Characterizing Organic Matter from Biotic and Abiotic Sources via Laser Desorption Mass Spectrometry

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0. Abstract

Astrobiology is the study of the distribution, evolution, and origin of life in our solar system. Life detection strategies currently aim to detect biosignatures, such as organic molecules, whose likely origin (based on knowledge of life on earth) requires a biological entity. One major challenge in astrobiology is distinguishing which (if any) organic compounds in a given sample are biologically derived. This is in part due to the fact that there is a large area of overlap in the type and complexity of organic molecules in abiotic and biotic sources, so the line is blurred. In order to better understand this area of overlap, this study focuses on carbonaceous chondrites as an abiotic endmember and a terrestrial shale as a biotic endmember. The working hypothesis for this study is: there is a statistically significant difference between the diversity of organic compounds observed in carbonaceous chondrites and terrestrial oil shale as indicated by multiple chemical indices that incorporate measures of the population of organics detected via Laser Desorption Mass Spectrometry (LDMS).

0.1 Plain Language Summary

Astrobiology aims to study life in the solar system. There are multiple strategies to detect life outside of Earth, many of which rely heavily on detecting the presence of molecules indicative of life, such as organic molecules. Detecting organic molecules on an extraterrestrial object does not automatically indicate life, as there are many natural chemical processes that can produce these types of molecules with no biological intervention. A substantial challenge in the astrobiology field is distinguishing the organics made from non-biological processes and those made by biology (e.g., living organisms). What makes this difficult is that there is a large area of overlap in the types of organics seen in samples with and without biological input. In an attempt to distinguish these molecules, this study uses carbon-rich meteorites to serve as an endmember that lacks biological origin and an oil shale from Earth to serve as an endmember of biological origin, investigating if there is a significant difference in the organic material in these two types of samples by looking at the diversity, distribution, and structure of these molecules using Laser Desorption Mass Spectrometry (LDMS).

1. Introduction

Planetary science is a field that encompasses many of the natural and physical sciences including geology, astronomy, biology, chemistry, and physics. Planetary science is the study of planets, moons, comets, asteroids, and other celestial bodies in the universe and how they interact with one another. This includes studying the composition, formation, history, dynamics, and many other components of each body. The Planetary Science and Astrobiology Decadal Survey (National Academies of Sciences, 2023) is a comprehensive document that prioritized science objectives and mission concepts for the next decade based on community suggestions. This document is organized into questions that the community is in the process of answering, each focused on a different aspect of planetary science. Among these prioritized foci are: i) what and how organic building blocks are synthesized in the Solar System; and ii) what is the extent of organic chemical evolution, potentially leading towards life (National Academies of Sciences, 2023). Studying and better understanding these areas gives the base knowledge required for effective life detection missions on other bodies in the solar system. In order to potentially find life outside of Earth, the nature of abiogenesis, the idea that life arose from nonliving sources, must be properly understood to avoid false positives and negatives.

As of now, there are a number of active and future missions with life detection as either a primary or secondary objective, the most notable being Mars 2020 and the *Perseverance* rover, onboard ExoMars and the *Rosalind Franklin* rover. *Perseverance* is part of the ongoing Mars Sample Return (MSR) mission and it has collected geological and atmospheric samples (Green, 2021) for future study, including possible life detection. While not yet launched, ExoMars and Mars Organic Molecule Analyzer (MOMA) in the rover payload aims to detect signs of past or present life on Mars by using a mass spectrometer to analyze drill samples acquired from up to two meters below the surface (Goesmann et al., 2017). This is a significant depth, as instruments onboard the currently active rovers generally only penetrate a few centimeters. The ExoMars mission will search for signs of past life on Mars with the MOMA instrument as a crucial component of the payload, and is currently scheduled to be launched in 2028 (Vago & Spato, 2022).

A hurdle in any mission looking for extraterrestrial life is determining exactly what qualifies as a sign of life, or biosignature, which is currently a highly debated topic in the planetary science community. The Ladder of Life Detection is a reference that provides a strawman hierarchy by which observations might be classified as “high” or “low” fidelity biosignatures. This document lists Darwinian evolution as the strongest evidence for life, and environmental habitability as the weakest. Organic molecules, as a whole, fall in the middle of the spectrum, making organics alone insufficient to claim as life. This paper also explicitly states that a biological explanation must be the last-resort hypothesis, so as to avoid a false positive scenario, creating a null hypothesis of non-biological processes (Neveu et al., 2018).

