DCT 221

Impacts of Bird Droppings and Deicing Salts on Highway Structures: Monitoring, Diagnosis, Prevention

By

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Impacts of Bird Droppings on Highway Structures

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Abstract:

Results of this study indicate that the chemical composition of bird droppings can vary dependent of sources. Generally urban birds appear to discharge feces that contain more variety of elements than the farm-grown. Results also show that the biological properties of bird droppings are different dependent on the sources. Various species of bacteria and fungi are found and identified in the two bird fecal samples. The urban birds appear to have feces contain both bacteria and fungi whereas the farm-grown birds have uniformly shown more fungi than bacteria species. The following fungal species have been identified: *Geotrichum.* The following bacteria have been observed. For Sample A, they are *Staphylococcus-lentus* and *Corynebacterium-glutamicum***.** For Sample B, they are *Bacillus-pumilus* and *Staphylococcus-xylosus***.** For Sample C they are *Citrobacter-amalonaticu* and *Stenotrophomonas-maltophilia.* The occurrence of bacteria in bird feces is not surprising. As the bird are feeding on various food sources available to them. As such it is expected that birds, especially pigeons can be easy vector for the transmission of germs in the environment. Therefore, it is recommended that precautions be observed during cleanup of bridges for the removal of bird droppings. This is most important during time of epidemics. The corrosion of concrete materials is a slow process. Preliminary results did show the impacts of bird droppings on the concrete.

1.0 Statement of Problems

Transportation structures are keys to economic development while serving the general infrastructural needs of society. Billions of dollars are being spent on the maintenance and improvement of these vast systems due to deterioration from processes such as freeze-thaw, deicing salts, and sulfate attack. One of the major issues is the diffusion of chemicals from bird droppings and deicing salts into the concrete of highway structures leading to cracks, weakening and corrosion of materials. Due to the high chemical reactivity of bird droppings and high concentration of salts such as sodium and magnesium, the accumulation and leaching of these components into concrete and steel structures is having a detrimental effect on the construction materials and the health of the infrastructures. Many of these chemicals in deicing salts and bird droppings are suspected to have played a role in the collapse of several bridges including the Minnesota Bridge in 2007. There is no study on the impacts of bird droppings on transportation structures and life cycle cost analysis. Very little is known on how much role bird droppings play on the life cycle of the structures.

Objectives

This research project was to develop data and tools necessary for decision-making process at DelDOT in transportation structure monitoring and corrosion prevention due to bird droppings. Using the information gathered during research a life cycle cost analysis can be made. Specifically, the project was to achieve the following objectives:

(1) Study the chemistry and the biology of bird droppings as to identify pertinent variables contributing to the health of highway structures
(2) To study the chemistry and biology of bird droppings as to gain insights into the pathogenic nature, and
(3) To study the dissolution of concrete materials in the presence of bird dropping.

Historically, man's outlook on pigeons has changed multiple times while the world continues to evolve structurally and socially. During WWII carrier pigeons, also known as "homing pigeons", were valuable resources of military operations. They were sent long distances to carry messages, therefore at the time, acting as a method of communication. As the world evolved and more industrial and urban areas were being set up much of the wildlife that once acted as home to many animals disappeared. With the evolution of time came different modes of communication and the carrier pigeon was no longer needed. Furthermore, with rapid urbanization development, virtually every city deems these once valuable creatures with names such as "rats with wings."

The problem with the overabundance of pigeons in the urban areas is the desecration of cultural icons such as statues, architecture, and art. While it is not only unsightly, there is visual proof that droppings of these birds are having corrosive effects on structures. There is little information on how much corrosion of infrastructures is taking place. This has prompted us to quantify the detrimental effects the droppings may have on highway structure, i.e., bridge.

The droppings were characterized for chemical, biological, and physical properties. Information on the characteristics of bird droppings allows us to gain better understanding of what the droppings are made of and how they could harm not only highway structures but also to humans. It is known that when the droppings are removed from structures they often become airborne which can cause illness in humans. Some of the most common pathogens, bacteria, and fungi, found in pigeon droppings include: *histoplasmosis, cryptococcus, psittacosis, E. coli, bacillus pumilus, staphylococci,* and *streptococc*i. Chemically, the composition of anions and cations is a concern with respective to the structural integrity of highway structures.

Two of the most dangerous ions are chloride and magnesium which were present in most deicing salts. Magnesium can react with concrete paste which is made of calcium-silicate-hydrate (C-S-H) and calcium hydroxide. The C-S-H increases bond strength in concrete while calcium hydroxide contributes alkalinity that resists acidic corrosion. When magnesium reacts with C-S-H, calcium chloride and magnesium-silicate-hydrate are formed, which in turn will lower the bond strength therefore weakening the structure. When magnesium reacts with calcium hydroxide it produces magnesium hydroxide and calcium chloride. This reaction is also detrimental to highway structures because it reduces the pH allowing chlorides to infiltrate and corrode the reinforcing steel in the inner structure. This can be related to pigeon droppings because it is believed that there are similar chemicals components in the bird droppings.

The bird droppings samples used in this research were obtained from two different locations. One was a privately owned bird farm in Bear, Delaware called Thompson's Bird Farm and the other was from the undercarriage walkways and maintenance areas of bridge number 1-693 on the E. 4th Street over the Christina River in Wilmington, Delaware. For the sack of simplicity the bird droppings from Thompson's Bird Farm as termed "sample A" and the sample from E. 4th Street as "sample B". A third sample (sample C) was the same as sample A, only it was fresher specimen. The diet of birds at sample A site consisted of a multigrain feed called Purgrain European Supreme Feed whereas the diet of birds at sample B site was somewhat unknown. It is assumed that birds at sample B, fed on a mixture of human foods, berries, nuts and insects. The diets of the birds at these two sites varied and contrast greatly and their living conditions different, it is expected that there will be different in properties and environmental impacts between these samples.

2.0 Materials and Methods

2.1 Bird dropping

The droppings used for this research were obtained from two different locations. One was a privately owned bird farm in Bear, Delaware called Thompson's Bird Farm and the other was from the undercarriage walkways and maintenance areas of bridge number 1-693 on E. 4th Street over the Christina River in Wilmington, DE (Sample A). For the duration of this project and for simplicity the bird droppings from Thompson's Bird Farm were called Sample A and the sample

from E. 4th Street was Sample B. Also a third sample (Sample C) the same as Sample A was only fresher specimen. The bird diet of Sample A consisted of a multigrain feed called Purgrain European Supreme Feed whereas the diet of Sample B was unknown. It is assumed that birds of Sample B, being of this species and from the city of Wilmington, ate a mixture of human foods, berries, nuts and insects. The diets of the two specimens contrast so greatly and their living conditions were very different, our research has thoroughly explored each relevant aspect.

