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Taphonomic Degradation of Chicken Feathers by Bacteria and Fungi in Varying Sediments

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ABSTRACT

The purpose of this study was to identify the relationships between the early taphanomic degradation of chicken feathers and the environmental/biological variables that affected the feather's physical characteristics. The variables tested included sediment type, length of burial, and bacteria. We examined the physical changes that occurred in Rooster Chinchilla Rounds from the species *Gallus gallus* when they were inoculated with *Bacillus subtilis* bacteria from local soil and buried in two different sediments. The sediments tested were local silt from St. Joseph River and ash deposits from Mount St. Helen's 1980 eruption. We analyzed for changes in barbule count, macrostructure, for fossilization of feathers.

INTRODUCTION

Feathers are an incredibly diverse and multifunctional anatomical structure that have been the center of an ongoing evolutionary debate among paleontologists. The lack of a substantive fossil record to support varying evolutionary theories has only emphasized the importance of finding said fossils (Prum et al., 2002). In light of this missing historical evidence studies have begun to focus on the question of decomposition of organic matter and the validity of dating (Hokanson, 1991). Both extinct and extant avian and non-avian feathers are all primarily composed of the protein beta-keratin and made up of the same basic parts. The beta-keratin protein can be the target of hydrolysis and keratinolytic activity in varying bacteria and fungi species, yielding faster degradation (Cai et al., 2008). The majority of taphonomic feather research today looks into what organisms and biological variables degrade the feather rather than how they affect the fossilization process. My research is unique in that I am exploring both the effect of different variables on feather degradation and how it applies to fossilization. This research and further research like it could be useful to further paleontologists understanding of bird feathers, fossilization, waste removal in the poultry industry, and the biological sciences as a whole.

MATERIALS AND METHODS

Sample Selection

This experiment was conducted using Rooster Chinchilla Rounds from one bird species, *Gallus gallus*. One-Hundred feathers measuring from 5-15 cm long were obtained from www.zuckerfeather.com of which 60 were randomly selected for experimentation. Likewise two sediment types were obtained: silt from St. Joseph River in Berrien Springs, Michigan, and ash

from Mount St. Helen in Skamania County, Washington. Multiple soil samples were taken from a chicken coup at the Green Farm near Cassopolis, Michigan, and the Koudele Farm in Berrien Springs, Michigan in order to culture and grow bacteria that could be found on or around live specimens. Bacteria were isolated from these cultures and identified to be *Bacillus subtilus* in order to be used on the feathers later in the experiment.

Experimental Set Up

Both the silt and ash were sifted through a fine grate to remove any unwanted material such as grass, wood chips, leaves, twigs, bugs, etc.. Both sediment types were poured into 6 separate aluminum bins measuring 14 x 10.5 x 3 inches each to roughly halfway full then autoclaved at 121^{o}C and 15 psi for 30 minutes. To insure minimal cross contamination, researchers hands were washed before each bin was autoclaved and each bin was covered with a plastic sheet immediately following autoclaving. A small sample of each sediment was put aside to test both the organic content and pH. The organic content was tested by weighing a crucible then filling it with one of the sediment types and weighing it again. Then the crucible was superheated by a Type 1500 Thermolyne Furnace to 950^{o}C for 30 minutes and weighed after cooling. The pH of the soil was obtained using Hydrion Spectral Micro Essential 9800 pH Strips. Each sediment was diluted into sterile water and a pH strip was dipped and analyzed.

Prior to burying the feathers, each feather was dried overnight in a Blue M STABLE-THERM gravity oven to remove any moisture. A small fragment roughly 1cm x 1cm was cut from each feather's tip for analysis under an SEM. The photographs of these samples served as the controls for the micro-analysis of the feathers post burial for each trial. Further photographs were taken through the lenses of a light microscope and a dissecting microscope to compare the macro structure of each feather.

To set up each bin we first prepared 1200 ml of distilled sterilized water in an autoclave in order to be used for both the control and experimental feather bins. Half of the sterilized water was inoculated with *Bacillus subtilus*. We dug a small trench in each bin and poured in 100ml of sterilized distilled water in three of the six bins for each sediment type and poured 100 ml of inoculated distilled sterilized water in the remaining bins. We placed five feathers spaced equally apart in each bin and buried them at roughly equal depth concave side up. 100 ml of sterile water was poured on top of each bin after burial then covered with plastic wrap in order seal in the moisture and prevent cross contamination. Six bins, three silt and three ash (without bacteria), served as the control groups and the remaining six served as experimental groups.

Once each bin was fully prepared, they were left in room where humidity and temperature are relatively stable during the changing weather. Two bins of each sediment type, one control and one experimental, were unburied every thirty days for 90 days straight.

