EVALUATION OF THE EFFECT OF MICROBIAL ACID ATTACK ON CONCRETE BRIDGES IN TEXAS

THESIS

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FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Dedicated to my mother,

Teresa Estrada

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1.0 Introduction

The Texas Department of Transportation (TxDot) oversees several thousand Texas roadways and bridges (Trejo et al., 2008). With headquarters located in Austin, TxDot is responsible for providing safe, reliable, and cost effective infrastructures for Texans (Trejo et al., 2008). Recently, numerous East Texas concrete columns associated with East Texas bridges have been identified as having surface deterioration (Trejo et al., 2008). Aside from safety concerns it is of interest to investigate the factors that contribute to column deterioration because replacing these bridges is a lengthy and costly process (Trejo et al., 2008).

There are several factors that contribute and accelerate concrete degradation (Trejo et al., 2008). Previous research shows that concrete degradation can be accelerated by mechanical, chemical, and microbial factors. Factors that contribute to and or accelerate concrete degradation include freeze thaw, corrosion, cracking of concrete, initial composition, and an alkali-silica reaction (Belie et al., 1996; Tittleboom et al., 2010). Cracks present in the concrete column can expand from freeze thaw cycles leading to further expansion of existing channels creating a path for chemicals that can degrade the cement paste and reinforcement bar (Idiart et al., 2011; Tittelboom et al., 2010; Trejo et al., 1998). In addition, reactions such as the alkali-silica and alkali-carbonate reactions can also lead to further expansion (Kosmatka et al., 2002). This type of degradation occurs when the minerals present in the aggregate react with alkali hydroxides (Kosmatka et al., 2002). Although these reactions are uncommon and the process is prolonged, they are important because they can lead to further expansion of the concrete (Kosmatka et al., 2002). The initial concrete composition is also important especially when the concrete is subject to extreme environmental conditions (Kosmatka et al., 2002). For example, concrete bridges that are exposed to sea water must have optimum mix designs and low permeability in order to resist sulfate and chloride damage to both the paste and reinforcement bar (Kosmatka et al., 2002).

There are several species of microbes that can contribute to the degradation of concrete (Belie et al., 2004). Under anaerobic conditions, sulfur reducing bacteria produce hydrogen sulfide which then provides an ideal environment for sulfur oxidizing bacteria (Belie et al., 2004; Okabe et al., 2007; Satoh et al., 2009; Yamanaka et al., 2002). The end product of the synergism of these two organism types is hydrated sulfur trioxide that lowers the pH of the concrete and provides optimum environmental conditions for other organisms (Belie et al., 2004). Microbial induced concrete corrosion involves a complex ecosystem of organisms where end products are sulfuric acid (Belie et al., 2004). For example, the colonizing organism *Desulfovibrio* can reduce sulfur components into hydrogen sulfide thereby providing an ideal environment for other organisms that have been known to be associated with concrete degradation which include *Thiobacillus neapolitanus*, *Thiobacillus thiooxidans*, and *Thiobacillus ferrooxidans* (Belie et al., 2004; O'Connell et al., 2010; Okabe, 2007; Sand, 1987). Previous studies have shown that heavily degraded concrete has elevated populations of *Thiobacilli* species while noncorroded concrete has a lower population (Kulpa and Baker, 1990; Sand, 1987). *Thiobacillus neapolitanus* requires a pH within the range of 7.0 to 4.0 in order to grow by obtaining sufficient energy from the oxidation of elemental sulfur, hydrogen sulfide, and thiosulfate (Kulpa and Baker, 1990; Okabe et al., 2007). An acidic environment also provides the necessary pH for *Thiobacillus thiooxidans* which uses the same energy sources as the previous organism (Kulpa and Baker, 1990; Okabe et al., 2007). *Thiobacillus ferrooxidans* is also capable of oxidizing elemental sulfur, hydrogen sulfide, and iron which is especially important as this organism can oxidize iron on the reinforcement bar and cause further damage to the durability of concrete (Kulpa and Baker, 1990). Production of sulfuric acid can react with calcium hydroxide in the concrete to yield gypsum and ettringite (Belie et al., 2004). Gypsum formed on the surface of concrete can induce stress thereby causing cracking (Bassuoni and Nehdi, 2007). In addition, ettringite is formed when gypsum reacts with calcium aluminate intermediates in the cementitious material thereby causing further cracking of the concrete (Bassuoni and Nehdi, 2007). The loss of concrete strength occurs when the sulfuric acid reacts with calcium silicate hydrate causing decalcification (Bassuoni and Nehdi, 2007; Malhotra and Carino, 2004). Concrete corrosion due to sulfur producing sulfur oxidizing bacteria reaction is summarized below (Bassuoni and Nehdi, 2007).

$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4 \bullet 2H_2O$$

$$CaSiO_2 \bullet 2H_2O + H_2SO_4 \rightarrow CaSO_4 + Si(OH)_4 + H_2O$$

$$3CaO \bullet Al_2O_3 \bullet 12H_2O + 3(CaSO_4 \bullet 2H_2O) + 14\ H_2O \rightarrow 3CaO \bullet Al_2O_3 \bullet 3CaSO_4 \bullet 32H_2O$$

Therefore it is of interest to identify these species and assess microbial populations as well as determine the pH of concrete pillars (Okabe et al., 2007; Satoh et al., 2009).

Fluorescence in situ hybridization (FISH) is a technique that utilizes a fluorescently labeled rRNA targeted oligonucleotide probe to quantitate microbial communities in an environment (Hahn et al., 1992). The methodology of FISH involves using a biological marker that is inserted into a specific area of DNA (Hahn et al., 1993). Fluorescently labeled rRNA targeted oligonucleotide probes will serve as indicators as to which microbes are present by using stains and molecular probes (Hahn et al., 1992). The stain 4′, 6-diamidino-2-phenylindole (Dapi) intercalates into DNA and thus detects all organisms present while the probe Cy3 is specific for the target organisms which are bound to the rRNA targeted oligonucleotides (Hahn et al., 1992). Once the probe is bound to complementary single stranded DNA it can be visualized by epimicroscopy (Hahn et al., 1992).

In order to assess the factors that contribute to concrete degradation and the impact of microbes and the impact of sulfate and chloride ions on concrete pillars will be determined. Sulfate and chloride are both ions that can be easily detected with ion chromatography (Cunico et al., 1998; Fritz and Gjerde, 2000). Ion chromatography is a separation method which uses a charged stationary phase for the separation of particular ions of interest (Cunico et al., 1998; Fritz and Gjerde, 2000). Separation occurs due to the positively charged functional groups located on the stationary phase. Ions with higher negative charge will have a stronger affinity for the stationary phase than ions having a single negative charge (Cunico et al., 1998; Fritz and

Gjerde, 2000). For example, if an anion column is used for the separation of a mixture containing sulfates, chloride, and sodium, the sodium ions will be eluted first from the column due to positive charge repulsion from the stationary phase, chloride ion will be eluted second and the sulfate ion will be eluted last due to a higher charge (Cunico et al., 1998). These ions can then be characterized by a conductivity detector (Fritz and Gjerde, 2000).

In addition to chemical and biological assessment of concrete it is also important to determine the level of deterioration of concrete. This can be done both visually and quantitatively (Biczók, 1967). Visual inspection of the concrete specimen of interest and determination of chemical composition, changes in concrete strength, and changes in length and volume are a few ways that can be done (Biczók, 1967; Kosmatka et al., 2002). The compressive strength test for hardened concrete is a common method that can be used to assess the extent of concrete deterioration. A number of protocols are commonly employed (Biczók, 1967; Kosmatka et al., 2002). The first protocol involves utilizing cured specimens that have been molded according to ASTM C31 or C192 (Kosmatka et al., 2002). A second protocol utilizes hardened concrete that has been prepared according to ASTM C42 and is used for in situ testing (Kosmatka et al., 2002). Finally, testing can be conducted on specimens made from cast in place cylinder molds utilizing ASTM C873 (Kosmatka et al., 2002). Cast in place cylinders are prepared during the normal preparation of concrete; once the concrete cures a section is then removed (Kosmatka et al., 2002). The length and diameter of the cylinders must be at least three times as much as the course aggregate in the concrete (Kosmatka et al., 2002). In addition, concrete samples should be prepared according to ASTM C617/C1231 in order to avoid additional influences from the ends of the cores (Kosmatka et al., 2002). This can be avoided by grounding or capping the ends of the core samples (Kosmatka et al., 2002). The compressive strength test is done in order to determine concrete quality and degree of corrosion (Biczók, 1967; Kosmatka et al., 2002).

The purpose of this investigation was to determine the chemical and microbial factors that contribute to or accelerate the degradation of concrete columns associated with Texas bridges. As the concrete column undergoes chemical and microbial attack the end result weakens the strength of the column which provides support for the bridge. Various concrete engineering, microbiological, and analytical methods will be exploited in order to determine the extent of concrete degradation. Several samples including water samples, concrete scrapings, and core samples were collected at numerous bridges identified by the TxDot.

2.0 Experimental

2.0.1 Field sampling

Several locations were identified by the Texas Department of Transportation as areas of interest for this investigation. All the sites had bridges spanning waterways and there was a concern that some of the bridge pillars may have lost their integrity. Figure 1 illustrates the general location of all of the bridges.

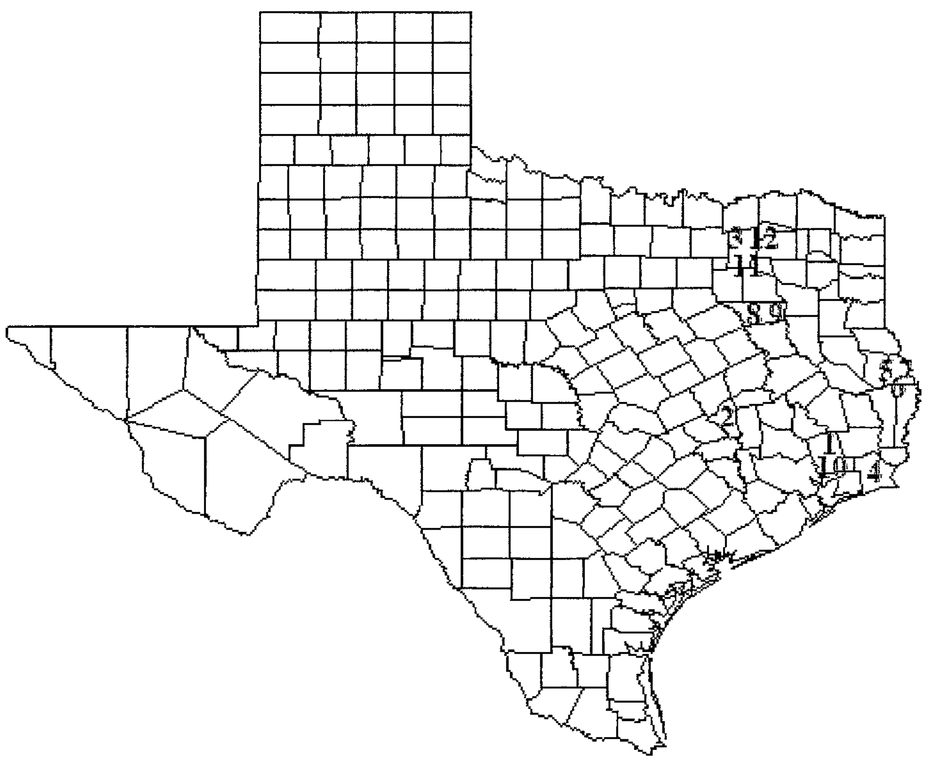

Figure 1: Locations of the bridges

2.0.1.1 Tarkington Bayou (Liberty County)

The first bridge is located on FM 787 at Tarkington Bayou (Liberty County). The overall bridge site has low deterioration. Two water samples were obtained with one underneath the bridge and the second 100 yards upstream from the bridge. Concrete scrapings were taken on three different columns at various elevations from the ground. On the first column samples were taken at 6 inches and 22 inches from the ground; the second column, a sample was taken at 20 inches; and on the third column, a sample was taken at zero inches from the ground. This bridge site was later revisited and two water samples were collected, one near the bridge and the second upstream from the bridge. A mud sample was also collected near the bridge. Concrete scrapings were obtained from wall 1 and column 1 at various elevations from the ground. The first set of concrete scrapings was obtained from bridge 1 span wall 1 at 6, 18, and 42 inches from the ground. The second set of concrete scrapings was obtained from bridge 1 span 1 column 1 at 6, 18, and 42 inches from the ground. Figure 2 shows a picture taken from the west side of the bridge, there are two spans labeled 1 and 2. Each span consists of a row of seven columns. Columns were identified from the north to south and spans were identified from the west to east. Samples from other bridge locations following the same identification convention mentioned above. All surface core samples were obtained by TxDot personnel. Core samples were extracted from Tarkington Bayou, Navasota River, Lake Tawakoni, and Alligator Bayou using a HILTI DD 120 Diamond Coring System with 1.5 inch core bit. The first set of cores obtained from the initial visit at Tarkington Bayou was extracted from the southwest end of a 1970's concrete precast limestone aggregate column at 6 and 22 inches from the ground. The second set of cores was extracted from a 1970's west end expansion site cast wall at 20, 27, and 34 inches from the ground. The third set of cores was extracted from a 1930's west end middle span section concrete column taken at 21, 28, and 38 inches from the ground. The final set of cores was taken from a 1970's precast limestone aggregate on the southwest side of the column at 10, 14, and 26 inches from the ground. Samples collected from the 1970's concrete were extended based on the original bridge which was built in 1930's. Table 1 summarizes sampling and bridge site information.