A commonly sought-after material to focus on finding are organic molecules, chemical compounds with at least one carbon atom covalently bonded to other elements. Simple organic compounds, such as amino acids and hydrocarbons, are not qualified to be biosignatures on their own, however. These types of molecules can be synthesized from various chemical processes in space or in a lab, as demonstrated by the infamous Miller-Urey experiment (Parker et al., 2014). In this experiment, Stanley Miller and Harold Urey used a custom glass apparatus to simulate the conditions of Earth’s early atmosphere and surface, assuming reducing conditions for the atmosphere. After some time, the atmospheric constituents began reacting with a spark discharge (simulated lightning), resulting in the formation of amino acids and other simple organics in the apparatus, demonstrating that organic compounds can be created without any biological activity. That experiment has been recreated many times with varying refinements and changes to the simulated atmosphere including using different mixtures of gasses and different methods of inducing the reaction (Parker et al., 2014). Organic molecules created without biological intervention are considered to be abiotic organic matter, indicating that their creation is purely the result of chemistry and not self-sustaining biology. Conversely, molecules that are biologically self-sustaining, as is the case for many organic molecules found on Earth, are termed biological or biotic organic matter.

As illustrated in Figure 1, the organic compounds in abiotic sources (e.g. meteorites, asteroid parent bodies, and the interstellar medium) tend to be simpler and smaller by mass compared to those found in biotic sources (e.g. organic-rich terrestrial samples). Organic molecules in biotic sources span the full range of complexity, from the simplest building blocks such as amino acids, to functional molecules such as DNA. Despite limitations on the complexity range of abiotic molecules, there is a notable area of overlap in size and complexity that organic compounds from both abiotic and biotic sources share. This overlap area is of notable interest to astrobiologists as it is a promising area of study to understand abiogenesis, or the point in which abiotic material becomes self-sustaining and biotic, and where Darwinian evolution begins to take effect (Kumar et al., 2020). Further, distinguishing which organic molecules require biological intervention to form/polymerize/activate is of high value to avoid false positives on life detection missions.