2.2 Physical chemical characterization

The physical chemical properties of bird droppings were characterized for (1) moisture content, (2) water extractable chemicals (both cations and anions) and (3) surface chemical elements (using EDX).

2.1.1 Moisture content

The dry weight was determined gravimetrically. Briefly, the following steps were followed: (1) Weigh three aluminum dishes with weights record (W_1). (2) Add one gram bird droppings to each dish, weigh, and record the weight (W_2). (3) Heat the sample with the aluminum dish in oven at 105^{o}C for 1 hour. (4) Remove sample from oven, weigh and record the weight (W_3). (5) Calculate dry weight of sample by subtracting the dish weight (W_1) from the dish weight + dry droppings (W_3). (6) Calculate water weight by subtracting the dish weight + dry droppings, W_3, from the dish weight + wet droppings, W_2. (7) Put dishes in oven at 550^{o}C for 30 minutes. (8) Remove dishes, weigh and record weight (W_4). (9) Calculate the weight of the ash by subtracting the weight of the dish (W_1) from the weight of the dish + ash (W_4).

2.1.2 Water extractable chemicals

Water extractable chemicals were analyzed by leaching the bird droppings with (distilled deionized water) DIW. Six grams of droppings were added 120 mL of DIW water in a beaker and mixed for 18 hours with 1" magnetic stirring stick. The solution was centrifuged at 3500 rpm for 15 min as to separate the solid from the solution. The supernatant was filtered with 2.5 μm-filter paper under suction to collect the filtrate which was filtered again through 0.2 μm-Nylon membrane using syringe filter. The filtrate was collected and stored in refrigerator until use. The chemical composition of major anions, namely, chloride, nitrate, sulfate and phosphate were analyzed using ion chromatography (Model: Dionex DX500) equipped with analytical/guard column: Dionex AS16 and GS16, AS40 automated sampler, LC30 chromatography oven, ED40 electrochemical detector, and GP50 gradient pump.

Major cations, namely, Ca. Mg were analyzed using atomic absorption spectrophotometer. Ammonia was analyzed using the phenate method. Briefly, an aliquot solution containing the bird dropping was diluted 1000x then added phenol reagent. Once color is stable for 24 hours measure absorbance in spectrophotometer at 640 nm and record. Sample A was mixed on June 16, 2010, Sample B was mixed on June 21, 2010, and Sample C was mixed on July 19, 2010.

2.1.3 Surface observations

Bird droppings were observed under scanning electron microscopy (SEM) for morphological properties and X-ray spectrophotometry (EDAX) for surface elements. Samples were air dried and attached to aluminum stubs, then sputter-coated with gold-palladium on a Denton vacuum bench top Turbo III. For concrete samples use double sided tape on bottom of concrete to stick to plate. Place in the microscope with the appropriate rod and holder. A Hitachi S-4700 Field Emission scanning electron microscope was used to image the samples with the secondary electron detector at different working distances for each image based on area being captured. For EDX after capturing images analyze them with the appropriate computer software for elemental composition taking certain sections at a time. SEM/EDX-Field Emission

2.2 Biological characterization

The biological properties of bird droppings were characterized using FAME (Fatty Acid Modifying Enzyme Analysis) and DNA techniques.

2.2.1 Fatty Acid Modifying Enzyme Analysis

Grow bacteria/fungi colonies for FAME analysis:
Bectan Dickinson nutrient agar-base was used to cultivate nonfastidious microorganisms for FAME assay. The recipe for the preparation of TSBA (trypticase soy broth agar) standard media for aerobes was 30 g TSBA, 15 g granulated agar, and 1 L distilled water. Nutrient agar was prepared by mixing 3 g beef extract, 5 g peptone and 15 g agar.

Preparation of FAME reagents:
Four reagents are required to cleave the fatty acids from lipids: Reagent 1 for saponification (45g NaOH, 150 mL methanol, and 150ml distilled water), Reagent 2 for methylation (325 mL certified 6.0 N hydrochloric acid and 275ml methyl alcohol), Reagent 3 for extraction (200 mL hexane and 200 mL methyl tert-butyl ether), and Reagent 4 for sample cleanup (10.8 g NaOH dissolved in 900 mL distilled water.) Because we were unsure of how much sample to use, we tried three different amounts of sample in the bottom of each test tube as well as a blank with just the reagents in it. Since it is a qualitative test, however, the amount of sample did not have much significant influence on the results. Add 1 mL of reagent #1 to each test tube, cap, and vortex for 5-10 seconds. Put in metal heating basket and heat in boiling water for 5 minutes. Remove and vortex for another 5-10 seconds then place back in boiling water bath for another 25 minutes. Remove and cool the test tubes, then add 2 mL of reagent #2 to each. Vortex for 5-10 seconds, caps, and put in hot water bath in test tube rack at 80^{o}C for 10 minutes. Remove and cool, then add 1.25 mL of reagent #3. Cap test tubes and mix in rotator for 10 minutes. Remove bottom phase of solution from test tubes and discard. Then add 3 mL reagent #4 to test tubes and rotate 5 minutes. Remove top phase (approximately 1 mL) and put in GC vials. Cap vials. Load calibration standard into position 1 and samples subsequently. Identify samples on sequence table in same order. Turn on carrier gas (H_2) and FID gas (N_2, H_2, air). Press start sequence and wait for samples to finish running. Click analysis and go to 2D plot and Dendrogram.

FAME procedures:
Mix 5 mL sterile water with small amount of bird droppings and then vortex to dissolve. Make solution as normal with DI water and decant. Save residual sludge for analysis. In microbiology hood pipet 100 microliters of sterile samples as well as sludge samples into individual sets of TSBA and Agar petri dishes. (Use a different set of petri dish agar and TSBA for each sample). Using a spreader cover the surface of nutrient agar or TSBA with sample. Close and seal with parafilm around edges, then label. Put in incubator at 28^{o}C and allow them to incubate for at least 24 hours.

Harvesting/Streaking Cultures:
Under a clean microbiology hood place incubated petri dishes with cultures in the hood along with other sterile streaking and extraction devices. Take a new petri dish with nutrient agar or TSBA and label it correspondingly with the grown dish. Using a steel extractor/streaker dip in ethanol and then flame until an orange glow is attained. Dip this in the side of the new petri dish to ensure cooling and not to kill the bacteria being harvested. Gently skim and pick up enough culture to streak the first quadrant. From this quadrant all others will be streaked. Dip in ethanol, flame, and cool between streaking. Follow this method for all dishes. Seal transferred cultures and incubate at 28^{o}C for at least 24 hours. The newly grown bacterial cultures can be harvested from the 2nd and 3rd quadrant confluent cells and used for FAME analysis.