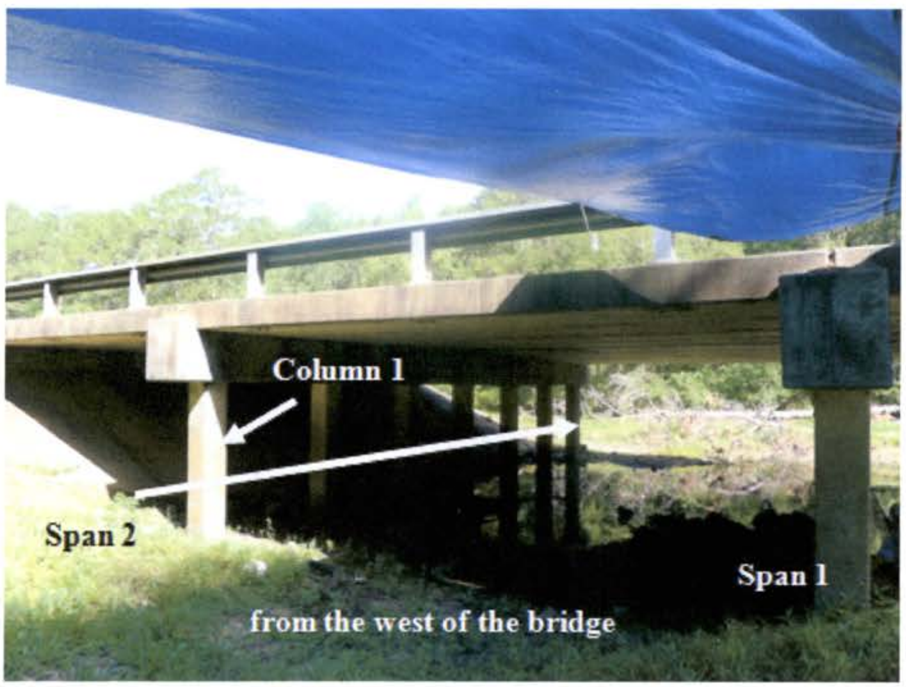

Figure 2: Column and span naming convention for FM 787 at Tarkington Bayou (Liberty County)

Table 1: Date of visit, general and sampling information for FM 787 at Tarkington Bayou (Liberty County)

Date of Visit	11/5/2009
General Information	Built in 1930, widening 1973; overall bridge site has low deterioration
Visual Inspection	Condition of the bridge appears to be good
Chemical analysis sampling information	Water 1 and 2 were collected next to the bridge, water 3 collected upstream Concrete scrapings: column 1 at 6 and 22 inches from the ground, column 2 at 20 inches from the ground, and column 3 at 0 inches from the ground
Chemical analysis sampling information revisit	Water 1 collected next to the bridge and water 2 was collected upstream from the bridge One mud sample was collected Concrete scrapings: wall 1 at 6, 18, and 42 inches from the ground and on column 1 at 6, 18, and 42 inches from the ground
Core sampling information	First set of cores: 6 and 22 inches Second set of cores: 20, 27, and 34 inches Third set of core: 21, 28, and 38 inches

2.0.1.2 Navasota River (Robertson-Bryan District)

Figure 3, shows the level of deterioration of the second bridge located on SH 21 at the Navasota River (Robertson-Bryan District). A visual inspection of a precast column indicated no apparent deterioration, paste appeared to have decreased, and the bottom of the wall span appeared to have been repaired. Water samples were collected at the north and south end of the bridge. A mud sample was also collected next to the column on the south side of the bridge. Concrete scrapings were taken on the north and south end of the bridge at various elevations. On the first column, scrapings were taken at 36 inches; on the second column, concrete scrapings were taken at 12 inches; for the third column, scrapings were taken at 36 inches; and on the fourth column, concrete scrapings were taken at zero inches. Core samples were extracted from three columns at various elevations from the ground. The first sets of concrete cores were extracted from column 1, close to the center wall span, heights are unknown. The second set of concrete cores was extracted from the center of the wall span of column 2 at 0, 18 and 32 inches. The last set was extracted away from the center wall span from column 3, heights are unknown. A summary of all samples collected and general bridge site information is presented in Table 2.

Figure 3: Overview of the level of deterioration at SH 21 at Navasota River (Robertson-Bryan District)

Table 2: Date of visit, general and sampling information for SH 21 at Navasota River (Robertson-Bryan District)

Date of Visit	11/19/2009
General Information	A visual inspection of a precast column indicated no apparent deterioration, paste appeared to have decreased, and the bottom of the wall span appeared to have been repaired.
Visual Inspection	Deterioration level is low
Chemical analysis sampling information	Three water samples were collected, mud sample was collected on the south side of the bridge; concrete scrapings were taken from wall 1 at 6, 18, and 42 inches and column 1 at 6, 18, and 42 inches from the ground
Core sampling information	First set of cores: elevations unknown Second set of cores: 0, 18 and 32 inches from the ground Third set of cores: elevations unknown

2.0.1.3 Lake Tawakoni (Hunt County)

The third bridge was located at SH 276 at Lake Tawakoni (Hunt County) and was built in 1959. Samples were taken from column 131; this column was labeled with the letter T. The surface of the column closest to the water line appeared to have medium level deterioration. Two water samples were collected next to the bridge and two concrete scrapings were obtained at 6 and 24 inches from the water. Six core samples were extracted from column 131 at various elevations. The first corroded core sample was extracted from the northwest end of column 131 at 6 inches. The second semi corroded sample was extracted from the northwest end of column 131 at 24 inches from the ground. The third corroded core sample was taken from the southeast side of column 131 at 6 inches from the ground. The fourth semi corroded core sample was taken from the southeast side of column 131 at 24 inches from the ground. The fifth non corroded core sample was taken from the southeast side of column 131 at 4 centimeters from the ground. The sixth non corroded core sample was taken from the southeast end of column 131 at 5 centimeters from the ground. Table 3 presents a summary of sampling information for SH 276 at Lake Tawakoni (Hunt County).

Table 3: Date of visit, general and sampling information SH 276 at Lake Tawakoni (Hunt County)

Date of Visit	12/10/2009
General Information	Built in 1959; samples were taken from column 131
Visual Inspection	The surface of the column closest to the water line appeared to have medium level deterioration
Chemical analysis sampling information	Two water samples were collected next to the bridge Concrete scrapings were taken from column 3 at 6 and 24 inches from the ground
Core sampling sample information	All cores were extracted from column 131 Northwest, corroded 6 inches and semi corroded 24 inches Southeast, corroded at 6 inches and semi corroded 24 inches Southeast, non corroded at 4 and 5 feet

2.0.1.4 Alligator Bayou (Jefferson County)

Figure 4 shows the level of deterioration of the fourth bridge located on SH 82 at Alligator Bayou (Jefferson County) and built in 1952. Severe deterioration was evident on this bridge as the main reinforcement was exposed. There was surface deterioration and rebar corrosion; however, the concrete cores samples that were extracted appeared to be in good condition. Two water samples and two mud samples were obtained at this site. The first mud sample was obtained from underwater underneath span 1, and the second mud sample was obtained from the bank. Four concrete scraping samples were also obtained. The first sample was from span 0, 34 inches above the ground on column 5. This was a non corroded concrete sample. The second sample was a surface organic sample and was obtained from span 1, column 3 at 6 inches below the water level. The third sample was a corroded concrete sample obtained from span 1, column 6 at 6 inches above the water level. The last sample was a corroded concrete sample obtained from span 1, column 5 at 3 inches above the water line.

Eight core samples were taken from two columns: column 6 span 1 and column 3 span 1. The first set of core samples was extracted from column 6 span 1 at 34, 45, and 85 inches from the ground. The second set of core samples was extracted from column 3 span 1 at 4, 8, and 28 inches from the ground. The last set of core samples was extracted from column 3 (closest to the ground) span 1 at 16 and 40 inches from the ground. Table 4 presents a summary of sampling information for SH 82 at Alligator Bayou (Jefferson County).

Table 4: Date of visit, general and sampling information SH 82 at Alligator Bayou (Jefferson County)

Date of Visit	4/30/2010
General Information	Built in 1952
Visual Inspection	Bridge site showed severe deterioration with surface deterioration, exposed main reinforcement, and rebar corrosion.
Chemical analysis sampling information	Two sets of water and mud samples were collected Concrete scrapings were taken column 3 6 inches below the water level, column 5 3 and 34 inches above, and column 6 at 6 inches above
Core sampling information	First set of cores: column 6 span 1 at 34, 45, and 85 inches from the ground The second set of cores: column 3 span 1 at 4, 8, and 28 inches from the ground Third set of cores: column 3 span 1 at 16 and 40 inches from the ground.

Figure 4: Overview of the level of deterioration at SH 82 at Alligator Bayou (Jefferson County)

2.0.1.5 Patroon Bayou (Sabine County)

The fifth bridge of interest was located at FM 276 at Patroon Bayou (Sabine County) and built in 1967. The bridge appears to have deterioration reaching approximately 8 feet from the bottom of the pier. The bridge has exposed aggregates and is highly scaled. The water level is low and the foundation is exposed. From 0-60 inches from the bottom of the pier the column is corroded, from 60-68 inches from the bottom of the pier the column is semi corroded, and above 68 inches the column is non corroded. Two water samples were obtained, one from the west side of the bridge and the other from the east side of the bridge. Two mud samples were obtained, one from the west and the second from the east side of the bridge. Concrete scrapings were obtained from two columns at various elevations from the ground. For column 1, concrete scrapings were obtained at 12 and 72 inches from the ground; and for column 2, concrete scrapings were obtained at 24 and 65 inches from the ground. Table 5 presents a summary of bridge site information for FM 276 at Patroon Bayou (Sabine County).