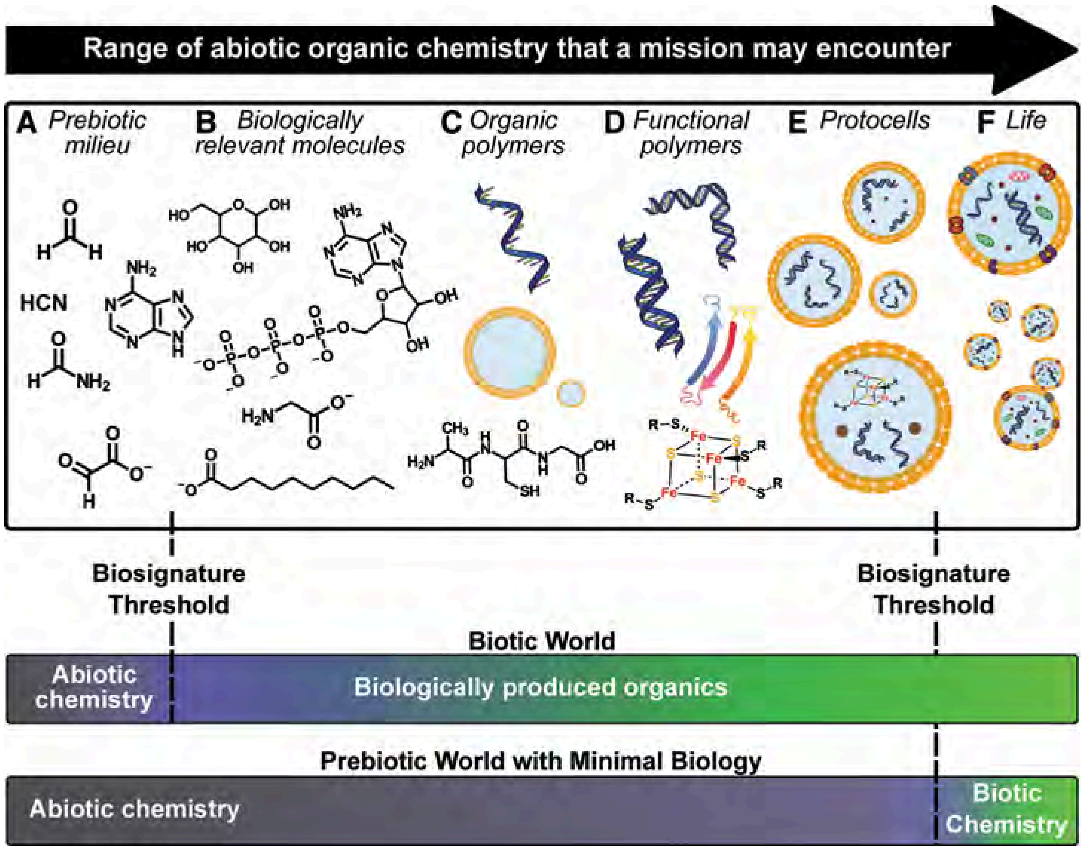


Figure 1: Sketch of the illustrated range of complexity of organic molecules. The colored bars at the bottom represent the expected distribution of organics in the above categories and their likelihood of being biologically derived. The biotic world (top bar; e.g. Earth) shows more molecules being produced biologically and the prebiotic world (bottom bar; e.g. Titan) shows many of the same organics being produced abiotically (Barge et al., 2022).

Among the most prominent and accessible abiotic sources are meteorites, broken-off parts of asteroids that have fallen onto the Earth (as well as other bodies in the solar system). There are multiple categories of meteorites, some of which have little to no endogenous organic material. Stony meteorites, as the name implies, are primarily made of silicate rock (as opposed to metal alloys found in iron meteorites). Of the stony meteorites, chondrites are among the most primitive materials to come from the solar nebula (Brearley, 1997), the ancient gas cloud that formed the solar system >4.5 billion years ago. Some of these meteorites closely approximate the composition of the solar photosphere, a representation of the composition of the solar system as a whole (Scott & Krot, 2003). The abiotic organic material in these meteorites is commonly sequestered in the matrix rather than individual mineral grains and can experience various degrees of thermal and aqueous alteration while in space, which makes carbonaceous chondrites ideal targets for the study of primitive abiotic materials (Mullie & Reisse, 1987). This study focuses solely on organic-rich carbonaceous chondrites in order to obtain the best possible data within the scope of the project.

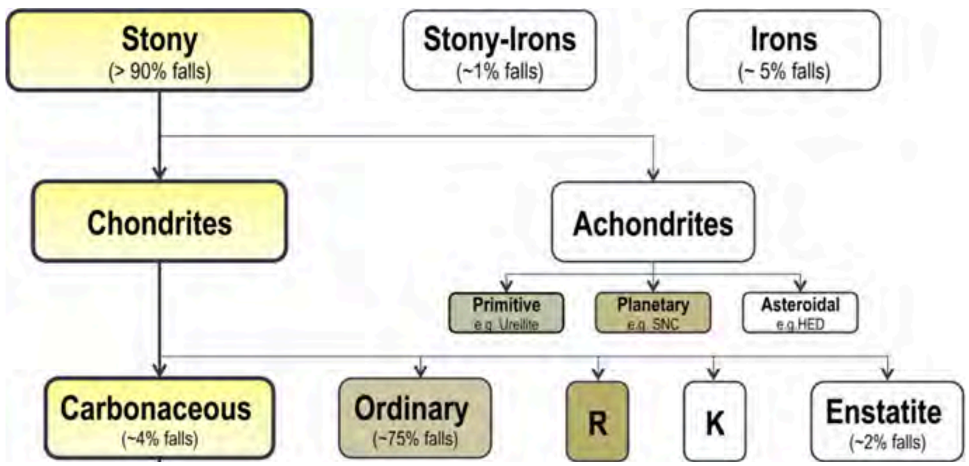


Figure 2: Visual representation of the different types of meteorites and the relative abundance of organic material in them. Yellow boxes represent categories with the most organic material, brown with fewer organics, and white with little to no organic material (Elsila et al., 2016).