2.2.2 DNA analysis

Extraction:
Using 2.5 mL capped tubes add 1 mL DNA extraction buffer. Then using metal streaking loop pick up a small amount of DNA from the bacteria plates and dip in buffer allowing it to fall off the loop into the buffer. Flame loop in between each sample. Add 10 µL proteinase-K and 20 µL lisozyme. Incubate for 15 minutes at 37^{o}C. Incubate for 15 min at 37^{o}C and then freeze at -80^{o}C (two times). Incubate at 37^{o}C until thawed. Add 100 µL of 10% Sodium dodecyl sulfate. Invert to mix. Incubate for 1 h in 65^{o}C water bath. Add 1 mL phenol: chloroform: isoamyl alcohol (25:24:1) to each microtube then vortex. Centrifuge at 3000 rpm at 4^{o}C for 5 min. Transfer top layer to new tube and repeat last two steps. Transfer top layer to new tube and add 600 µL of room temperature 100% isopropanol. Invert gently to mix and then incubate at room temperature overnight. Centrifuge at 13000 rpm for 30 min. Pour off buffer and isopropanol. Add 1 mL of 70% Ethanol. Invert several times. Then spin at 13000 rpm for 10 min. Pour off ethanol and repeat the previous step. Pour off ethanol and dry pellet. Resuspend in 30 µL of filtered TE. Allow pellet to dissolve for 1 h in refrigerator. Aliquot 25 µL to new tube and place in -80^{o}C.

Clean up:
Transfer DNA to provided Zymo spin column in collection tube. Centrifuge at13000 rpm for 30 seconds. Discard the flow through. Add 200 microliters DNA wash buffer, then centrifuge at 13000 rpm for 30 seconds and repeat once. Put 10 microliters sterile water into column**.** Transfer column to 1.5-milliliter microcentrifuge tube and centrifuge at 13000 rpm for 30 seconds. Dilute purified DNA to 10 nanograms/ microliter. The specimens are now ready for PCR amplification and then can be sequenced.

2.3 Concrete exposure experiments

Concrete samples were exposure to bird droppings and loss of mass monitored the surface roughness determined using AFM.

2.3.1 Static and dynamic exposure
Preparing the Concrete:
Crush concrete with large workshop crusher. To further crush it use a hand held bowl crusher. Sieve so there is uniform granular size between a 150(bottom) and 250(top) sieves. Average granular size will be 194 micrometers.

Static tests:
Control (blank)- concrete with no bird droppings. Concrete with smeared layer of wet bird droppings. 24" Osram Sylvania Gro-Lux/Aquarium 20 Watt Light (Sunlight 400-700 nm), Temperature maintained at 37^{o}C.

Use nine pieces of concrete to smear wet Sample C bird droppings onto half of each piece. At intervals of 1, 3, and 5 weeks gently scrape/rinse bird droppings off 3 cement samples with DI water, dry for 30 min at 105^{o}C. Then observe under microscope both the side which was exposed to bird droppings, and the side that was not on each piece for physical damage. Samples will be kept under UV light 24 h a day.

Dynamic tests:
Control (blank): 1 gram crushed concrete in DIW. Samples+ 1 g crushed concrete in solution prepared with solutions prepared by filtration of bird droppings water extracts (8- µm Whatman ashless filter paper) Temperature maintained at 23^{o}C. Rotational Speed: 7.5 rpm

Crush concrete to granular size and put through sieve for granular size between 150 (Mesh No. 100) and 250 (Mesh No. 60) microns. Weigh 24 1-g (to four decimal places) amounts of crushed concrete and distribute into 24 test tubes, individually. To 6 of these test tubes add 10 mL DI water. To 6 of these test tubes add 10 mL Sample A solution. To 6 of these test tubes add 10 mL Sample B solution. To 6 of these test tubes add 10 mL Sample C solution. Mix in rotational mixer constantly. Determining Total Suspended

Solids Repeat the following steps at 1, 3, and 5 weeks. Run DI water through filter then put in aluminum weighing dish and dry for 1 h at 105^{o}C. Weigh then put back in for 30 min and weigh and record to ensure difference below 4%. Filter samples following with three 20 mL successive DI water washes. Store liquid filtrate. Put filter with solids in aluminum weighing dish and heat for 1 h at 105^{o}C. Weigh filter and concrete after and record. The difference between this weight and weight of dried filter is total suspended solids.

2.3.2 Atomic Force Microscopy
Tip and sample Preparation:
Twist tip off the gel pack and position in tip holder with the largest area pointing upwards. To load tip in the holder, push the holder down on a flat surface and insert tip until it rests against the back support. Attach four pinholes on back of tip holder to four pinholes on scanner.

Aligning the Laser:
Starting at the top height lower manually using the tip deflection to determine the correct height to scan at, then when at correct height press engage. Tilt the condenser on microscope back and load sample using appropriate clamp. Move the scanner over and align pins, check sum value to see it is still present. Bring samples into focus and use the navigator button to move to an area of interest. Make sure the horizontal and vertical values have not drifted. Now when you have found an area of interest press engage. To capture an image select capture, flatten the image and then name the file and save. To export files go to the Utility menu and select Tiff Export.

3.0 Results and discussion

3.1 Physical-chemical properties

The moisture content of the bird droppings was high for both samples, >99%. The birth droppings also were high in volatile organic content, >99% for both samples. The pH value was neutral for both samples also. Table 1 summarizes eh physical characteristics and pH value of both samples.

3.2 Chemical properties

3.2.1 Surface element contents
Tables 2 and 3 show the results of surface analysis of nonmetals and metals, respectively, in the two bird dropping samples. Generally, carbon and oxygen are major elements in all bird dropping samples which is typical of organic materials such as bird droppings. Bird droppings from pigeons in urban environment appeared to have less chemical elements compared to gram grown ones. In addition to C, N, O, P, Cl and S, farm grown pigeons also have Si, and F in their feces.

Urban pigeons also seem to have less number of chemical elements in their feces. Metals are not major constituents of bird droppings as they constitute the ash content which is small compared to the organic and volatile fraction. Potassium and calcium are the major metallic elements. It must be mentioned that gold and palladium did not belong to the bird feces; rather they are background reading from the mount that holds up the sample during EDX analysis. Three separate bird feces samples from the farm grown showed significant concentration of silver. Figure 1 shows typical EDX spectra of bird dropping samples.

3.3 Biological properties

3.3.1 SEM images
Figures 2 and 3 show the SEM images of the bird droppings for Sample A and Sample B, respectively. Results clearly show the presence of bacteria and fungi species. It is also seen that the Sample A (wild animals) show the presence of both fungi and bacteria, mostly bacillus whereas Sample B, farm grown, show more uniformly in the presence of fungal spores. In order to better identify the bacteria and fungi species present in the bird feces, FAME and DNA technique were used.