Table 5: Date of visit, general and sampling information for FM 276 at Patroon Bayou (Sabine County)

Date of Visit	12/3/2010
General Information	Built 1967
Visual Inspection	The bridge appears to have deterioration reaching approximately 8 feet from the bottom of the pier. The bridge has exposed aggregates and is highly scaled. The water level is low and the foundation is exposed. From 0-60 inches from the bottom of the pier the column is corroded, from 60-68 inches from the bottom of the pier the column is semi corroded, and above 68 inches the column is non corroded.
Sampling information	Two sets of water and mud samples were obtained from the west and east side of the bridge Concrete scrapings were taken from column 1 at 12 and 72 inches from the ground and the second set of samples were taken from column 2 at 24 and 65 inches from the ground

2.0.1.6 Carrice Creek (Sabine County)

A sixth bridge was located at SH 21 at Carrice Creek (Sabine County) and built in 1967. It is a small bridge with four spans having high levels of deterioration. On column 3, the rebar is exposed and corroded while additional corrosion is observed on the south end of the bridge. There is the possibility of poor construction on this bridge site because there is not enough mortar covering the original concrete. Two water and two mud samples were obtained on the west and east side of the bridge. Concrete core scrapings were obtained from two columns at various elevations: on column 1, at 6 and 96 inches from the ground; and on column 3, at 12 and 96 inches from the ground. Table 6 presents a summary of bridge site sampling information for SH 21 at Carrice Creek (Sabine County).

Table 6: Date of visit, general and sampling information for SH 21 at Carrice Creek (Sabine County)

Date of Visit	12/3/2010
General Information	Built 1967
Visual Inspection	Bridge site had only four spans having high levels of deterioration. Column 3 has the rebar exposed. Additional corrosion was also observed on the south end of the bridge site.
Sampling information	Two sets of water and mud samples were collected on the west and east sides of the bridge site Concrete scrapings were collected on column 1 at 6 and 96 inches from the ground and on column 3 at 12 and 96 inches from the ground

2.0.1.7 Palo Gaucho Bayou (Sabine County)

The seventh bridge was located at FM 3121 at Palo Gaucho Bayou (Sabine County) and built in 1968. The degree of deterioration runs from the bottom of the column to the top of the column, approximately 7 feet. There is no visual rebar exposure or corrosion; the columns of interest are on the east side of span 1, the north side of column 1, and on the south side of column 3. Two water samples and two mud samples were collected on the north and south side of the bridge. Four concrete scraping samples were obtained from two columns at various elevations. On column 1, sample scrapings were taken at 6 and 90 inches from the ground; on column 3, sample scrapings were taken at 6 and 90 inches from the ground. Table 7 presents a summary of bridge site sampling information for FM 3121 at Palo Gaucho Bayou (Sabine County).

Table 7: Date of visit, general and sampling information for FM 3121 at Palo Gaucho Bayou (Sabine County)

Date of Visit	12/3/2010
General Information	Built 1968
Visual Inspection	The degree of deterioration runs from the bottom of the column to the top of the column, approximately 7 feet. There is no visual rebar exposure or corrosion.
Sampling information	Two sets of water and mud samples were collected Concrete scrapings where obtained from two columns; Column 1 samples were taken at 6 and 90 inches and column 2 samples were taken at 6 and 90 inches

2.0.1.8 SH 31 West Bound at Kickapoo Creek (Henderson County)

Figure 5: Overview of the level of deterioration of SH 31 West Bound at Kickapoo Creek (Henderson County)

Figure 5 shows the level of deterioration of the eighth bridge located at SH 31 West Bound at Kickapoo Creek (Henderson County); 3.2 miles west of the Smith County line near Chandler, Texas. The bridge was built in 1930 and has 2 spans. A visual inspection of the bridge indicated that the bridge appears to have moderate scaling with approximately ¼ - ½ inches of penetration along the waterline of the concrete pillars. There is severe scaling of column 4 and column 5 at the water line with a penetration of approximately 3 inches. The columns, from 0 to 30 inches above the water line, show scaling of the surface cement paste and exposure of course aggregate. At 30-42 inches above the waterline, the cement paste has mild scaling and column 4 has rebar corrosion. The following columns were tested for span 1 from the west side (the water level was 3 feet deep): column 2 from the north side, and column 4 from the south. Two water samples and two mud samples were taken at the north and south end of the bridge. Eight concrete scraping samples were taken from two columns at various elevations from the ground. The first set of concrete scrapings was taken from span 1 column 2, at 6, 18, 30, and 54 inches from the ground. The second set of concrete scrapings was taken from span 1 column 4 at 6, 18, 30, and 54 inches from the ground. Table 8 presents a summary for samples collected at SH 31 West Bound at Kickapoo Creek (Henderson County).

Table 8: Date of visit, general and sampling information for SH 31 West Bound at Kickapoo Creek (Henderson County)

Date of Visit	4/14/2011
General Information	Built approximately 1930; west bound bridge site consisting of 2 spans
Visual Inspection	Moderate to severe deterioration.
Sampling information	Two sets of water and mud samples were collected on the north and south end of the bridge Concrete scrapings were taken from two columns from span 1; Column 2 span 1 taken at 6, 18, 30, and 54 inches from the ground; Column 4 span 1 taken at 6, 18, 30, and 54 inches from the ground

2.0.1.9 SH 31 East Bound at Kickapoo Creek (Henderson County)

The ninth bridge location was at SH 31 East Bound at Kickapoo Creek (Henderson County), 2.8 miles East of Smith County line near Chandler, Texas (east bound). The bridge was built in 1970 and contains 7 spans. Upon visual inspection of the bridge, there appears to be moderate scaling and approximately ¼ - ½ inches of penetration along the water line of the concrete pillars. On the columns, from the water line to 18 inches above the waterline, there is scaling and aggregate is not bound to the concrete. Approximately at 18-42 inches above the waterline, there is mild scaling but no rebar corrosion. The following columns were tested: span 2, from the north side of column 3, and from the north side, column 4. The water level was approximately 1 foot deep on span 1. Two water samples and two mud samples were collected on the north and south side of the bridge. Eight concrete scrapings were collected from two columns at various elevations. The first set of concrete scrapings was collected from span 1 column 3 at 6, 18, 30, and 54 inches from the ground. The second set of concrete scrapings was obtained from span 2 column 4 at 6, 18, 30, and 54 inches from the ground. Table 9 shows a summary of sampling information for SH 31 East Bound at Kickapoo Creek (Henderson County).

Table 9: Date of visit, general and sampling information for SH 31 East Bound at Kickapoo Creek (Henderson County)

Date of Visit	4/14/2011
General Information	Built approximately in the 1970s, east bound consisting of 7 spans
Visual Inspection	Moderate to severe deterioration.
Sampling information	Two sets of water and mud samples were collected on the north and south end of the bridge Concrete scrapings were obtained on two columns on span 2; Column 3 span 2, scrapings were taken at 6, 18, 30, and 54 inches from the ground. Second set of scrapings were taken from column 4 span 2 at 6, 18, 30, and 54 inches from the ground

2.0.1.10 FM 787 at Tarkington Bayou (Liberty County)

The tenth bridge location was at FM 787 at Tarkington Bayou (Liberty County). Little Tarkington Bayou Relief Bridge was built in 1930 and the columns appear to be in good condition. From the ground level to 6 inches from the ground was previously covered by water which has receded and area has some degree of deterioration. Two water samples and two mud samples were collected on the north and south side of the bridge. Three concrete scrapings were collected on the first span at 6, 42, and 90 inches from the ground. Table 10 presents a summary of sampling information for FM 787 at Tarkington Bayou (Liberty County).

Table 10: Date of visit, general and sampling information for FM 787 at Tarkington Bayou (Liberty County)

Date of Visit	7/20/2011
General Information	Built 1930; Little Tarkington Bayou Relief Bridge
Visual Inspection	Columns appear to be in good condition.
Sampling information	Two sets water and mud samples were collected on the north and south end of the bridge Concrete scrapings were collected on span 1 at 6, 42, and 90 inches above the ground

2.0.1.11 FM 751 at Duck Creek (Hunt County)

The eleventh bridge was located at FM 752 at Duck Creek (Hunt County). Bridge was built in 1959 and the columns appear to be in good condition. Rebar exposure is not exposed however there is organic material growing on the surface of the column. Two water samples were collected on the north and south end of the bridge site. Three concrete scrapings were collected on span 2 at 6, 30, and 66 inches above the ground. Table 11 shows a summary of sampling information for FM 752 Duck Creek (Hunt County).

Table 11: Date of visit, general and sampling information for FM 752 at Duck Creek (Hunt County)

Date of Visit	7/21/2011
General Information	Built in 1959; located on the south side of 429
Visual Inspection	Columns appear to be in good condition.
Sampling information	Water samples were collected on the north and south end of the bridge Concrete scrapings were collected on span 2 at 6, 30, and 66 inches above the ground

2.0.1.12 FM 751 at S. Fork Sabine River (Hunt County)

The twelve bridge is located at FM 751 at S. Fork Sabine (Hunt County). Bridge was built in 1959 and the columns appear to be in good condition. Rebar exposure is not exposed however there is organic material growing on the surface of the column. Two water samples were collected on the north and south end of the bridge. Three concrete scrapings were obtained on the first span at 6, 30, and 54 inches above the ground. Table 12 presents a summary of sampling information for FM 751 at S. Fork Sabine (Hunt County).

Table 12: Date of visit, general and sampling information for FM 751 at S. Fork Sabine (Hunt County)

Date of Visit	7/21/2011
General Information	Built in 1959; located on the south side of 429
Visual Inspection	Columns appear to be in good condition, rebar exposure is not observed.
Sampling information	Water samples were collected on the north and south end of the bridge Concrete scrapings were collected on span 1 at 6, 30, and 54 inches above the ground

2.0.2 Chemical Analysis

2.0.2.1 Sample preparation

For chemical analysis all samples consisting of mud or concrete scraping were pulverized into a fine powder using a mortar and pestle. Each sample was extracted for approximately 48 hours at a 1:2 ratio of sample to water or 1:2 ratio of sample to 5N HCl (Mallinckrodt Baker, Inc., Paris, Kentucky). The water extract from the mud and concrete scraping was filtered using a 15cm diameter MFS Microfiltration System (Sierra Court, Dublin, California). All samples were centrifuged using an Allegra X-12 Centrifuge (Beckman Coulter, USA). Samples were centrifuged at 1500 g's for 15 minutes at 19°C and the supernatant removed from all samples, filtered, then stored at 4°C prior to analysis by ion chromatography and pH measurements.

2.0.2.2 Determination of pH

Determination of pH was conducted by utilizing phenolphthalein or a pH meter. Approximately 50 mg of phenolphthalein ($C_{20}H_{14}O_4$), J.T. Baker, Phillipsburg, N.J.) were weighed into a 100-mL volumetric flask and dissolved in 50 mL of 95% ethanol (Aper alcohol distillery, Lexington, Kentucky), then filled to the mark with distilled water. Phenolphthalein was dropped on the surface and interior perimeter of the core samples that were obtained from Tarkington (Liberty County), Navasota River (Bryan District), Lake Tawakoni (Hunt Country), and Alligator Bayou (Jefferson County). The depth of penetration was measured for each surface core and outliers were discarded using the Dixon Q test method (Ellison et al., Rorabacher, 1991) In addition, an Orion Research model 201/digital pH meter with Orion combination pH electrode (Orion

Research; Taiwan) was used; the pH meter was calibrated at 4, 7, and 10 with appropriate calibration buffers. The pH was measured for water, mud, and concrete scrapings at a 1:2 ratio of sample to distilled water (Czerewko et al., 2003).

2.0.2.3 Sulfide determination

A 0.1 M Na_2S stock solution was prepared by weighing 2.4049 g of sodium sulfide 9-hydrate (ACS reagent grade, Mallinckrodt AR) into a 100mL volumetric flask and then filling to the mark with distilled H_2O. Appropriate dilutions from the sodium sulfide stock were made in order to prepare a standard series ranging from 10^{-2} to 10^{-6} M Na_2S. Millivolt response measurements were taken using a Beckman Coulter Phi 510 Electrochemistry Meter (Beckman Coulter, Inc., Fullerton, CA). A silver/sulfide ion selective electrode Orion Series A meter was used (ThermoScientific, USA). An initial measurement of 10 mL sample water from SH 21 at Navasota River was tested at 25°C, followed by 10µL spikes with 10^{-2}M Na_2S standard in order to determine the concentration of sulfide in the water sample. Table 13 shows the concentration of the sodium sulfide curve series.