In contrast to the abiotic organic matter found in meteorites, samples from the Marcellus Shale represent an archetypal example of biotic organic material. This Devonian organic-rich shale unit is located in the Appalachian Basin and displays a range of thermal maturities, which is a measure of the degree of heat-induced degradation in the organic material found in the rock. Higher thermal maturity incurs more degradation of the organics in the sample compared to lower maturity. Like carbonaceous chondrites, the organic material in the Marcellus Shale is sequestered in the matrix rather than its mineralogy. Additionally, both the Marcellus Shale and carbonaceous chondrites come from water-rich environments, and both have analogous amounts of kerogen, or insoluble organic material (Matthewman et al., 2013). Insoluble organic material is generally observed to be more complex than soluble organics and can, by extension, yield a better representation of the range of organic matter in the sample. Insoluble organic material does not dissolve in water while soluble organic material is removed from the sample upon being soaked in water.

This study resulted in the analysis of three carbonaceous chondrite samples as well as three samples from the Marcellus shale in order to characterize the insoluble organic inventory in abiotic versus biotic sources, respectively. The meteorite samples represent two subgroups within the carbonaceous chondrite category: CM and ungrouped. All selected meteorites are from the same petrologic grade, indicating a similar geologic history. This study tested two working hypotheses: i) the organic diversity in carbonaceous chondrites is statistically different than that found in the Marcellus Shale; and, ii) there is a significant difference in the organic diversity of CM and ungrouped meteorite samples. These hypotheses were tested using a number of chemical indices that leverage observations of structure (degree of unsaturation), population distribution (mass and mass range), and molecular diversity (different molecular formulas) as shown in Figure 3.

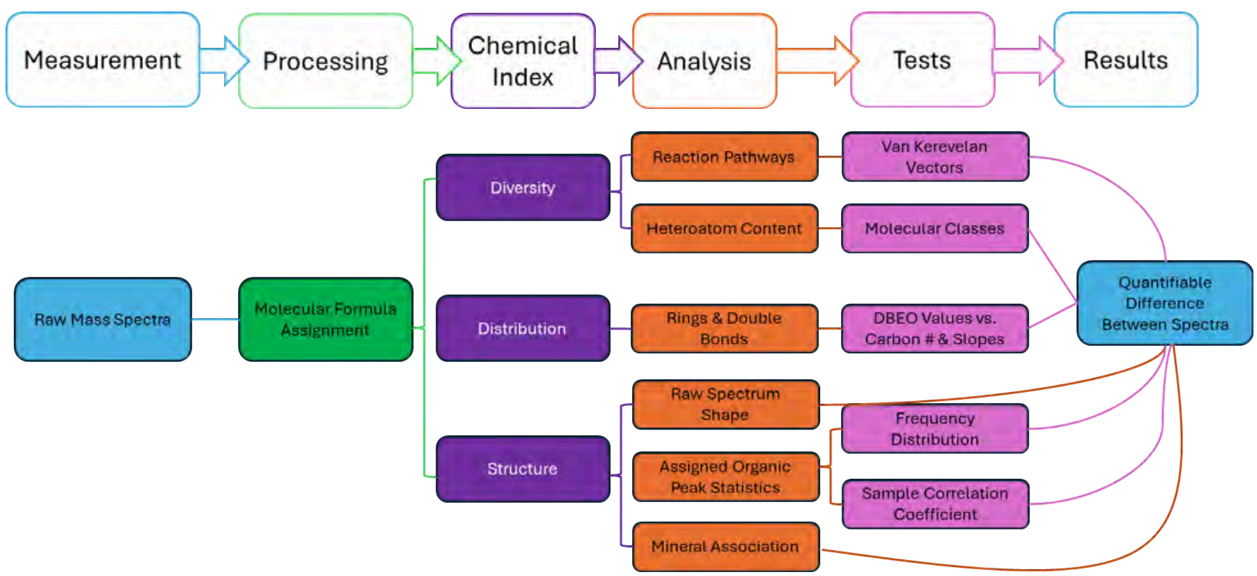


Figure 3: Visualization of the different ways the hypotheses were tested for this study. Statistics were also applied to identify any robust distinctions where appropriate.