3.3.2 FAME analysis
Based on results from FAME analysis, the following microorganisms were identified. Cultures for FAME analysis were shown in Figure 4. The following are results from FAME studies of the bird feces.

Fungi:
Sample C:
Identified: *Geotrichum*
All the other plates were identified as *Gliocladium* even though there were some variations in color.

Bacteria: Sample A:
Identified: *Staphylococcus-lentus, Corynebacterium-glutamicum*

Sample B:
Identified: *Bacillus-pumilus, Staphylococcus-xylosus*

Sample C:
Identified: *Citrobacter-amalonaticus, Stenotrophomonas-maltophilia*

3.3.2 DNA Testing
The results obtained through the DNA sequencing were very similar to the results of the Fatty Acid Modifying Enzyme (FAME) tests performed. This means that the data is reliable and accurate. What is seen in the data is some similarity between the different samples as well as a pattern of slight differences. Some of the species common between the samples include *Bacillus sp., Bacillus safensis,* and *Bacillus pumilus*. These bacteria are very routinely found in bird droppings as well as in the natural environment, which shows that they are not all that significant. As for the difference between the samples it is seen that the strains of many of the different bacilli, *Cornyebacterium,* and Staphylococcus are different from each other. The difference in bacteria and other pathogens in the pigeon droppings are due to diet, living conditions, and degree of human exposure.

Geotrichum is a common fungus in the natural environment and is also many times found on spoiled fruit, meat and other foods. In the household it is sometimes even found in the carpet and damp rotting walls. While *Geotrichum* has low pathogenicity it has been reported to cause disease in people with weakened or suppressed immune systems. Geotrichosis, caused by this fungus can also cause bronchial, oral, and vaginal infections, the main transfer of this infection being airborne. *Gliocladium,* another fungus with no known pathogenicity to humans or animals, is often used in pesticides as a method of protection from harmful fungi and rots. It is often described closely in relation to Penicillium. Some of the bacteria found in the droppings prove to be non-pathogenic as well.

Corynebacterium-glutamicum is a bacterium with no toxicity that does not produce spores. This bacterium produces a number of useful enzymes and compounds sometimes used in the production of amino acids such as lysine. One of the most commonly found bacteria in the studied samples was *Bacillus Pumilus*. This bacterium is naturally occurring in soil and dead plant matter and like *Gliocladium* is sometimes used as an ingredient in pesticides. It has not shown any harmful effects to humans or the environment as of now. A few of the more pathogenic components of the bird droppings may be causation for a bit more caution during exposure.

Staphylococcus lentus and *staphylococcus xylosus* along with other species of this bacteria were found to be present in the samples analyzed, however, *S. lentus* and *S xylosus* were the most prominent. These bacteria are pathogenic species that has shown to cause mild to fatal dermatitis in gerbils and mice. As far as its threat to humans it has been seen to cause pyelonephritis which is an ascending urinary tract infection that reaches the pelvis and kidneys. Another bacterium known to cause urinary tract infections as well as other infection is *Stenotrophomonas maltophilia*. This bacterium is known to colonize in hospital fluids such as irrigation fluids, patient secretions, as well as intravenous fluids. While it is of low

virulence to people with health immune systems those with deteriorated immune systems are at risk for such infection. It does not normally cause infection in people with healthy immune systems due to the fact that it is normally only transferred through invasive devices such as in the medical field. The last bacteria with we have found with sufficient assurance is *Citrobacter Amalonaticus.* This organism is an anaerobic organism, however, is aero tolerant. Like most of the other bacteria we have found it has generally low pathogenicity and will normally cause only mild infections including urinary tract infections in those with healthy immune systems. However, people with weakened immune systems can encounter severe infections because it is an opportunistic species of bacteria.

Taking a broader view of the bacteria and fungi species found in the bird droppings it seems that they are not routinely dangerous to humans. It appears that often those found are normally harmful when one has a weakened immune system or an open area of flesh. This does not mean that caution should not be taken in the removal and cleaning of bird droppings in public areas. Teams that remove large amounts of droppings and are exposed to these bacteria on a day-to-day basis should wear eye protection, nose and mouth cover, and gloves at the bare minimum. There are bacteria regularly found in bird droppings that are more pathogenic than those found in this research and should be reason enough for concern.

3.4 Concrete dissolution

Corson of concrete materials in the presence of bird feces is shown in loss of weight (Figure 5). Generally concrete materials exposed to bird feces lost weight to a greater extent than the control.

The degree of damages to concrete by bird droppings was investigated by observing the changes of surface roughness using AFM (Figure 6). Based on the analysis of the roughness of both the control and experimental data is seems that the control pieces are rougher than the experimental pieces with bird droppings on them as shown in Figure 7. However, upon looking at other aspects of this analysis such as the phase (softness of the material), we have reason to believe that there is a logical explanation for this. When comparing the phases of the control and experimental pieces of concrete, the experimental seems to be softer. We believe this is due to the components of bird droppings that were able to make their way into microcracks in the concrete which would fill them causing it not only to become smoother but filled with material much softer than that of the concrete. The benefit of the AFM was that we were able to look at the concrete on a surface level and could determine any faulty information. Overall the data presented from this experiment shows that bird droppings are able to penetrate the microcracks of concrete and looking at the leaching process from the dynamic concrete test we can make the assumption that the dried droppings can have an effect on the strength of the material. When this weakend concrete is put under stressors such as carloads and freeze-thaw of precipitation it is more likely to succumb to corrosion and deterioration.

Looking at the results for the dynamic concrete testing it seems that the test tubes with bird dropping solution showed a steady decrease in suspended solids. The bird droppings had a corrosive effect on the concrete that eroded it to a size smaller than the pore size of the filtration paper being used. Although it can be seen that distilled water also eroded the concrete to a smaller particle size we have reason to believe that the actual suspended solid weight of the concrete with specimen in them were actually less than measured. This is due to the fact that the specimen solution over time may have crystalized in small amounts on the concrete particles.

Looking at corrosion on a larger scale such as city bridges and highways bird droppings accumulate in much larger quantities and with more frequency. The difference between the experimental methods and real life situations is that on these highway structures there is more renewal and leaching of the corrosive components in these bird droppings. This can be attributed to regular rainfall and constant build up of fresh bird droppings, which in turn will greatly increase the corrosion and deterioration of the concrete, which we have only seen on a small scale in my experiments. Overall bird droppings should be taken into consideration when maintaining and constructing highway structures. Some types of precautions that can be taken in the construction process currently being tested are using epoxy coated rebar, replacing rebar

with a plastic composite, and concrete that is less permeable. Often newer concrete is more susceptible to corrosion because it is not fully hydrated and is therefore more absorbent. To treat this situation there is a siloxane sealer that has been tested and proved to completely prevent the absorption of chlorides by concrete.