2.0.2.4 Sulfate and Chloride Analysis

The stock eluent solutions were 100 mM $NaHCO_3$ (ACS reagent grade, Mallinckrodt Baker, Inc., Paris Kentucky) and 100 mM Na_2CO_3 (ACS reagent grade, Fair Law, New Jersey). The standard concentration for sulfate and chloride stock solutions was 1000 mg/L (NSI Solutions, Raleigh, NC). For the analysis of sulfates and chlorides, a QuickChem 8500 Lachat Instruments ion chromatograph with a Lachat rapid anion column from Loveland, Colorado was used. A Lachat Instruments CM-100 Conductivity module was used for the detection of anions. Sodium carbonate and sodium bicarbonate stocks were diluted to yield a final concentration of 9.0 mM $NaHCO_3$ and 0.5 mM Na_2CO_3 and were run at 1.4mL/min for ion chromatograph (Karmarkar and Bahowick, 1996; Pfaff, 1993). The standard curve range for chloride was 1.5-150 mg/L and for sulfate was 2.5-250 mg/L (Karmarkar and Bahowick, 1996; Pfaff, 1993). Table 13 presents the standard curve range for sulfate and chloride that was used for sample analysis (Karmarkar and Bahowick, 1996; Pfaff, 1993). All samples were analyzed using the Environmental Protection Agency Protocol 300.0 (Pfaff, 1993).

Table 13: Standard curve range for sulfate, chloride, and sodium sulfide

Curve range for SO_4^{-2}, mg/L	Curve range for Cl^- mg/L	Curve series for Na_2S, M
1.5	2.5	10^{-2}
3	5	10^{-3}
12	20	10^{-4}
30	50	10^{-5}
90	150	10^{-6}
150	250	--

2.0.3 Microbial Analysis

2.0.3.1 Sample Preparation

One gram of concrete was dissolved in 1 mL(need to be consistent in using mL or ml in text) of 2-4% paraformaldehyde (PFA) solution (pH 7.2, Fisher Scientific, Fair Lawn, New Jersey). The extracted microbial cells samples were pulverized into a fine powder using a mortar and pestle. Cells were then fixed in fixation buffer prior to hybridization. The following reagents were utilized for the fixation procedure: phosphate buffer (PBS solution) that consisted of 1.3 M NaCl (Mallinckrodt Baker Inc., Paris, Kentucky), 70 mM Na_2HPO_4, and 30 mM NaH_2PO_4 (Carolina Biological Supply Company; Burlington, N.C.), anhydrous ethanol suitable for histology (EMD Chemicals Inc., Gibbstown, N.J.), and sodium pyrophosphate ($NaP_2O_7 \bullet 10H_2O$, Fisher Scientific; Fair Lawn, New Jersey). The microbial cells were fixed for 16 hours at 4°C prior to fluorescence in situ hybridization. After the fixation procedure, the slide was rinsed in either 0.1% pyrophosphate or PBS and 50% ethanol/PBS.

2.0.3.2 Reagents for in situ hybridization

The following reagents were utilized for fluorescence in situ hybridization: sodium pyrophosphate ($Na_4P_2O_710H_2O$, Fisher Scientific, Fair Lawn, New Jersey) ; 0.1% lysozyme (1 mg corresponding to 37,320U, Fluka, Buchs, Switzerland) dissolved in 1 mL of 100 mM Tris/HCl(pH 7.5) and 5 mM EDTA (the tris (hydroxymethyl)aminomethane ($NH_2C(CH_2OH)_3$ for molecular biology was obtained from EMD Chemicals, Inc., Gibbstown, NJ); formamide (low conductivity biotechnology grade, Amnesco, Solon, Ohio); hybridization solution and washing buffer that consisted of 0.9 M NaCl (Mallinckrodt AR Mallinckrodt Baker Inc., Paris, Kentucky), 5 mM Na_2EDTA (ethylenediaminetetracetic acid ferric sodium salt, $C_{10}H_{12}N_2NaFeO_8$, Sigma Chemical Co., St. Louis, MO), and 20 mM Tris/HCl(pH 7.0, Tris/HCl Omni Pur, EMD Chemicals, Inc., Gibbstown, NJ); 0.01% SDS ($CH_2(CH_2)_{11}OSO_3Na$, Electrophoresis Grade, Assay 99% min., Fisher Scientific, Fair Lawn, New Jersey); blocking agent was obtained from Roche, USA; Citiflour Mountant media #0 was obtained from TED Pella, Inc., Redding, CA; EUB 338 5′-(Cy3) GCTGCCTC CCGTAGGAGT-3 desalted (EMG Operon); DAPI (4-6-diamidino-2-phenylindoledihydrochloride, Polyscience, Inc., Warrington, PA); anhydrous ethanol suitable for histology (EMD Chemicals, Inc., Gibbstown, N.J).

2.0.3.3 Microbial Analysis Protocol

For the fluorescence in situ hybridization experiments, 90 µL of 0.1% pyrophosphate and a 10 uL aliquot of sample were placed in a polypropylene Eppendorf tube and vortexed. 10 µL of solution was then placed onto a 8 well gelatin slide and incubated for 15 minutes at 43°C. 10% lysozyme stock was diluted in a 1:10 ratio with tris buffer. 10 µL of this solution was added to each well. The slide was placed in a humidity chamber and incubated for 1 hour at approximately 37.5°C. After hybridization, the slide was rinsed in 50%, 70%, 96% ethanol for 3 minutes each. A solution containing 30% formamide and 70% hybridization solution was placed on a Kim wipe and inside a 50 ml polypropylene conical vial which prevents fixed cells from drying out during the hybridization procedure. A solution containing 45 µL of formamide, 105 µL hybridization solution, and 15 µL of blocking agent was prepared. Nine µL of previously prepared solution was placed into each well and incubated for 30 minutes. After incubation, 1 µL of the specific probe of interest (Cy3-EUB338) was added to each well and incubated for 1.5 hours at 37.5°C in a humidity chamber. After hybridization, the slide was rinsed with distilled water and placed in washing solution for 20 minutes. The slide was rinsed with distilled water and allowed to air dry. Citifluor was evenly added to the slide and 20 individual randomized counts for DAPI and Cy3 were conducted. Samples were visualized with a Nikon Eclipse 80i with X-cite series 120Q EXFO microscope at 100X.

2.0.4 Compressive strength

2.0.4.1 Mortar sample preparation

Mortar cubes with five mix designs were prepared. The compositions for all mix designs were as follows: water to cement ratio was 0.45, fine aggregate (sand) to binder ratio was 2.50, air entrainer 90 was 2.3 (fl oz/cwt), and supplementary cementitious material varied depending on mix design. Mix design 1 was used as a reference mix containing type I portland cement. All other mix designs were based on mix design 1 with different types of cement and cementitious material. . Mix design 1 was coated with a concrete preservation treatment solution (CPT-2000; Rockwall, TX). Mix design 2 utilized type 5 Portland cement instead of type 1 Portland cement which consists of high sulfate resistant cement. Mix design 3 utilized class C fly ash as a filler which replaced 30% of type 1 Portland cement by weight. Mix design 4 utilized class F fly ash which replaced 30% of type 1 Portland cement and mix design 5 utilized silica fume in place of 10% of type 1 Portland cement by weight. ASTM C109 was followed to prepare and test the concrete cubes.

2.0.4.2 Solution preparation and laboratory exposure

96% sulfuric acid (Acros Organics, New Jersey, USA) was used to prepare various concentrations of sulfuric acid with final concentrations of 1% and 3% (v/v); these percentages of sulfuric acid were prepared in order to accelerate the scenario involving microbial induced degradation (MID). These samples were used for both laboratory and field studies. Exposure changes of these samples were examined by utilizing compressive strength analysis.

2.0.4.3 Field exposure

For field exposure, the selected site was FM 787 at Tarkington Bayou (Liberty County). Field exposure involved three different scenarios and the first one involved placing specimens directly into the water at Tarkington Bayou after a minimum of a 248 day curing period. For the second scenario, specimens were cured for a minimum of 191 days following an exposure to 1% sulfuric acid solution for 57 days. These samples were then placed in Tarkington Bayou field water for the duration of the investigation. In the last scenario, specimens were cured for a minimum of 295 days and subsequent exposed to 3% sulfuric acid solution for 15 days. These samples were also placed in Tarkington Bayou field water for the duration of the investigation. Deterioration of specimens under different exposures were examined by utilizing compressive strength analysis.

2.0.4.4 Compressive strength analysis

A Test Mark model CM-0030-RT (Test Mark, East Palestine, OH) was used to conduct strength test analyses for mortar cubes of various compositions and exposures to sulfuric acid. Test Mark was set to 2”X 2” cubes, 50-100 psi/s at 50% strength loss; the threshold was set to 10,000 lbs. in order to measure the strength of each sample cube. Compressive strength analysis was done after concrete sampling according to ASTM C192 (ASTM International Standard, 2007).

3.0 Results

3.0.1 Chemical analysis

The following tables present the depth of penetration using phenolphthalein indicator, pH, sodium sulfide concentration ranged 10^{-2} M Na_2S to 10^{-6} M Na_2S, percent sulfate and percent chloride values for water (W), mud (M) and concrete (C) scrapings. The depth of penetration refers to the depth at which the phenolphthalein is colorless indicating that the concrete is no longer basic and has been affected by the environment. For each sample, the designated number-letter-number-number refers to the location-type of sample-concrete column-inches above ground respectively. The latter two designations only apply to concrete column samples. For example (1-C-1-6) refers to a sample that was acquired at FM 787 at Tarkington Bayou, bridge 1 from a concrete column, number 1 at 6 inches above the water line.

3.0.1.1 Chemical analysis for FM 787 at Tarkington Bayou (Liberty County)

Table 14 shows the depth of penetration using phenolphthalein indicator for various surface concrete cores obtained from FM 787 at Tarkington Bayou (Liberty County). Most of these samples have organic material on the top surface of the core and the color change from colorless to pink occurred at 12 millimeters or less with a low standard deviation. For all bridge sites measurements taken for each core depended on the overall circumference of each core, measurements were taken at every 10 mm.

Table 14. Depth of Penetration for Bridge Columns at FM 787 at Tarkington Bayou (Liberty County)

Sample Identification	Number of measurements taken	Average, mm	Range, mm	Standard Deviation	Organic Material on top surface	Approximate percent coverage of organic material	Approximate height of organic material, mm
1-C-1-6	13	4.15	1-10	3.29	Y	80%	1
1-C-1-22	10	1.60	1-5	1.35	Y	75%	1
1-C-1-20	9	2.56	1-5	1.51	Y	40%	1
1-C-1-27	12	1.67	1-4	0.9848	Y	10%	1
1-C-1-34	6	1.50	1-3	0.84	N	--	--
1-C-1-0	11	1.64	0-4	1.6895	N	--	--
1-C-1-90	13	3.38	1-15	4.407	Y	40%	1
1-C-1-20	12	4.17	1-12	3.62	Y	30%	1

Table 15 represents pH, sulfate and chloride concentration data from FM 787 at Tarkington Bayou (Liberty County); the pH of the water was slightly acidic while the pH of the concrete was essentially neutral. The concentration of sulfate and chloride for the water samples were generally low.

The concrete scrapings had at least 5 times the amount of sulfate when compared with the water samples with sample 1-C-1-22 having the highest amount (approximately 50 fold more than the average concentration found in the water samples. The highest concentration of chloride was found in the sample collected on 1-C-1-6 having approximately 17 times the concentration of chloride as in the water samples.