2. Methodology

2.1 Samples of Abiotic Organic Material: Carbonaceous Chondrites

The carbonaceous chondrites used in this study are CM2 Aguas Zarcas, CM2 Jbilet Winselwan, and C2-ung Tarda. In order to reduce variables, all three samples are of petrologic type two, indicating moderate aqueous alteration based on water content and mineral proportions (van Schmus et al., 1967). CM meteorites have been demonstrated to possess the highest abundance of organic material among meteorites, motivating the selection of this subgroup for study (Elsila et al., 2016). Characterization of an ungrouped chondrite allows investigation into the distribution, diversity, and structure of organic matter between different parent bodies. These classifications stem from the parent body that the meteorites are thought to have originated from. They are grouped based on chemical composition, oxygen isotope composition, and mineralogy, leading to a strong likelihood that samples within the same group share a parent body (Callahan et al., 2011). Ungrouped meteorites are not much different in this regard, the only difference between them and established groups is that scientists have not yet discovered five meteorites with similar data from the aforementioned categories. Two of the three samples are falls, Jbilet is a find, but it was reportedly retrieved shortly after it fell (Ruzicka et al., 2015). Such limited exposure time to surface conditions on Earth should theoretically reduce the extent of terrestrial contamination. Identifying potential terrestrial contamination is not an exact science, and typically includes searching the data for peaks known to be likely contaminants or peaks that are unlikely to be found in the sample (e.g. uncharacteristically large high-mass peaks in the abiotic endmember). Unexpected peaks may still be natural, and are assigned a formula based on the mass to charge ratio in order to determine whether the peak potentially represents contamination. All three of these samples were provided by distributors in the Meteoritical Society's list of trusted sources.

2.2 Samples of Biotic Origin: Marcellus Shale

The Marcellus Shale is a Devonian formation deposited in the Appalachian Basin in Pennsylvania and the surrounding states. The unit consists of black oil-shale that displays a range of thermal maturities (Zagorski et al., 2012). The thermal maturity directly affects the organic material in the shale; higher maturity corresponds to more degradation of the organic material.

The Marcellus Shale samples were provided by Dr. Jay Kaufman and range across three thermal maturities (as determined by previous work): oil-bearing, wet gas, and dry gas. In the context of this study, these maturities refer to the degree of degradation the organic molecules have undergone as a result of thermal influence. Immature samples display the least amount of degradation, while overmature samples display the most. The samples were in the form of drill cores, the primary area of interest in the cores being that they share common total organic carbon (TOC), ^{13}C and ^{34}S across thermal maturities, suggesting that the organic material came from the same provenance. This is to show the progressive degradation of organic material that can be found in biotic sources in order to gain a more comprehensive dataset on biotic organic material.

2.3 Measurement Techniques

The primary analytical method leveraged to characterize the organic inventory in the samples was laser desorption mass spectrometry (LDMS). This technique utilizes a pulsed laser source to ionize the powdered sample material. Ions generated during laser irradiation are steered toward a mass analyzer for separation of m/z (mass to charge ratio). This experiment used a Thermo Scientific Q Exactive™ Orbitrap™ interfaced with a custom Spectroglyph laser desorption source and sub-atmospheric sample chamber. The Orbitrap mass analyzer characterizes organic and inorganic material with ultrahigh mass resolving powers (m/dm > 100,000 FWHM at m/z 400) and ppm-level mass accuracy. A schematic diagram of the custom interface is provided in Figure 4 below.