Data Collection

The feathers in each of the four bins selected at a time were gently removed and shaken of to remove excess sediment. We analyzed for qualitative changes in microstructure and macrostructure using pre-prepared scales for each as well as quantitative measurements of barbule counts. The macrostructure scale was as follows: 1- Feather intact, 2-Frayed edges, 3- Between 25-50% missing, 4- Between 50-99% missing, 5 – Entire feather missing. The microstructure scale was as follows: 1- Barbules uniform, 2- Up to 25% compressed or spread out, 3- Between 26-50%, 4-Between 51-57%, 5- More than 75%. Comparative T-tests were used to analyze all data from each different analysis method. PH and organic content results were

calculated and compiled. Lastly, we tested for keratinolytic activity in both *Bacillus subtilus* and fungus species using feather meal agar.

RESULTS

It was shown that no significant data was found for all tests run except for the barbule count for feathers buried in ash giving a p-value of 0.0360 (table 1, figures 1-3). Of the 60 feather samples, all of them were confirmed to have unexpected fungal growth. pH tests showed both soils went from a pH of 7 to a pH of 6 after 3 months of burial. Organic content test showed silt having an organic content of 6.48% and ash having 0.78%. Both the *Bacillus subtilus* and fungus showed keratinolytic activity by thriving and utilizing the feather meal agar (Photo 2).

DISCUSSION

Unfortunately these results show no significance for any of the data sets except for feathers buried in ash giving a p-value of 0.0360. There are multiple reasons why the data turned up the way it did. First was that our data was not normally distributed and the data sets ended up being much smaller than they were supposed to be. This was because some of the feathers were too difficult to interpret under a microscope due to the fact that they were heavily coated with sediments, making some microscope work hard to read and thus had to be omitted.

Although there was no significant difference in any of the unpaired t-tests that we ran, that does not mean the data does not tell a story. We see multiple trends in our data and experiment that point towards further research. The first is that in our graphs (figures 1-3) we see that if the barbule count is high for any of the sets of data, then the corresponding micro and macro scales were low, and vice versa. Unfortunately, we experienced a fungal contamination. We also

noticed that the feathers that showed the most degradation had the most fungal growth around them in their aluminum pans. This was interesting because after we tested for keratinolytic activity using feather meal agar and found that both the fungus and the bacteria had utilized the agar as the carbon source, the fungus seemed to grow and utilize it more (picture 2). Because of this, we believe that the fungus is the primary contributor to the degradation of the chicken feathers. That being said, we believe that the bacteria and fungus most likely work together to degrade the feather faster than if they were on their own. Lastly, when testing for organic content we found that silt had an organic content of 6.48% and ash had 0.78%. The feathers that showed the most degradation were the ones buried in ash. This would be expected because the less the organic content, the less other materials the bacteria and fungus would have to feed on, forcing them to utilize the feather as a carbon source instead of other material.

Previous research by Hokanson (1991) found that feathers exposed to damp/wet climates with low organic content showed the most feather degradation over time. We found similar results in our data showing that feathers buried in the ash with the lowest organic content had the most degradation. Hokanson also found that environments with higher pH sediments had better degradation, unfortunately our pH's stayed consistent for both soils, giving us no variation.

This experiment encountered a lot of technical and experimental issues that we had to work with in order to continue our project. That being said, future research in this particular topic and others like it are wide open for various studies. Future research could re-run this same experiment and split the fungus and bacteria and see how they work independently due to the fact that we did not plan for fungal growth. Future research could also identify the fungus that grew on our sediment samples and see if any other research has been conducted on that particular species keratinolytic activity and processes

LITERATURE

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Table 1- Chart portraying the result of 6 different t-tests that analyzed barbule count, macro scale, and micro scale for each sediment type.

	Untreated Mean	Treated Mean	t-value	D.F.	P-value
Barbule Count Silt	134.42	151.75	0.8177	24.53	0.4213
Barbule Count Ash	157.55	96.45	2.381	11.21	0.0360
Macro Scale Silt	2.67	2.20	1.606	22.33	0.1223
Macro Scale Ash	2.47	3.27	2.028	25.94	0.0529
Micro Scale Silt	2.93	2.27	1.426	28.0	0.1648
Micro Scale Ashe	1.77	2.79	2.066	19.67	0.0522