Table 15. pH, sulfate, and chloride concentrations at FM 787 at Tarkington Bayou (Liberty County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^- unit?	Cl^- (ppm)
1-W-1	5.40	--	5.23	--	--	8.96	--	--
1-W-2	5.55	--	5.68	--	--	6.50	--	--
1-W-3	5.25	--	5.22	--	--	7.47	--	--
1-C-1-6	--	0.2522	1.01	4.00E-5	40.05	3.37	1.34E-4	133.6
1-C-1-22	--	0.2531	7.02	2.77E-4	277.36	1.44	5.69E-5	56.89
1-C-2-20	6.95	1.0169	2.93	2.88E-5	28.8	3.91	3.84E-5	38.45
1-C-3-0	7.84	1.0170	3.86	3.80E-5	38.0	5.12	5.03E-5	50.3

3.0.1.2 Chemical analysis for a revisit location for FM 787 at Tarkington Bayou (Liberty County)

Table 16 represents the data from a revisit of the bridge at FM 787 at Tarkington Bayou (Liberty County). The water samples had a slightly acidic pH. The pH of the mud and concrete scrapings collected close to the ground had a relatively neutral pH and the other samples collected at higher elevations had a pH that was neutral to slightly basic.

The mud sample had approximately 40 times more sulfate than the two water samples. The concrete scrapings collected on the wall, 1-C-W1-6, had approximately nine times more sulfate than the remaining samples collected on the wall. This sample also had a 137 fold increase in sulfate concentration when compared to the amount of sulfate found in the water. In addition, sample 1-C-W1-6 also had the highest concentration of chloride with approximately 4 times as much chloride as the water samples. Sample 1-C-1-18 had the highest concentration of sulfate having approximately 165 times more sulfate than the concentration found in the water samples. For chlorides, samples taken at the lowest elevation had the highest concentration with approximately 5 times more chloride than the water samples. As the elevation at which samples were extracted from column 1 increased, the chloride concentration decreased significantly.

Table 16: pH, sulfate, and chloride concentrations at FM 787 at Tarkington Bayou (Liberty County) Revisit

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^-	Cl^- (ppm)
1-W1	6.12	--	2.70	--	--	45.1	--	--
1-W-2?	6.35	--	2.80	--	--	48.8	--	--
1-M	7.16	5.0021	53.5	1.07E-4	107	27.3	5.46E-5	54.6
1-C-W1-6	7.17	5.0019	189	3.78E-4	378	103	2.06E-4	206
1-C-W1-18	7.80	5.0070	18.4	3.67E-5	36.7	1.06	2.12E-6	2.12
1-C-W1-42	7.80	5.0050	25.7	5.13E-5	51.3	1.06	2.12E-6	2.12
1-C-1-6	6.90	5.0079	118	2.36E-4	236	128	2.55E-4	255
1-C-1-18	7.22	5.0044	227	4.54E-4	454	22.5	4.50E-5	45
1-C-1-42	7.94	4.9972	165	3.30E-4	330	9.61	1.92E-5	19.2

3.0.1.3 Chemical analysis for SH 21 at Navasota River (Robertson-Bryan District)

Table 17 shows the depth of penetration using phenolphthalein indicator for various surface concrete cores obtained from SH 21 at Navasota River (Bryan District). Most of these samples have organic material on the top surface of the core and the color change from colorless to pink occurred at 38 millimeters or less with a low standard deviation.

Table 17: Depth of Penetration for Bridge Columns at SH 21 at Navasota River (Robertson-Bryan District)

Sample	Number of measurements taken	Average, mm	Range, mm	Standard Deviation	Organic Material on top surface	Approximate percent coverage of organic material	Approximate height of organic material
2-C-1R-0	12	24	10-38	8.16	Y	10	1
2-C-2R-0	13	0.38	0-3	0.9693	Y	10	1
2-C-1-6	9	5	3-8	1.5	Y	<10	1
2-C-2-6	12	3.75	0-6	2.34	Y	<10	1
2-C-1-22	8	3.25	1-8	3.24	Y	80	1
2-C-2-22	8	0.75	0-3	1.03	Y	50	1
2-C-1-56	12	16.17	12-20	2.4058	N	--	--

R: the wall on this column was repaired and date of repair is unknown

A sodium sulfide calibration curve prepared for 10^{-2} M Na_2S to 10^{-6} M Na_2S ranged from -760 mV to -130.6 mV in measured potentials. A water sample taken from this site had an initial millivolt reading of -144 mV. After one 10 μL addition of 10^{-2} M Na_2S standard into the 10 mL water sample the change was -403 mV. Nine additional 10 μL aliquots were made into the sample water and the final potential was -605 mV indicating that the concentration was beyond the lower limit of the calibration curve. Table 18 represents the data for SH 21 at Navasota River (Robertson-Bryan District). The pH of the water and the mud were slightly acidic with the third water sample having approximately 5 times less sulfate and twice as much chloride as the other two water samples. The pH of the concrete scrapings collected at various elevations was neutral to relatively basic.

When taking into consideration the sample height of the concrete shavings and the concentration of sulfate, there was less sulfate present at 12 inches (samples 2-C-2-12) when compared to 36 inches above the water line (2-C-3-36).

Table 18: pH, sulfate, and chloride concentrations at SH 21 at Navasota River (Robertson-Bryan District)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
2-W-1	6.05	--	53.5	--	--	48.9	--	--
2-W-2	6.10	--	55.6	--	--	43.1	--	--
2-W-3	6.35	--	11.2	--	--	88.0	--	--
2-M-1	6.77	5.0168	64.9	1.29E-4	129	16.1	3.21E-5	32
2-C-1-36	7.72	5.0037	55.4	1.107E-4	111	27.4	5.48E-5	55
2-C-2-12	8.95	5.0191	19.6	3.91E-5	39.1	88.9	1.77E-4	177
2-C-3-36	7.69	5.0052	104	2.08E-4	208	109	2.18E-4	218
2-C-4-0	7.70	5.0250	55.1	1.10E-4	110	84.8	1.69E-4	169

3.0.1.4 Chemical analysis for SH 276 at Lake Tawakoni (Hunt County)

Table 19 shows the depth of penetration using phenolphthalein indicator for various surface concrete cores obtained from SH 276 at Lake Tawakoni (Hunt County). Most of these samples have organic material on the top surface of the core and the color change from colorless to pink occurred at 40 millimeters or less with a low standard deviation.

Table 19: Depth of Penetration for Bridge Columns at SH 276 at Lake Tawakoni (Hunt County)

Sample	Number of measurement taken	Average, mm	Range, mm	Standard Deviation	Organic Material on top surface	Approximate percent coverage of organic material	Approximate height of organic material, mm
3-C-1-6	12	0.5	0-5	1.44	Y	60	1
3-C-1-24	12	21.92	10-36	8.73	Y	10	--
3-C-1-6	10	3.7	0-10	4.64	Y	90	<1
3-C-1-24	11	6.45	0-12	4.1319	Y	50	<1
3-C-1-4	11	11.64	0-25	9.61	N	--	--
3-C-1-5	11	8.18	0-40	14.71	N	--	--

Table 20 presents the data for SH 276 at Lake Tawakoni (Hunt County). The pH of the water was slightly basic with a low concentration of sulfate and chloride. Concrete scrapings taken closest to the ground had approximately 10 times more sulfate and 8 times more chloride than a sample taken at higher elevation. In addition, sample 3-C-3-6 also had a higher concentration of sulfate and chloride than the water samples.

Table 20: pH, sulfate, and chloride concentrations at SH 276 at Lake Tawakoni (Hunt County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
3-W-1	7.35	--	11.8	--	--	7.52	--	--
3-W-2	7.40	--	11.4	--	--	7.32	--	--
3-C-3-6	7.45	5.0051	1110	0.0022	2200	126.2	2.52E-4	252
3-C-3-24	8.43	5.0042	106	2.12×10^{-4}	212	15.3	3.06E-5	30.6

3.0.1.5 Chemical analysis for SH 82 at Alligator Bayou (Jefferson County)

Table 21 shows the depth of penetration using phenolphthalein indicator for various surface concrete cores obtained from SH 82 at Alligator Bayou (Jefferson

County). Most of these samples have organic material on the top surface of the core and the color change from colorless to pink occurred at 20 millimeters or less with a low standard deviation.

Table 21: Depth of Penetration for Bridge Columns at SH 82 at Alligator Bayou (Jefferson County)

Sample	Number of measurement taken	Average, mm	Range, mm	Standard Deviation	Organic Material on top surface	Percent coverage of organic material	Approximate height of organic material, mm
4-C-1-8	8	5.375	3-7	1.30	Y	30	3
4-C-1-28	7	5	4-6	0.577	Y	65	1
4-C-1-85	9	5.78	5-9	1.39	Y	60	2
4-C-1-40	11	3.09	1-5	1.25	N	--	--
4-C-1-4	6	7.33	1-11	3.78	Y	45	1
4-C-2-4	11	8.55	3-20	4.74	Y	35	3

Table 22 presents pH, sulfate and chloride concentrations for SH 82 at Alligator Bayou (Jefferson County). The water in contact with the concrete columns yielded a pH that was slightly basic but with a high concentration of sulfate and chloride. For one of the mud samples located near span 1 taken underwater (sample 4-M-1) the percent sulfate was approximately three times as much as that for a mud sample which was collected on the bank (sample 4-M-2).

Table 22: pH, sulfate, and chloride concentrations at SH 82 at Alligator Bayou (Jefferson County)

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
4-W-1	7.50	--	148	--	--	189	--	--
4-W-2	7.40	--	148	--	--	192	--	--
4-M-1	7.95	5.0000	261	5.22×10^{-4}	522	43.1	8.62E-5	86.2
4-M-2	7.65	4.9999	75.6	1.51×10^{-4}	151	48.1	9.62E-5	96.2
4-C-3-(-6)	8.55	4.9937	75.5	1.51×10^{-4}	151	28.5	5.71E-5	57.1
4-C-6-6	8.10	5.0046	314	6.27×10^{-4}	627	220	4.40E-4	440
4-C-5-3	8.20	4.9909	390	7.81×10^{-4}	781	290	5.81E-4	581
4-C-5-34	7.40	5.0017	1136	0.0023	2300	70.8	1.41E-4	141

For sulfate and chloride analysis, comparing the corroded concrete (samples 4-C-5-3 and 4-C-6-6) to the concrete coated with a biofilm (sample 4-C-3-(-6)), the corroded concrete had approximately four times as much sulfate present. The non corroded sample (5-C-5-34) had the highest concentration of sulfate when compared to the other samples obtained. For the chloride analyses, the two corroded samples, 4-C-6-6 and 4-C-5-3, had approximately four times as much as the non-corroded sample 4-C-5-34 and nine times as much as the sample with the biofilm 4-C-3-(-6). Also, the non-corroded sample had the lowest pH and the highest concentration of sulfate.

3.0.1.6 Chemical analysis for FM 276 at Patroon Bayou (Sabine County)

Table 23 presents the data for FM 276 at Patroon Bayou (Sabine County). The pH of the mud and water was acidic with a relatively low concentration of sulfate and chloride. When considering the concrete scrapings, the samples taken from the first column at 12 inches above the water line (5-C-1-12), had nearly the same concentration of sulfate as the sample taken at 72 inches (5-C-1-72). The concrete scrapings had at least 6 times more sulfates than the water and mud samples. For chlorides, samples taken at lower elevation were approximately nine times as concentrated as the sample taken at higher elevation. For column 2, the sample taken at 24 inches (5-C-2-24) had twice as much sulfate as the sample taken at 65 inches (5-C-2-65); the chloride amount for both of these samples was low and approximately the same.