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5.0 Appendices

5.1 Appendix A: Implementation Plan

Results of this study indicate that the chemical composition of bird droppings can vary dependent of sources. Generally urban birds appear to discharge feces that contain more variety of elements than the farm-grown. Results also show that the biological properties of bird droppings are different dependent on the sources. Various species of bacteria and fungi are found and identified in the two bird fecal samples. The urban birds appear to have feces contain both bacteria and fungi whereas the farm-grown birds have uniformly shown more fungi than bacteria species. The following fungal species have been identified: *Geotrichum.* The following bacteria have been observed. For Sample A, they are *Staphylococcus-lentus* and *Corynebacterium-glutamicum***.** For Sample B, they are *Bacillus-pumilus* and *Staphylococcus-xylosus***.** For Sample C they are *Citrobacter-amalonaticu* and *Stenotrophomonas-maltophilia.* The occurrence of bacteria in bird feces is not surprising. As the bird are feeding on various food sources available to them. As such it is expected that birds, especially pigeons can be easy vector for the transmission of germs in the environment. Therefore, it is recommended that precautions be observed during cleanup of bridges for the removal of bird droppings. This is most important during time of epidemics. The corrosion of concrete materials is a slow process. Preliminary results did show the impacts of bird droppings on the concrete.

5.2 *Appendix B: Sample FAME Calibration*

Prof Method	Prof Time	Flag	Reason	Comment	Library	SI	Identification	Created By
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6		NO MATCH	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.11	Photorhabdus-luminescens-luminescer	
TSBA6	8/19/10 4:46 PM	TRUE	Excessive RMS RI Drift	ECL SHIFT OR DEVIATION EXCEED	TSBA6		NO MATCH	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.66	Escherichia-fergusonii-GC subgroup A	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.66	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6		NO MATCH	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.7	Enterobacter-hormaechei	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.62	Escherichia-fergusonii-GC subgroup A	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.63	Klebsiella-oxytoca-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.19	Grimontia-hollisae	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6		NO MATCH	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.77	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.7	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.7	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.49	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	TRUE	Low Total Response	Total response less than 50000.0. Co	TSBA6	0.57	Staphylococcus-xylosus-GC subgroup	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.77	Escherichia-coli-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6		NO MATCH	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.75	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.62	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.81	Bacillus-filicolonicus"	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6	0.8	Bacillus-pumilus-GC subgroup B	
TSBA6	8/19/10 4:46 PM	FALSE			TSBA6		NO MATCH	

5.3 Appendix C: DNA Sequencing Results

Sample A
4-Bacillus Pumilus
8-No relevant match
17-Corynebacterium glutamicum, arthrobacter
26-uncultured bacterium, sporosarcina
27-uncultured bacterium, staphylococcus lentus, staphylococcus intermedius
28-Alcaligenes sp., Alcaligenes faecalis, uncultred bacterium
30-Bacterium, alcaligenes, bacillus aryabhattai
31-Bacterium, alcaligenes, bacillus aryabhattai
34-No relevant match
35-Staphylococcus lentus, Staphylococcus intermedius, uncultred bacterium
36-Bacterium YWFR, Alcaligenes sp., Alcaligenes faecalis, uncultured alcaligenes
38-No relevant match

Sample B
7-Bacillus Pumilus
9-Bacillus Safensis
12-Bacillus sp., Bacillus Safensis, Bacillus Pumilus
13-Bacillus sp., Bacillus Safensis, Bacillus Pumilus
14-Bacillus sp., Bacillus Safensis, Bacillus Pumilus
15-Bacillales bacterium
16-Staphylococcus equorum
19-Bacillus pumilus, Corynebacterium glutamicum, arthrobacter
21-Bacillus aquimaris, bacillus sp. Bacillus marisflavi
22-Bacillus pumilus, bacillus sp., bacillus safensis
24-Bacillus thuringiensis, bacillus cereus, bacillus anthrascis

Sample C
37-Bacterium YWFR, Alcaligenes sp., Alcaligenes faecalis, uncultured alcaligenes