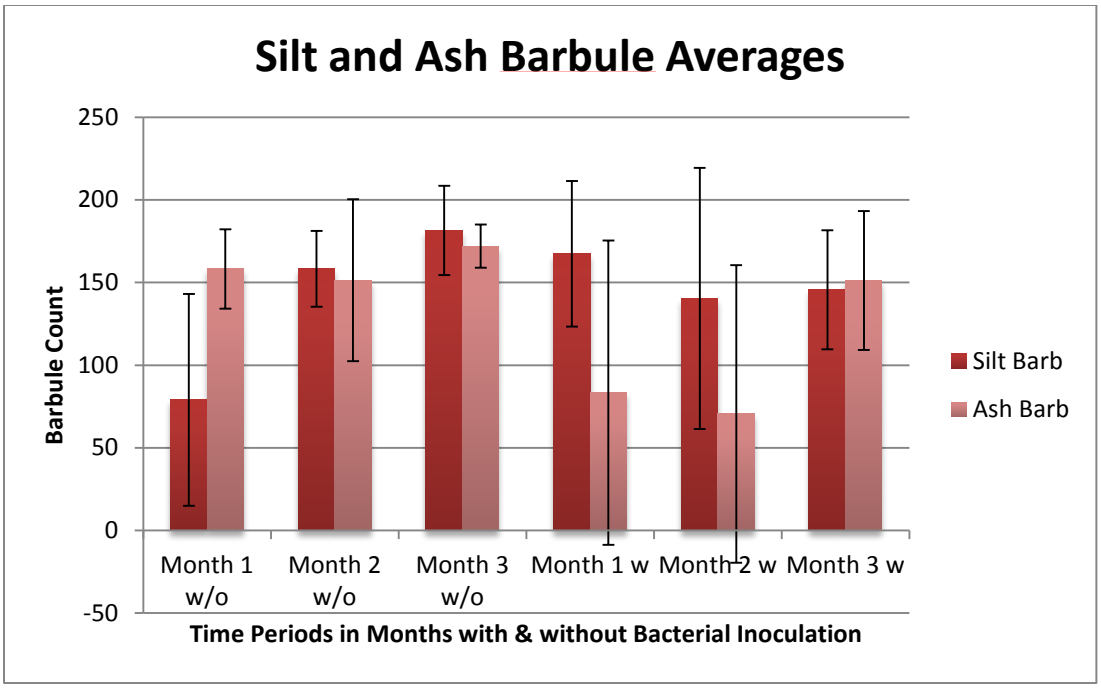


Figure 1- Bar graph displaying the average barbule count for all feathers present in each treatment for each sediment type. Error bars represent standard deviation.

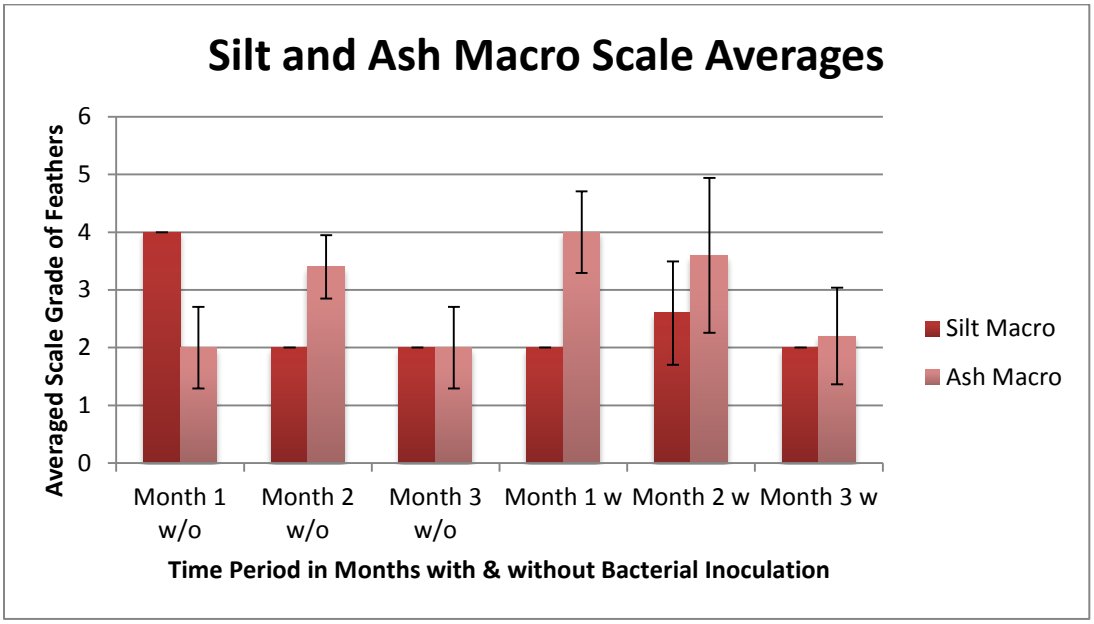


Figure 2- Bar graph displaying the average macrostructure scale value for all feathers present in each treatment of each sediment type. Error bars represent standard deviation.