Table 23: pH, sulfate, and chloride concentrations at FM 276 at Patroon Bayou (Sabine County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
5-W-1	5.90	--	20.7	--	--	19.2	--	--
5-W-2	5.55	--	18.6	--	--	17.4	--	--
5-M-1	6.20	5.0046	10.4	2.08×10^{-5}	20.8	0.361	7.21E-7	0.721
5-M-2	4.60	4.9966	8.17	1.64×10^{-5}	16.4	2.49	4.98E-6	4.98
5-C-1-12	7.60	5.0091	58.4	1.17×10^{-4}	117	9.47	1.89E-5	18.9
5-C-1-72	7.75	5.0040	63.8	1.27×10^{-4}	127	1.04	2.08E-6	2.08
5-C-2-24	8.15	5.0095	211	4.21×10^{-4}	421	18.4	3.67E-5	36.7
5-C-2-65	7.95	5.0023	119	2.38×10^{-4}	238	17.2	3.44E-5	34.4

3.0.1.7 Chemical analysis for SH 21 at Carrice Creek (Sabine County)

Table 24 represents results for SH 21 at Carrice Creek (Sabine County). The pH of the water and mud was slightly acidic, while the pH of the concrete was basic. When comparing the water, mud, and concrete samples, the concentration of sulfate and chloride were low with the exception of sample 6-C-3-12; this sample had approximately three times more sulfate and five times more chloride than the other samples.

Table 24: pH, sulfate, and chloride concentrations at SH 21 at Carrice Creek (Sabine County)

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
6-W-2	6.15	--	18.8	--	--	18.4	--	--
6-M-1	5.85	4.9955	8.21	1.64×10^{-5}	16.4	ND	--	--
6-M-2	6.25	5.0079	15.2	3.04×10^{-5}	30.4	5.84	1.17E-5	11.7
6-C-1-6	7.50	5.0038	24.2	4.84×10^{-5}	48.4	11.8	2.36E-5	23.6
6-C-1-96	7.75	5.0245	31.3	6.23×10^{-5}	62.3	ND	--	--
6-C-3-12	8.00	5.0252	90.8	1.81×10^{-4}	181	56.3	1.12E-4	112
6-C-3-96	7.75	5.0110	43.1	8.60×10^{-5}	86	7.26	1.45E-5	14.5

3.0.1.8 Chemical analysis for FM 3121 at Palo Gaucho Bayou (Sabine County)

In Table 25, the water and mud samples tested acidic, and the concrete samples taken at 90 inches above the water line had pH values well above 10, while the other concrete samples were slightly basic. Mud sample 7-M-1 had approximately 2.5 times more sulfate than mud sample 7-M-2. When comparing samples taken from column 1, the sample taken at 6 (7-C-1-6) had four times as much sulfate as the sample taken at 90 (7-C-1-90) and ten times as much chloride as the sample taken at higher elevation. The opposite occurred for column 3, with the sample taken at higher elevation (7-C-3-90) having approximately three times more sulfates than the sample taken at lower elevation (7-C-3-6).

Table 25: pH, sulfate, and chloride concentrations at FM 3121 at Palo Gaucho Bayou (Sabine County)

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount Cl^-	Cl^- (ppm)
7-W-1	6.15	--	19.3	--	--	15.7	--	--
7-W-2	6.10	--	18.9	--	--	15.3	--	--
7-M-1	5.35	4.9961	73.4	$1.47 X10^{-4}$	147	ND	--	--
7-M-2	5.70	5.0000	32.2	$6.44 X 10^{-5}$	64.4	12.4	2.48E-5	24.8
7-C-1-6	7.85	5.0186	219	$4.36 X 10^{-4}$	436	84.4	1.68E-4	168
7-C-1-90	>10	5.0039	52.6	$1.05 X 10^{-4}$	105	8.97	1.79E-5	17.9
7-C-3-6	7.95	5.0137	36.2	$7.22 X 10^{-5}$	72.2	12.6	2.51E-5	25.1
7-C-3-90	>10	5.0086	99.2	$1.98 X 10^{-4}$	198	11.5	2.30E-5	23

3.0.1.9 Chemical analysis for SH 31 at West Bound at Kickapoo Creek (Henderson County)

Table 26 presents pH, sulfate, and chloride concentration data for SH 31 West Bound at Kickapoo Creek (Henderson County). The pH of the water and mud samples was acidic, while the pH of the concrete samples was neutral to slightly basic. The sulfate and chloride concentration for the water samples was low, while the mud samples had approximately 3 times more sulfate and 2.5 times less chloride than the water samples. For concrete samples taken from column two and four, the sulfate and chloride concentration decreases as sample elevation increases. For both columns, samples taken closest to the ground have approximately 25 times more sulfate than the water samples and 10 times more than the mud samples. For chlorides, a similar trend is observed; sample 8-C-2-6 has 15 times more chloride than the water samples and sample 8-C-4-6 has 10 times more chloride than the water samples.

Table 26: pH, sulfate, and chloride concentrations at SH 31 West Bound at Kickapoo Creek (Henderson County)

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^-	Cl^- (ppm)
8-W1-S	5.58	--	55.3	--	--	38.1	--	--
8-W2-N	5.68	--	54.9	--	--	38.0	--	--
8-M1-S	5.75	4.9731	71.5	1.44E-4	144	8.40	1.69E-5	16.9
8-M2-N	4.88	5.0013	65.3	1.31E-4	131	6.88	1.38E-5	13.8
8-C-2-6	7.11	5.0055	690	0.0014	1400	290	5.79E-4	579
8-C-2-18	7.57	5.0086	274	5.47E-4	547	56.8	1.13E-4	113
8-C-2-30	7.08	5.0009	165	3.30E-4	330	69.9	1.40E-4	140
8-C-2-54	6.76	5.0043	221	4.42E-4	442	68.4	1.37E-4	137
8-C-4-6	6.97	5.0004	695	0.00139	1390	195	3.90E-4	390
8-C-4-18	7.16	5.0000	150	3E-4	300	46.1	9.22E-5	92.2
8-C-4-30	7.39	5.0016	55.7	1.11E-4	111	121	2.42E-4	242
8-C-4-54	6.95	5.0012	82.1	1.64E-4	164	75.8	1.52E-4	152

3.0.1.10 Chemical analysis for SH 31 at East Bound at Kickapoo Creek (Henderson County)

Table 27 shows the pH, sulfate, and chloride concentration data for SH 31 East Bound at Kickapoo Creek (Henderson County).The pH of the water and mud samples is acidic. The pH of the concrete scrapings was slightly neutral to basic with sample 11-C-3-54 having a pH well above pH 10.

Table 27: pH, sulfate, and chloride concentrations at SH 31 East Bound at Kickapoo Creek (Henderson County)

Sample	pH	Weight (g)	Sulfate (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amount of Cl^-	Cl^- (ppm)
9-W-1	5.55	--	27.8	--	--	35.3	--	--
9-W-2	6.09	--	24.2	--	--	37.8	--	--
9-M-1	3.95	5.0012	291	5.82E-4	582	21.6	4.32E-5	43.2
9-M-2	5.93	4.9994	196	3.92E-4	392	15.8	3.16E-5	31.6
9-C-3-6	7.67	5.0026	598	0.00120	1200	233	4.66E-4	466
9-C-3-18	7.73	4.9996	194	3.88E-4	388	116	2.32E-4	232
9-C-3-30	7.61	5.0025	98.2	1.96E-4	196	108	2.16E-4	216
9-C-3-54	11.8	5.0070	1.96	3.91E-6	3.91	78.7	1.57E-4	157
9-C-4-6	7.91	4.9998	487	9.74E-4	974	188	3.76E-4	376
9-C-4-18	7.53	5.0057	393	7.85E-4	785	38.2	7.63E-5	76.3
9-C-4-30	7.30	5.0029	199	3.98E-4	398	76.5	1.53E-4	153
9-C-4-54	8.10	5.0016	109	2.18E-4	218	1.06	2.12E-6	2.12

The concentration of sulfate and chloride for the water samples was generally low; however, the mud samples had approximately 16 times more sulfate than the water samples. For both columns, the sulfate and chloride concentrations are higher for samples taken at lower elevation and the concentration decreases as elevation increases. Concrete sample 9-C-3-6 had 40 times more sulfate than the water sample and approximately 2.4 times more sulfate than the mud and 11 times more chloride than the water and mud samples. Sample 9-C-4-6 had approximately 33 times more sulfate than the water and 2 times more sulfate than the mud. For chlorides, this sample also had 10 times more chloride than the water and mud samples.

3.0.1.11 Chemical analysis for FM 787 at Tarkington Bayou (Liberty County)

Table 28 presents the pH, sulfate, and chloride concentration data for FM 787 at Tarkington Bayou (Liberty County). The pH of the water and mud was neutral and the concrete scrapings had a relatively acidic pH with the exception of sample 10-C-2-42 which had a basic pH above 10. The water samples had a low concentration of sulfate and chloride and the mud samples had approximately 12.5 times more sulfate than the water samples. A higher concentration of sulfate was found in the sample taken at a lower elevation (10-C-2-6) and the concentration decreased as sample height increased. The concentration of chloride for concrete scrapings was low at all elevations. For this site visit, a higher concentration of sulfate is evident in the two mud samples and in the sample taken closest to the ground.

Table 28: pH, sulfate, and chloride concentrations at FM 787 atTarkington Bayou (Liberty County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^-	Cl^- (ppm)
10-W-1	6.78	--	17.2	--	--	5.73	--	--
10-W-2	7.19	--	16.4	--	--	5.71	--	--
10-M-1	7.10	5.0063	97.6	1.95×10^{-4}	194.95	2.92	5.433×10^{-6}	5.43
10-M-2	7.14	5.0261	151	3.00×10^{-4}	300.4	6.40	1.27×10^{-5}	12.73
10-C-2-6	5.63	3.0088	120	1.60×10^{-4}	160	3.84	5.10×10^{-6}	5.11
10-C-2-42	5.61	3.0070	0.740	9.84×10^{-7}	0.984	10.4	1.38×10^{-5}	13.83
10-C-2-90	>10	2.0076	0.167	3.33×10^{-7}	0.333	4.83	9.62×10^{-6}	9.62

3.0.1.12 Chemical analysis for FM 751 at Duck Creek (Hunt County)

Table 29 presents pH, sulfate, and chloride concentration data for FM 751 at Duck Creek (Hunt County).

Table 29: pH, sulfate, and chloride concentrations at FM 751 at Duck Creek (Hunt County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^-	Cl^- (ppm)
11-W-1	6.62	--	9.70	--	--	9.06	--	--
11-W-2	6.58	--	9.44	--	--	8.35	--	--
11-C-1-6	6.99	5.0023	93.8	$1.875 X 10^{-4}$	187.5	9.66	$1.93 X 10^{-5}$	19.3
11-C-1-30	6.07	5.0025	80.5	$1.61 X 10^{-4}$	160.92	14.8	$2.96 X 10^{-5}$	29.6
11-C-1-66	5.90	3.0019	25.8	$3.44 X 10^{-5}$	34.38	3.59	$4.78 X 10^{-6}$	4.78

The pH for the water and concrete scrapings was slightly acidic to neutral. Sulfate and chloride concentrations were low for both water samples and a higher concentration of sulfate was found on the samples closest to the ground (11-C-1-6 and 11-C-1-30). The concentration of sulfate decreases as sample elevation increases. Chloride concentrations were generally low for concrete scrapings taken at all elevations.

3.0.1.13 Chemical analysis for FM 751 at South Fork Sabine River (Hunt County)

Table 30 shows the pH, sulfate, and chloride concentration data for FM 751 at S. Fork Sabine River (Hunt County). The pH of the two water samples was slightly acidic and the pH of the concrete scrapings was acidic with the exception of sample 12-C-1-54 which had a neutral pH. The concentrations of sulfate and chloride for both the water and concrete samples were generally low with the exception of sample 12-C-1-54 which had the highest concentrations of sulfate and chloride.

