammell Crow Park
The Dallas World Aquarium
DEEP ELLUM
WEST DALLAS
Dallas


Flower Mound
Plano
Murphy
Wylie
Lavon
Carrollton
Addison
Richardson
Grapevine
Farmers Branch
Garland
Rowlett
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Euless
Heath
Irving
Sunnyvale
Dallas
Mesquite
Grand Prairie
Arlington
Forney
Balch Springs
Duncanville
Hutchins
Seagoville
Crandall
Cedar Hill
DeSoto
Lancaster
Wilmer
Combine

Commercial Account #00649800000000100

Location (Current 2020)

Address: 2301 N STEMMONS FWY
Market Area: 0
Mapsco: 32-D (DALLAS)

DCAD Property Map

View Photo

Electronic Documents (ENS)

Print Homestead Exemption Form

Owner (Current 2020)

DALLAS CITY OF
1500 MARILLA ST
DALLAS, TEXAS 752016318

Multi-Owner (Current 2020)

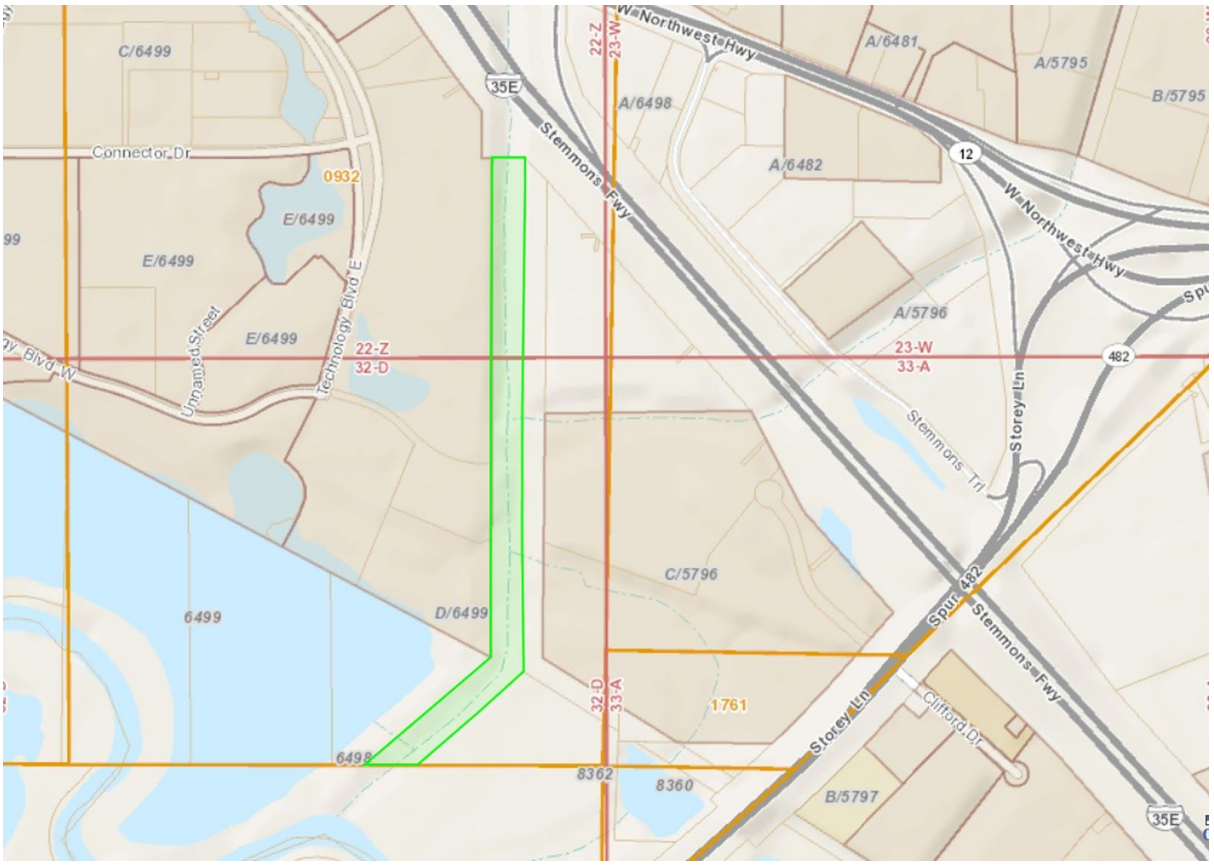
Owner Name	Ownership %
DALLAS CITY OF	100%