List of Figures

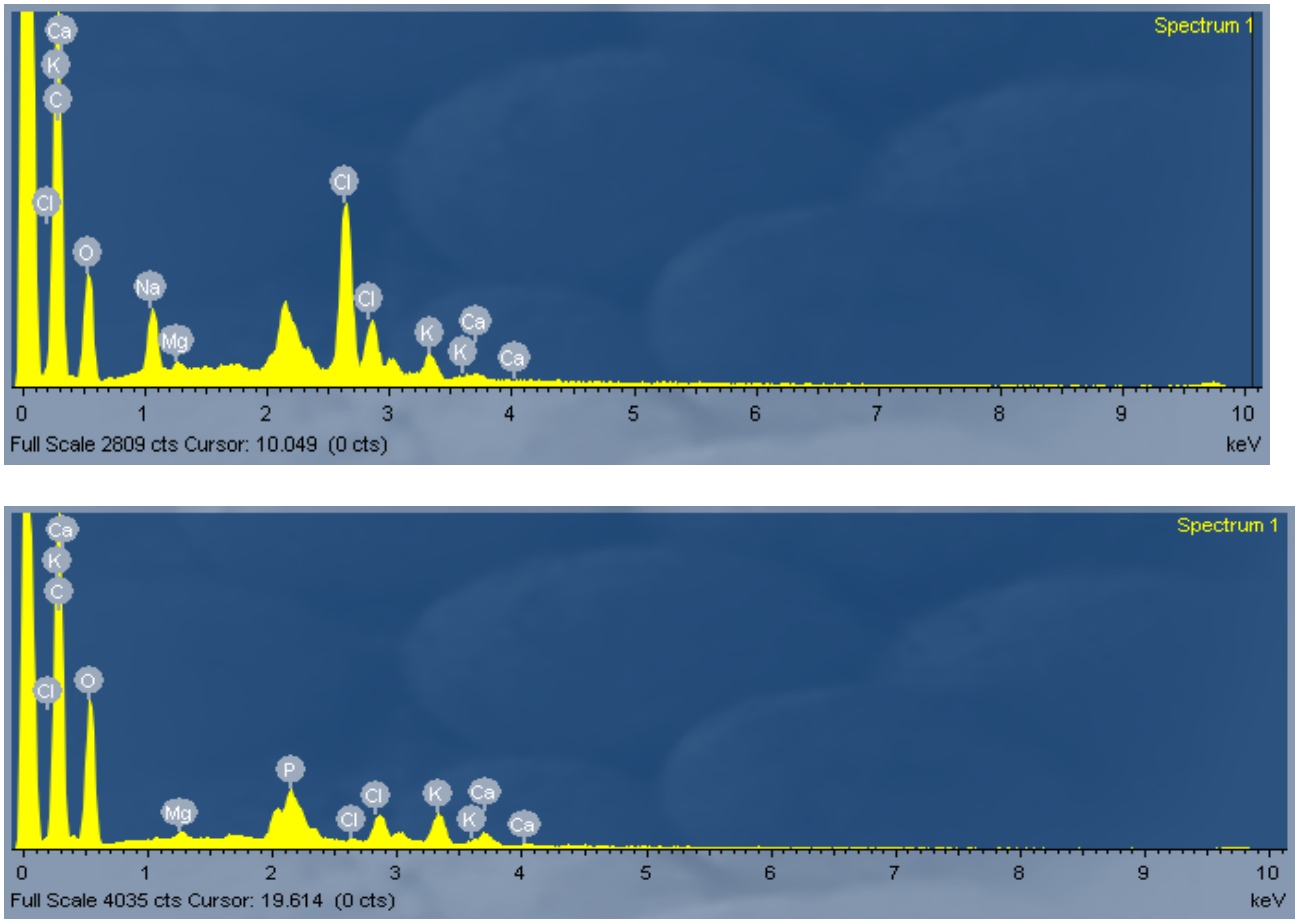

Figure 1 EDX spectra of bird droppings. Sample A (top); Sample B (bottom)