Table 30: pH, sulfate, and chloride concentrations at FM 751 at S. Fork Sabine River (Hunt County)

Sample	pH	Weight (g)	SO_4^{-2} (mg/L)	Mass SO_4^{-2}/mass of sample	SO_4^{-2} (ppm)	Cl^- (mg/L)	Amt Cl^-	Cl^- (ppm)
12-W-1	6.80	--	9.64	--	--	8.09	--	--
12-W-2	6.77	--	9.89	--	--	8.01	--	--
12-C-1-6	5.83	3.9925	13.4	1.34 X 10^{-5}	13.4	2.52	2.52 X 10-6	2.52
12-C-1-30	5.96	2.9837	5.31	8.90 X 10^{-6}	8.90	3.16	5.30 X 10^{-6}	5.30
12-C-1-54	7.50	5.0084	130	2.60 X 10^{-4}	260	116	2.32 X 10^{-4}	231.6

3.0.2 Microbial analysis

The following tables (See Tables 31-38) summarize the analyses of microbial population found at various bridge locations. Only percentages were reported because the weight of samples prepared after fixation could not be determined accurately. Once cell fixation and hybridization was complete, twenty randomized counts were done for both DAPI-stained cells and cell hybridized with Cy3-EUB338. An average and standard deviation was calculated for all Cy3-EUB338 and DAPI-stained cells which represent absolute numbers obtained from randomized counts. Percent ratio average of Cy3-EUB338/DAPI-stained was calculated by taking the first counts obtained out of the twenty for EUB338 and dividing that by the first individual count of DAPI and so forth. Percent standard deviations were also calculated based on these ratios.

3.0.2.1 Microbial analysis for FM 787 at Tarkington Bayou (Liberty County)

Table 31 shows microbial percentage ratio and absolute counts upon revisiting obtained at FM 787 at Tarkington Bayou.

Table 31: Microbial percentage ratio and absolute counts upon revisiting FM 787 at Tarkington Bayou (Liberty County)

Sample	Percent ratio average EUB338/DAPI	Percent ratio standard deviation	EUB 338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
1-C-1-0	38.382	39.526	28.2	27.14406	45.8	25.84081
1-C-1-18	44.6905	44.6549	19.8	16.28561	29	16.35784
1-C-1-42	42.7918	39.1679	40.4	31.00832	61.2	31.01375

The table illustrates the percent average Cy3-EUB338/DAPI of organisms found at this location as well as absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain. The DAPI stain intercalates into DNA and thus detects all organism present while the Cy3-EUB338 probe is specific for the Domain Bacteria. Therefore, the ratio EUB338/DAPI is an indicator of the average percent bacteria to total organisms present in a community. The average percent ratio for Cy3-EUB338/DAPI at this site was moderately high. Bacteria populations found with the Cy3-EUB338 probe were generally low however with the DAPI-stain there was a moderate amount of organisms found.

3.0.2.2 Microbial analysis for FM 276 at Patroon Bayou (Sabine County)

Table 32, shows the percent average Cy3-EUB338/DAPI of organisms as well as the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain.FM 276 at Patroon Bayou (Sabine County). For the biological samples taken on column 1, the percent average of Cy3-EUB338/DAPI ratio was higher at elevations closest to the ground. Higher numbers were also observed at elevations closest to the ground with absolute counts done with the Cy3- EUB338 probe and DAPI-stain decreased at higher elevations. For biological samples taken on column 2, the average ratio percent of EUB338/DAPI was moderate. Absolute individual counts for Cy3-EUB338 and DAPI-stain were high at lower elevations and decreased with increased elevation.

Table 32: Microbial percentage ratio and absolute counts at FM 276 at Patroon Bayou (Sabine County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
5-C-1-12	40.5115	16.5017	211.4	80.96549	543.8	154.17
5-C-1-74	18.3937	11.8441	77.6	73.85291	390.9	202.8528
5-C-2-10	31.165	17.9447	65.95	46.38792	200.9	120.135
5-C-2-75	59.5041	33.1284	33.8	19.8298	59	25.75594
5-C-2-85	48.1092	26.0895	31	21.98085	65.4	31.42309

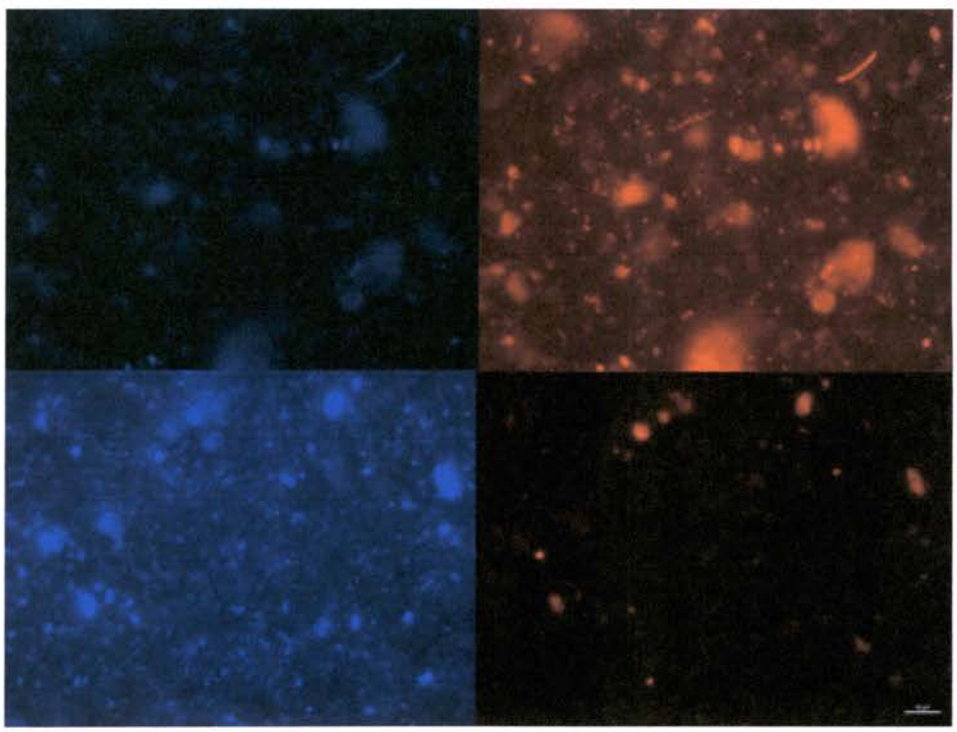

Figure 6: Dapi: Cy3 microbial populations of FM 276 at Patroon Bayou (Sabine County, top 5-C-1-12 and bottom 5-C-2-10

Figure 6 shows the microbial populations taken from sample 5-C-1-12; DAPI (top left-blue picture) shows all organisms found, and the Cy3 (top right-red picture) shows specific for the Domain Bacteria found with the EUB 338 probe.

3.0.2.3 Microbial analysis for FM 3121 at Palo Gaucho (Sabine County)

Table 33 presents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain found at Palo Gaucho (Sabine County). The percent average for Cy3-EUB338/DAPI for samples taken at various elevations were moderate. Absolute numbers for Cy3-EUB338 were generally low however the numbers found with the DAPI-stain where moderately high.

Table 33: Microbial percentage ratio and absolute counts at FM 3121 at Palo Gaucho Bayou (Sabine County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
7-C-1-6	33.7095	26.333	26	17.53193	95.4	51.03188
7-C-1-90	46.3683	20.8827	29.8	16.8448	73	35.65625
7-C-2-90	16.9786	14.7155	20.8	21.30876	118.8	79.54714

3.0.2.4 Microbial analysis for SH 31 West Bound at Kickapoo Creek (Henderson County)

Table 34 represents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain at SH 31 West Bound at Kickapoo Creek (Henderson County). The percent average of EUB338/DAPI was low for both columns. Absolute numbers observed with both the Cy3-EUB338 and DAPI stain were low as well.

Table 34: Microbial percentage ratio and absolute counts at SH 31 West Bound at Kickapoo Creek (Henderson County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
8-C-4-6	29.3097	35.1382	19.2	12.95173	42	15.60027
8-C-2-6	33.6069	41.9119	14	14.00752	23.8	22.00383
8-C-2-18	30.5644	42.9503	10.2	11.01482	20.25	12.76457

3.0.2.5 Microbial analysis for SH 31 East Bound at Kickapoo Creek (Henderson County)

Table 35 represents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain at SH 31 East Bound at Kickapoo Creek (Henderson County). The percent average for EUB338/DAPI was low for both elevations taken on column 4. Absolute numbers observed for Cy3-EUB338 and DAPI stain were moderately high for the sample taken closest to the ground (9-C-4-18).

Table 35: Microbial percentage ratio and absolute counts at SH 31 East Bound at Kickapoo Creek (Henderson County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
9-C-4-18	36.912	39.3815	48.8	26.9924	75.8	34.88266
9-C-4-54	5	22.3607	0.4	1.788854	0.4	1.788854

3.0.2.6 Microbial analysis for FM 787 at Tarkington Bayou (Liberty County)

Table 36 presents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain for FM 787 at Tarkington Bayou (Liberty County). The percent average for Cy3-

EUB338/DAPI was slightly high at all elevations. The absolute numbers observed for the Cy3-EUB338 and DAPI stain at both elevations were low.

Table 36: Microbial percentage ratio and absolute counts at FM 787 at Tarkington Bayou (Liberty County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
10-C-2-6	36.996	26.939	8.4	6.210348	28.6	19.86375
10-C-2-90	44.6061	41.749	5.2	6.100906	11	11.52571

3.0.2.7 Microbial analysis for FM 751 at Duck Creek (Hunt County)

Table 37 presents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain for FM 751 at Duck Creek (Hunt County). The percent average for Cy3-EUB338/DAPI was low. Absolute numbers observed for both the Cy3-EUB338 probe and DAPI stain were also low at all elevations taken on column 1.

Table 37: Microbial percentage ratio and absolute counts at FM 751 at Duck Creek (Hunt County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
11-C-1-6	22.5614	31.9803	10.2	15.70652	34.8	28.13801
11-C-1-30	7.75	23.31	8.4	17.30957	19	24.95891
11-C-1-66	17.0714	36.1477	1.8	2.745331	20	23.57519

3.0.2.8 Microbial analysis for FM 751 at S. Fork Sabine River (Hunt County)

Table 38 presents the percent average ratio of Cy3-EUB338/DAPI and the absolute numbers of organisms found using the Cy-3EUB338 probe and DAPI stain FM 751 at S. Fork Sabine River (Hunt County) The percent average for Cy3-EUB338/DAPI were moderate to low. The absolute numbers found for both the Cy3-EUB338 probe and

DAPI stain were generally low at all elevations taken on column 1 with the exception of the average for DAPI stain on column 12-C-1-6 which was slightly higher than the other DAPI counts for the other samples.

Table 38: Microbial percentage ratio and absolute counts at FM 751 at S. Fork Sabine River (Hunt County)

Sample	Percent average EUB338/Dapi	Percent standard deviation	EUB338 average	EUB338 standard deviation	DAPI average	DAPI standard deviation
12-C-1-6	35.8154	31.9227	16.2	13.82446	43.8	25.41363
12-C-1-30	37.5595	0.354607	4.8	5.287523	13.6	12.54214
12-C-1-54	17.0043	29.1874	6.2	9.666001	14	20.9058

3.0.3 Compressive strength analysis

The following Tables (See Tables 39-41) present the strength analysis for concrete cylinders for several types of mix designs aged at 1 and 3 months. For sample MD 1 Coated used the abbreviation CT.

3.0.3.1 Compressive strength analysis of concrete cylinders exposed in the field

Table 39 presents the compressive strength test for concrete cylinders having field exposure. Mix designs 1, 2, and 4 required more force to break.

Table 39: Compressive strength test for concrete cylinder's field exposure

Mix type	3 months
MD 1	6291 psi
MD 1 (CT)	5466 psi
MD 2	6106 psi
MD 3	4920 psi
MD 4	6041 psi
MD 5	5703 psi

Table 40 presents the compressive strength test for concrete cylinders having 1% sulfuric acid exposure, mix designs 2, 4, and 5 required more force to break.