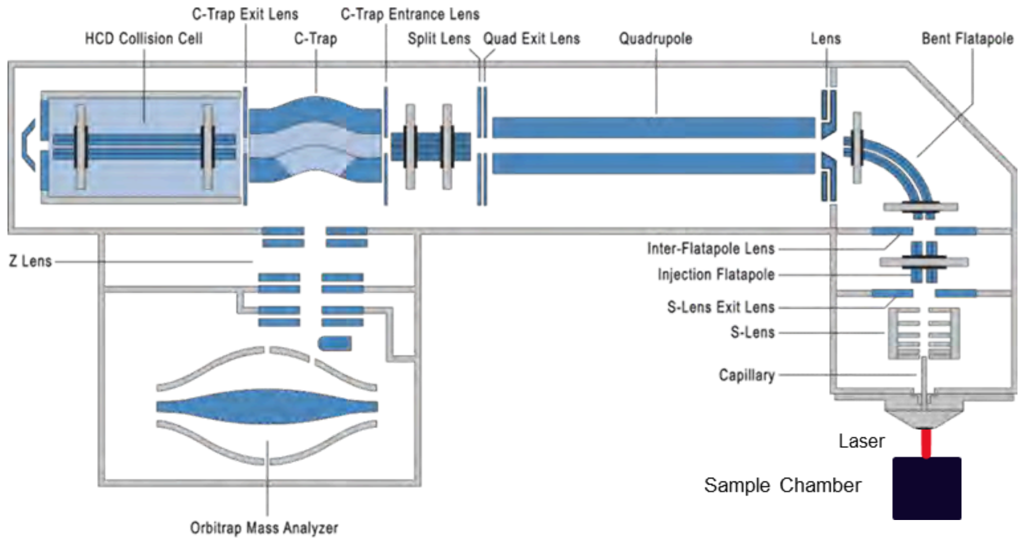


Figure 4: Schematic diagram of the custom Thermo Scientific Q Exactive™ Orbitrap™ used in this study (Thermoscientific, 2017).

2.4 Sample Preparation

All six samples were prepared following a standardized protocol developed during the Fall semester. Prior to contact with the sample, all tools were sequentially sonicated with acetone and methanol, which essentially removes all soluble organics to decrease any risk of further contamination. Fragments of about 3-5 mg were detached from each chondrite/shale sample via mechanical fracturing using a precleaned agate pestle. Each fragment was powdered using the same agate pestle and stored in a vial previously ashed to 500℃. Approximately 1 mg of crushed sample was placed on a 1 mm^2 piece of conductive copper tape applied to a glass slide compatible with the Q Exactive's sample chamber. The tape is necessary because the sample chamber is vertical. A "neat" piece of copper tape (with no sample on it) served as an intermediate blank and accounted for any organic contaminants derived from the adhesive. A more comprehensive blank consisting of UHV foil was also analyzed to support a progressive understanding of contamination sources.

2.5 LDMS Protocol

Each sample and blank was analyzed six times, with each analysis targeting a different zone of the sample to maximize the surface area of the material that is effectively characterized in this study (Figure 5). Each zone analysis consisted of roughly five hundred scans. Only a single sample and set of blanks (as described above) was placed inside the sample chamber at any one time. The instrument operated in positive mode, meaning the electrodes inside the Orbitrap manipulated positive ions rather than negative ones. Once the sample data were collected, a blank subtraction was applied to remove any contaminant signals derived from the copper tape and/or alumina and enable analysis of the data using the MFAssignR software (described further below).

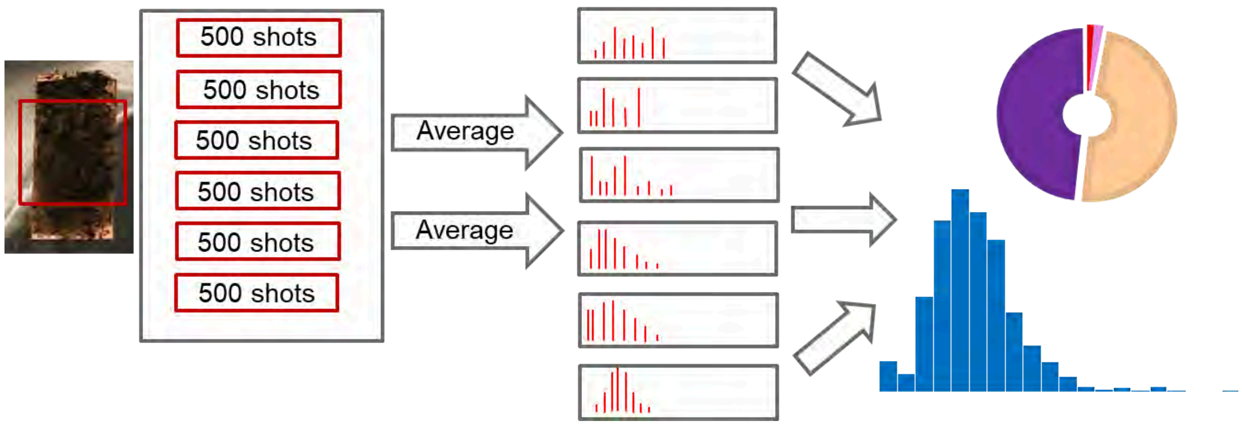


Figure 5. Schematic for how the data was collected and processed.