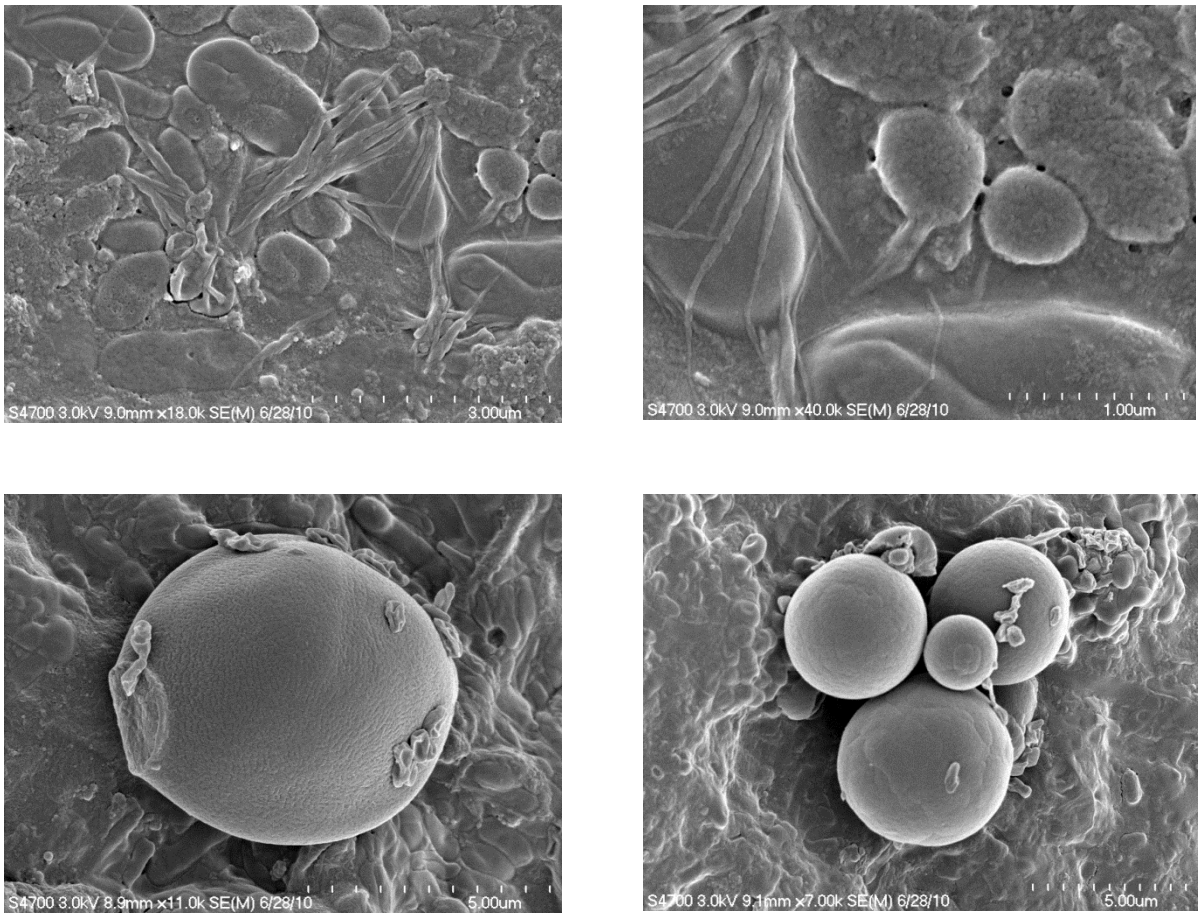

Figure 2. SEM images of bird dropping. Sample A

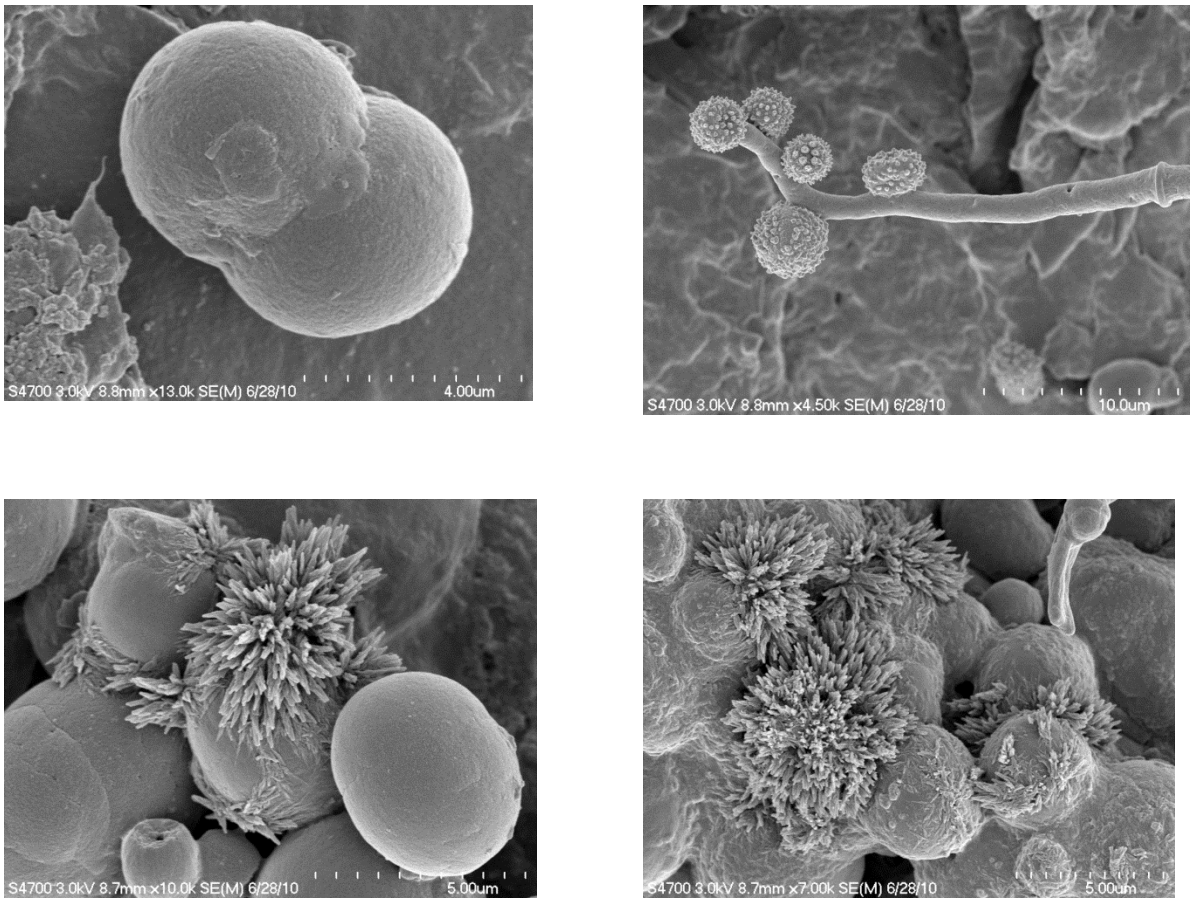

Figure 3. SEM images. Sample B

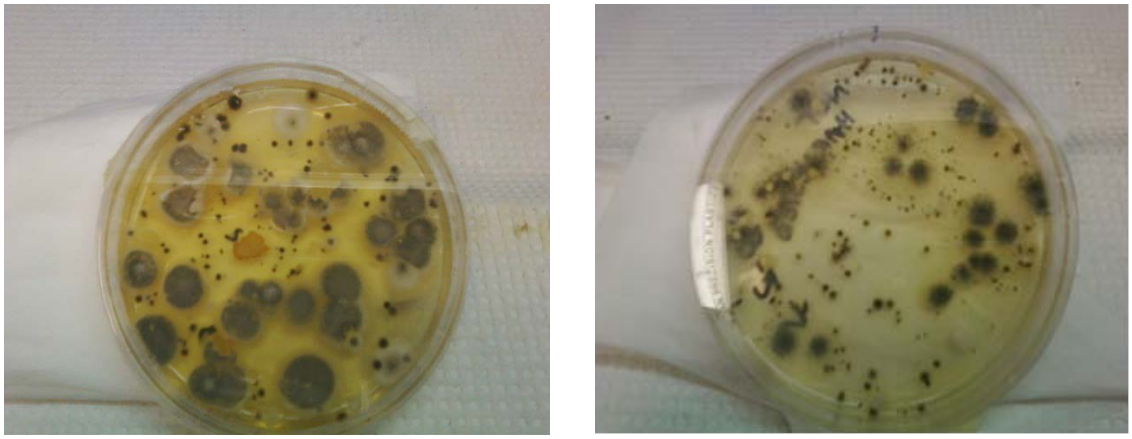

Figure 4. Typical culture of bird dropping re-grown in plate for FAME analysis. The beginning stages of bacteria and fungus plates grown in both TSBA and Nutrient Agar growing media.

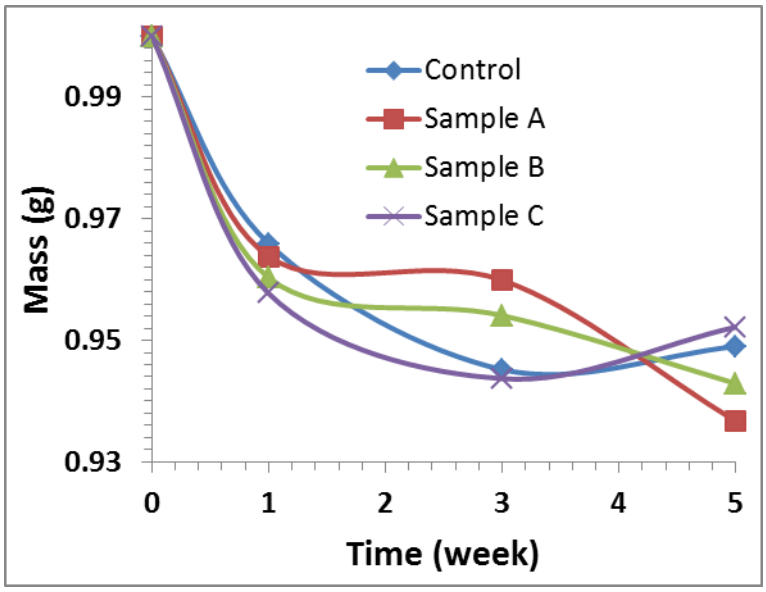

Figure 5. Weight loss in dynamic concrete corrosion test.