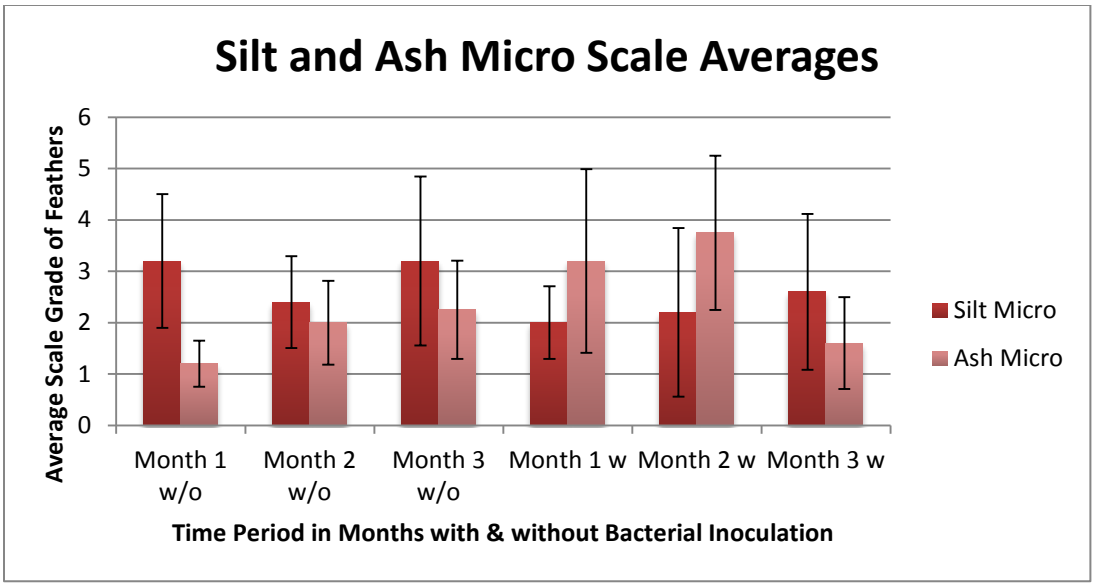
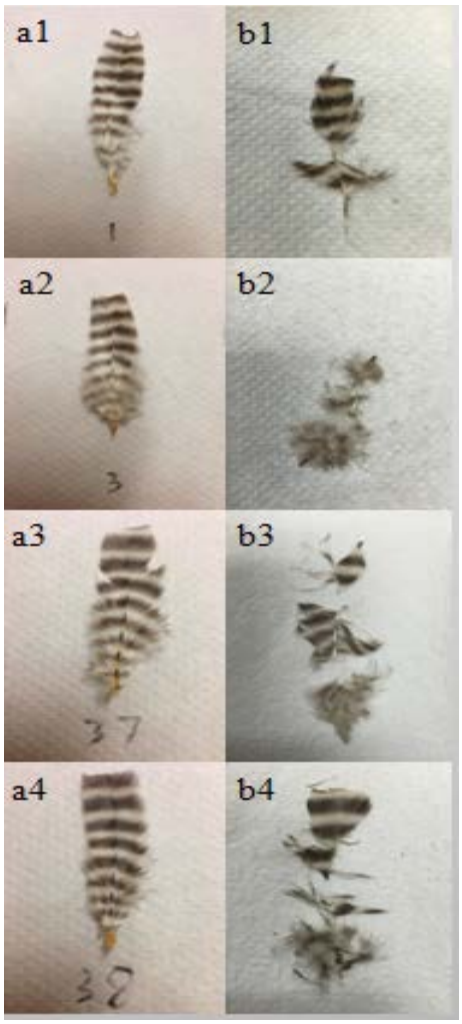


Figure 3- Bar graph displaying the average microstructure scale value for all feathers present in each treatment of each sediment type. Error bars represent standard deviation.



Photos. a1-b4- Each pair of pictures (a1:b1, a2:b2, a3:b3, a4:b4) represents the before (a) and after (b) picture for two silt treatments (1 & 2), and two ash treatments (3 & 4).

Photo 2 shows the feather meal agar and how the *Bacillus subtilis* and unidentified fungi utilized the agar. The left two plates (top left and bottom left) are the plates inoculated with bacteria. We see minor growth on them both, but not much. The right two plates (top right and bottom right) we see fungal growth and how well it utilized the agar.