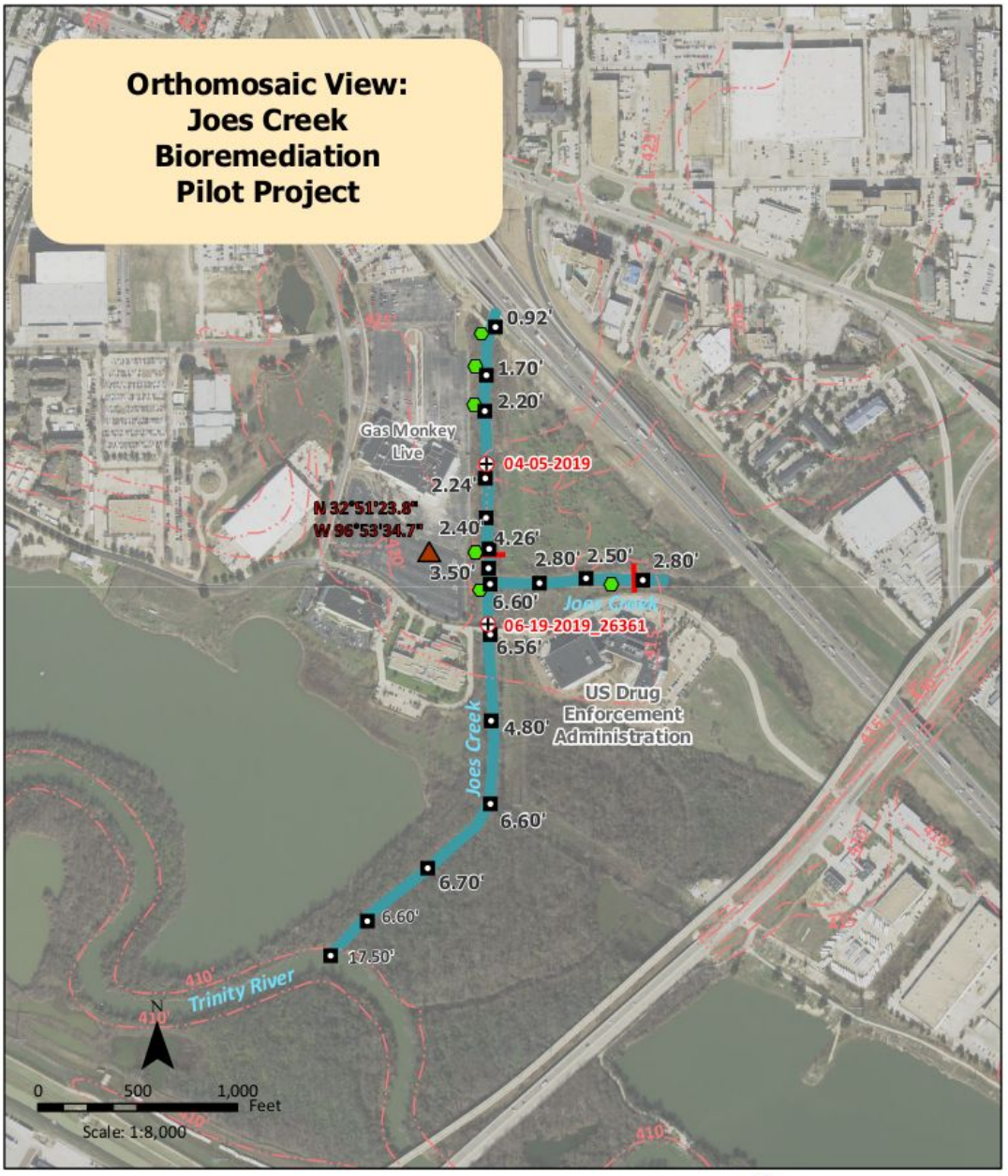
Legal Desc (Current 2020)

1: BLK 6498
2: SPLIT 1 ACS 11.049
3: DEAD ENDS INTO NATURE CENTER
4: VOL5415/243 DD09221960 CO-DC
5: 6498 000 000 2DA6498 000
Deed Transfer Date: 11/11/1900

Value

2019 Certified Values	
Improvement:	N/A
Land:	+ N/A
Market Value:	=N/A
Revaluation Year:	N/A
Previous Revaluation Year:	N/A





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Philadelphia Rod (Water Depths)
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- Storm Water Drains
- Water Quality Samples
- Water Depth Samples (6.70' - Water Depth)
- Sewer Pipeline Crossings
- Contours (5' Intervals)
- Joes Creek

YouTube address to the video of the Joe's Creek Bioremediation Pilot Plan Survey:

https://www.youtube.com/watch?v=ifK1OsE_rqI&feature=youtu.be

Thank you for your time. We count on your support. Water Is Alive has an accompanying curriculum for schools and interested groups to learn how to make these bio-filters. It is a good time to practice sustainability and clean up our waters. And teach others how to do the same.

Health and Happiness,

Virginia Kilgore
Founder of Water Is Alive
virginia@waterisalive.org