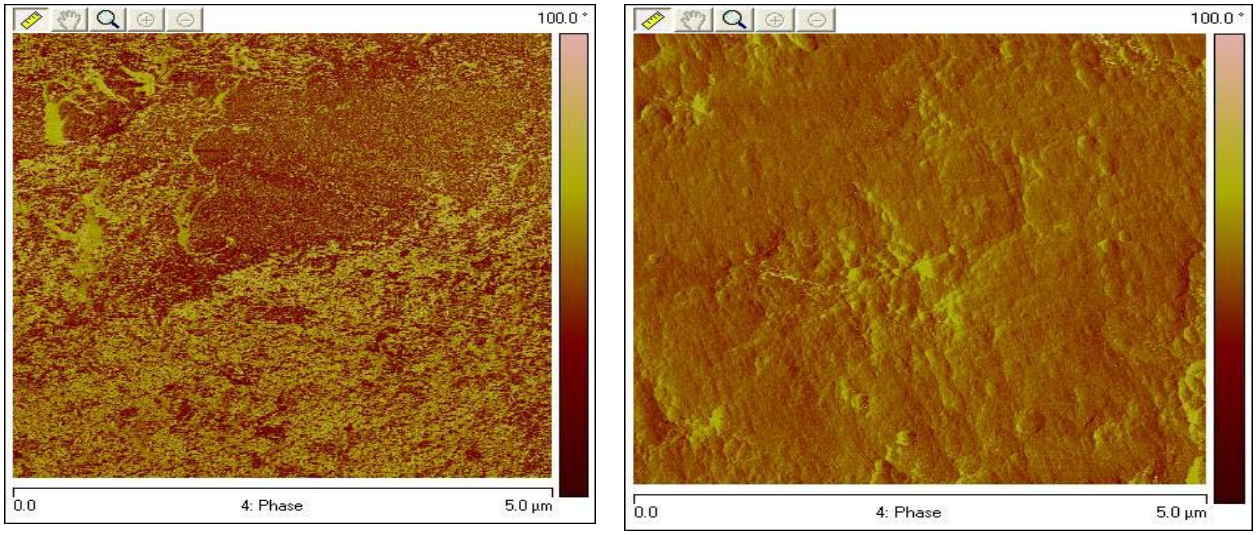

Figure 6. AFM images after five weeks. Control left); sample (right)

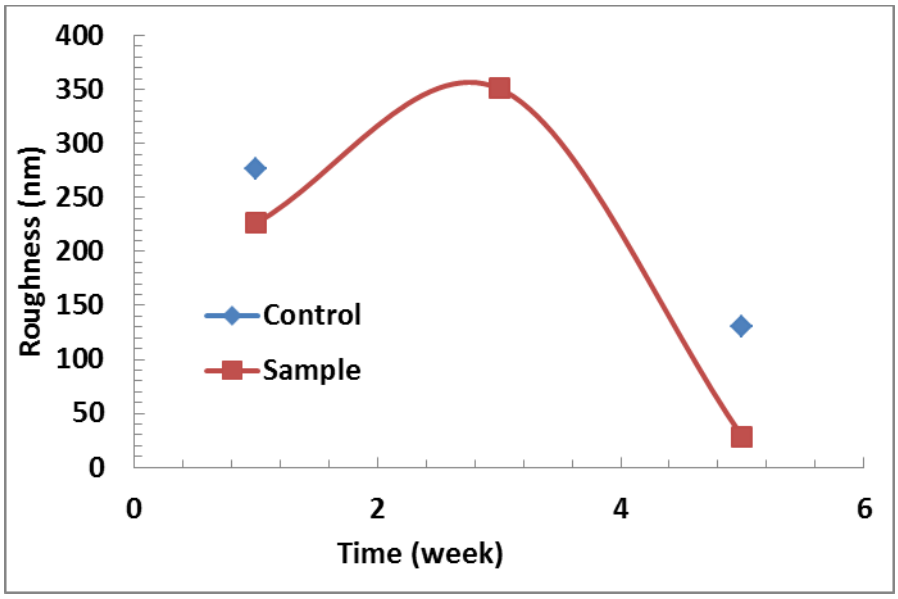

Figure 7. Changes in surface roughness of concrete exposed to bird feces solution.

List of Tables

Table 1. Moisture and Ash Content					
Sample	pH	Solid (%)	Moisture (%)	Volatile (%)	Ash(%)
A	6.71	0.81	99.19	99.46	0.54
A	6.71	0.92	99.08	99.50	0.50
A	6.71	0.90	99.10	99.55	0.45
Ave (A)		0.88	99.12	99.51	0.49
B	6.54	0.85	99.15	99.52	0.48
B	6.54	0.85	99.15	99.53	0.47
B	6.54	0.87	99.13	99.52	0.48
Ave(B)		0.85	99.15	99.52	0.48

Table 2a Non-Metal Content, Sample A (wt %)						
Sample No.	C	N	O	P	Cl	S
1	0.00		70.97	6.36	0.80	
2	26.75	33.58	26.48			
3	56.31		37.96	0.70	0.57	
4	54.34		36.26	3.70		
5	64.29		30.03	0.27	0.43	0.56
6	72.51		22.21		0.75	0.44
7	52.45		44.32		0.69	
8	59.58		32.73	2.37	0.39	0.25
9	52.55		41.21	0.73	0.63	
Ave	48.75	3.73	38.02	1.57	0.47	0.14

Table 2b. Non-metal content (Sample B) (wt %)

Sample No.	C	N	O	Si	P	Cl	S	F
1	57.34		28.94	1.87	1.15	5.11		
2	42.49	22.07	29.78		0.21	2.08		
3	56.02		31.93	1.27	0.25	5.94		
4	32.09		38.37	2.53		1.88		
5	59.87		30.8			4.11	0.62	
6	57.34		28.94	1.87	1.15	5.11		
7	13.65	11.79	13.91	0.4		0.77		8.92
8	24.74		27.78	17.24				4.67
9	28.58		34.19	18.98		0.81		5.22
10	40.64		40.9		2.29	1.9	3.82	
11	17.63		26.74	1.18	5.92	0.89	16.33	
12	28.98		45.28		12.3	1.1		
13	28.7		49.54		3.06	1.04	5.56	
14	5.15		3.69				26.29	1.74
15	34.2	29.65	31.7			1.66		
16	63.4		23.3			8.8		
Ave	36.93	3.97	30.36	2.83	1.65	2.58	3.29	1.28

Table 3a Metal Content, Sample A (wt%)

Sample No.	Mg	K	Ca	Pd	Au
1	3.55	11.39	1.94	12.31	13.02
2		8.51	4.67		
3	0.29	4.18			
4	2.34	3.35			
5		3.80	0.61		
6		4.10			
7		2.54			
8	1.13	2.24	1.31		
9	0.40	3.13	1.34		
Ave	0.86	4.80	1.10	1.37	1.45

Table 3b. Metal content (Sample B) (wt%)

Sample No.	K	Ca	Na	Mg	Al	Ti	Fe	Au	Ag	Pd
1	0.96	1.16	2.31	0.66	0.49					
2	1.76	0.52	1.07							
3	1.08	0.71	2.32	0.25	0.22					
4	0.43	0.34	0.8	0.19	0.62	1.21	0.54			
5	0.97	4.11	2.41	0.31						
6	0.96	1.16	2.31	0.66	0.49					
7			0.73					4.45	45.38	
8			0.69		1.5			10.27	5.15	7.96
9		0.19	0.43		2.99				8.62	
10	0.62	8.67	0.95	0.21						
11	0.4	30.54			0.37					
12	0.42	0.5	0.76	10.66						
13	0.3	11.24	0.57							
14	37.59	25.53								
15	1.13		1.37	0.28						
16	1.45	0.4	2.45	0.25						
Ave	3.00	5.32	1.20	0.84	0.42	0.08	0.03	0.92	3.70	0.50