Table 40: Compressive strength test for concrete cylinders 1% sulfuric acid exposure

Mix type	3 months
MD 1	3699 psi
MD 1 (CT)	4743 psi
MD 2	6331 psi
MD 3	4499 psi
MD 4	6757 psi
MD 5	5125 psi

Table 41 shows the strength analysis for concrete cylinders exposed to a 3% sulfuric acid mix; both mix designs required a relatively low amount of force to break.

Table 41: Compressive strength test for concrete cylinders 3% sulfuric acid exposure

Mix type	1 months
MD 1	2571 psi
MD 1 (CT)	3500 psi

4.0 Discussion

FM 787 at Tarkington Bayou (Liberty County-bridge 1, revisit, and bridge 10), are located near the Houston area (Figure 1). All three visits to the Tarkington Bayou site yielded samples with a low concentration of sulfate and chloride in the water; however when taking into consideration the concentration found in the concrete, it was different. Initially, for bridge 1, the highest concentration of sulfate was found in sample 1-C-1-22 with the other samples having a low concentration of sulfate present. Later, when the site was revisited, the concentration of sulfate was higher on the wall at lower elevations while samples obtained higher on the column also had a higher concentration of sulfate. Finally, bridge 10 had a higher concentration of sulfate at lower elevations. In both cases mud samples were obtained, and the concentration of sulfate was higher in the mud than in the water. It is clear that sulfate and chloride concentration can vary with the elevation above water, year, and environmental conditions. Variations could be attributed to environmental sulfate that could be present in the water and in the mud (Bair and Cann, 2005). Iron sulfide can be found in the natural environment and can be oxidized readily to water soluble sulfates in Texas aquifers (Skalny et al., 2010). Another possible source of variability is contamination from nearby chemical plants and agricultural fertilizer that can be dissolved either in the water or in precipitation (Skalny et al., 2010).

SH 21 at Navasota River (Robertson-Bryan District) located near Houston (Figure 1; bridge 2) had a higher concentrations of sulfate at all elevations except for a sample taken at 12 inches (2-C-2-12) which had less than half of the concentration of sulfate found in the other samples. For chlorides, the lowest concentration was found on column 1 at 36 inches (2-C-1-36).

SH 276 at Lake Tawakoni (Hunt County-bridge 3), SH 31 West Bound at Kickapoo Creek (Henderson County-bridge 8), and SH 31 East Bound at Kickapoo Creek (Henderson County-bridge 9) are all located near the Dallas area. These bridge locations all had a higher concentration of sulfate present at lower elevations and a decrease in sulfate concentration with elevation. The concentration of sulfate and chloride found in the water samples was generally low; however, it is interesting to note that the mud samples at these bridges had a higher concentration of sulfate present than in the water which can indicate a sulfate enrichment process.

There are many factors that can contribute to sulfate and chloride found in concrete. Concrete has a natural concentration of sulfate that is present when first prepared. In addition, groundwater can have dissolved sulfate that is found naturally in the environment (Skalny et al., 2010). There are many aquifers across Texas within all the sites visited which can affect the concentration of sulfate present in the concrete when it is exposed for an extended period of time to the ground water (Bair and Cann, 2005).

SH 82 at Alligator Bayou (Jefferson County) are located near the Houston area (Figure 1, bridge 4); three types of samples were taken from the concrete column: a biofilm sample, a corroded sample, and a non corroded sample. It is interesting to see that the corroded sample (4-C-5-3) had the lowest concentration of sulfate and chloride while the non corroded sample (4-C-5-34) had the highest concentration of sulfate in the concrete. The sulfate and chloride content was also high in both the mud and the water samples.

Figure 1 shows the relative locations for FM 276 at Patroon Bayou (Sabine County; bridge 5), SH 21 at Carrice Creek (Sabine County; bridge 6), FM 3121 at Palo Gaucho Bayou (Sabine County; bridge 7), and FM 751 at South Fork Sabine River (Hunt County; bridge 12). FM 276 at Patroon Bayou (Sabine County) concrete samples were taken from two columns; for the first column, the concentration of sulfate was moderate, while for the second column the sulfate and chloride concentrations were at least twice as much as the first column. The sulfate and chloride content in both the water and the mud samples were generally low with an acidic pH.

Samples were taken from two columns at SH 21 at Carrice Creek (Sabine County); column 3 had twice as much sulfate and chloride at lower elevations than column 1. The sulfate and chloride concentration in the water and mud were both low. Samples were taken from two columns at FM 3121 at Palo Gaucho Bayou (Sabine County); the first column had a concentration of sulfate and chloride that was higher closest to the ground while for column 3, the concentration of sulfate was higher at higher elevations. The absolute counts observed with the Cy3-EUB338 probe and DAPI stain for the Patroon Bayou location was high in comparison to the other sites visited. Even though this column has high population of bacteria they are not necessarily associated with concrete degradation by means of sulfur oxidizing and sulfur reducing mechanisms.

In addition, both Palo Gaucho and Carrice Creek have results for sulfates and chloride that are not in agreement with each other for example, where one column has high concentrations of sulfate and the second column has higher sulfates at higher elevations, these results could be attributed to how the samples were collected at the site visit.

Finally, for FM 751 at South Fork Sabine River (Hunt County) the concentrations of sulfates and chlorides in the water samples were generally low. However, the concrete had higher concentration of sulfate and chloride at higher elevations. These previous bridge sites that were discussed varied in the concentration of sulfate from column to column within the site. There are several possibilities for these variations. First, these bridges that have been exposed to a body of water at some time within their lifespan and water can easily extract sulfate that is not chemically bound from the lower elevations exposed to water.

Sulfur dioxide and hydrogen sulfide are byproducts of the petroleum and paper mill industry which can be absorbed in precipitation (Baird and Cann, 2005). Sulfur dioxide can be oxidized to sulfur trioxide and in the presence of water to generate sulfuric acid (Baird and Cann, 2005). Contaminated precipitation can also contribute by depositing onto the surface of a column (Baird and Cann, 2005).

$$2SO_2\ (g) + O_2 \rightarrow 2SO_3\ (g)$$

$$2SO_3\ (g) + H_2O \rightarrow H_2SO_4\ (aq)$$

$$H_2SO_4\ (aq) + H_2O \rightarrow HSO_4^- + H_2O \rightarrow SO_4^{-2}$$

Another important consideration is sulfate that is already present in concrete. Portland cement contains calcium sulfate which aides the rate of hydration of calcium silicate thereby strengthening the concrete (Skalny et al., 2002). Potassium sulfate is a water soluble sulfate found in the clinker which is easily extracted in water (Skalny et al., 2002). Alkali-calcium sulfates are another common component in cement which is water insoluble (Skalny et al., 2002). Therefore extractions involving water only extract sulfate that is water soluble and is not chemically bonded to the aggregate and cement paste (Skalny et al., 2002). Only non chemically bonded sulfate was analyzed for the course of this study. Future studies investigating microbial induced concrete degradation should include both non chemically bonded and aggregate bonded sulfate.

There are many factors that can contribute to the concentration of sulfate and chloride in concrete. Permeability of concrete is the penetration of chemicals dissolved in water (Kosmatka et al., 2002). There are several factors which influence the permeability of the portland cement concrete paste and aggregate quality, rate of hydration, and the length of time of moist curing (Kosmatka et al., 2002). The permeability of the paste is important because it provides the structure with the adhesive component (Kosmatka et al., 2002). The water-cement ratio influences the rate of permeability of the paste therefore water-cement ratio should be low (Kosmatka et al., 2002). For both rate of hydration and the length of time of moist curing longer duration of exposure is better (Kosmatka et al., 2002). With weakened cement paste the concrete is more susceptible to attack (Kosmatka et al., 2002). Sulfate from the environment or hydrogen sulfide produced by microorganisms can permeate through the column in the channels between the course aggregate and the cement paste thereby penetrating at higher elevations into the column where the cement paste is tightly bonded; this depends on concretes porosity (Kosmatka et al., 2002). Water can also play a role in the concentration of sulfate present in concrete columns by either extracting sulfates over the course of several years or by depositing sulfates from the environment (Kosmatka et al., 2002). Dissolved sulfur dioxide from nearby refinery can contaminate precipitate which can be oxidized to hydrated sulfate which can be in equilibrium with sulfate (Kosmatka et al., 2002). In regards to the concentration of chloride, sea water and deicers are possible sources (Kosmatka et al., 2002; Weritz et al., 2006). The reinforcement bar (rebar) is the support system of the concrete column providing it with durability and strength (Kosmatka et al., 2002). High quantities of chlorides pose a threat to the rebar due to the formation of an electrochemical cell on the steel bars (Kosmatka et al., 2002). One end of the steel bar becomes the anode and the other end becomes the cathode (Kosmatka et al., 2002). Hydroxide ions are formed due to the electric current that is at the anode end. The iron and hydroxide ions form iron hydroxide which oxidizes to iron oxide (Kosmatka et al., 2002). This process can lead to the expansion of the reinforcement bar by a factor of four and eventually cause the collapse of the column (Kosmatka et al., 2002). In the case of the bridges identified by the Texas Department of Transportation, the depth of color change using phenolthalein in a column is less than 40mm which is minimal when the entire width of the column is taken into consideration. Under non degradative conditions where concrete has a basic pH the reinforcement bar forms a protective oxide barrier around itself (Malhotra and Carino, 2004). In addition, in the event that chlorides and or calcium hydroxide reach the rebar there is a protective oxide barrier is degraded due to penetrating chlorides (Kosmatka et al., 2002; Malhotra and Carino, 2004). Calcium hydroxide reacts with carbon dioxide present in the pores of the concrete yielding sodium carbonate and water (Malhotra and Carino, 2004; Weritz et al., 2006). These two types of degradative processes do not occur until the corrosion threshold is reached (0.15% water-soluble cement) and pH drops rebar corrosion may begin (Kosmatka et al., 2002; Malhotra and Carino, 2004).

$$Ca\,(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

The probe used for bacterial identification was Cy3-EUB338 which is a general probe for identifying bacteria. Other probes need to be utilized for the characterization of bacteria that are associated with the sulfate reducing and or sulfate oxidizing bacteria. *Thiobacillus thiooxidans* is an organism that ends products is sulfuric acid the specific probe targeting this organism is Thio820 (Trejo et al., 2008). In addition, the probe S-S-T.int-04420a0A018 targets *Thiomonas intermedia* and *Thiomonas peromotabolis* these probes are a few of many that can be used for the detection of organisms associated with concrete degradation (Trejo et al., 2008) . However, these probes were not utilized because overall organisms detected within the Domain Bacteria were low. The organsims found on these bridges are bacteria that can be found in any environment. Perhaps the bacteria associated with sulfate reducing and or sulfate oxidizing mechanisms are present in these bridges but are present in a dormant state such as spores. In addition, there is no method to detect dormant states of bacteria only when the optimum environmental conditions arise can these dormant bacteria become vegetative and begin to colonize and make detection of these organisms possible.

5.0 Conclusions and Recommendations

Microbial induced deterioration (MID) of concrete bridge columns in Texas does not appear to pose a threat to their durability and strength. However, it is important to monitor the concentration of sulfate and chloride during the lifespan of a bridge column due to the potential damage that MID may pose on the concrete. Although the surface of various bridge sites appear to be deteriorated the columns overall are in excellent condition because the bacteria associated with sulfate reducing sulfate oxidizing are not present and chloride concentrations are generally low therefore these concentrations do not pose a threat to the integrity of the reinforcement bar. In addition, the depth of penetration for several site visits was less than 40mm suggesting that the concrete has a basic pH at depths greater than 40mm. This type of basic pH environment does not provide the ideal conditions for the microorganisms associated with MID (microbial induced concrete degradation). Therefore, MID corrosion does not pose a threat to these columns. Future microbial attack investigations should involve analyzing the concrete column's initial sulfate and chloride content. In addition, future extractions should be assisted by heating, sonication, and vortexing finely pulverized concrete, in an effort to extract all of the loosely-bound sulfates.

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