2.6 Measurement Analysis

LDMS is a semiquantitative technique that allows for the chemical characterization of both organic and inorganic material. Using an Orbitrap allows for mass-resolving powers and mass accuracies that enable molecular identification through exact mass determinations with significant digits in the third decimal place (Arevalo et al., 2023). However, the heterogeneity of the sample can manifest in the data in the form of 'hot spots', or individual scans that have significantly higher organic signals than others. In order to focus on the analysis of the bulk sample rather than shot-to-shot variability, the scans collected in each zone of each sample were averaged to produce a single mass spectrum whose peak distribution captures the composition of the analyzed aliquot of sample. The blank samples have been averaged in the same manner for internal consistency.

In order to assign molecular formulas, this study utilized an open-source code R package called MFAssignR (Schum et al., 2020). The assignment algorithm works with ultrahigh resolution mass spectrometry data by analyzing the elemental composition of each peak and separating those that include elements inconsistent with organic molecules from peaks that can be grouped into one of the following: CH, CHO, CHN, CHNO, CHNS, CHNOS. The code then mathematically assigns molecular formulas to the peaks that fit this criterion.

3. Results

3.1 Samples

As mentioned earlier, the Marcellus Shale is a black oil shale found in the Appalachian Basin. This study utilizes three samples from this unit, each from a different thermal maturity zone, the oil-bearing, wet gas, and dry gas zones (Figure 6). These samples were chosen specifically to display organic matter at different stages of degradation to retrieve the most complete data about terrestrial organic matter possible. One of the samples was received pre-powdered, and the remaining two had to be powdered manually.

This study also makes use of three different carbonaceous chondrites to give the most comprehensive data on abiotic organic material possible within the scope of this study. The chondrites chosen are CM2 Aguas Zarcas, CM2 Jbilet Winselwan, and C2-ung Tarda. All of these meteorites are relatively free of terrestrial contamination, making them ideal candidates for this study. Like the terrestrial samples, all three meteorites were powdered manually.

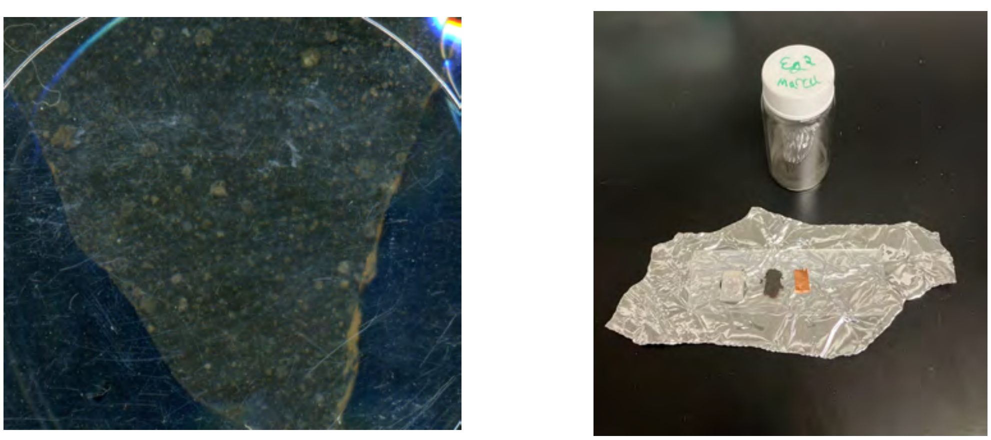


Figure 6: Images of CM2 Jbilet Winselwan (left) and mounted Marcellus Core EG with blanks (right). CM2 Jbilet Winselwan was photographed through its storage container due to contamination concerns, resulting in what looks to be scratches on the sample’s surface. These scratches are on the container, not the sample.

3.2 Chemical Diversity

A graphical method to observe structural differences between the samples is the Van Krevelen diagram. Using this method results in plots of molar H/C ratio on the y-axis and molar O/C ratio on the x-axis and has been historically used with sedimentary samples to classify the degree of thermal alteration in a sample (Burnham, 2018).

In order to better visualize the information given in these plots, heat maps were laid on top of the Van Krevelen diagrams using code in R (pictured in Figure 7). This code makes use of Kernel Density Estimation, which is used to produce a smooth estimate of the probability density function (Węglarczyk, 2018). For the purposes of this study, the location of the 'hot' area near the y-axis is the primary area of interest. Samples with more degradation of organic matter (higher maturity) show the 'hot' area closer to the y-axis than less mature samples. This is due to high maturity samples having fewer heteroatom-containing molecules and comparatively more hydrocarbons.

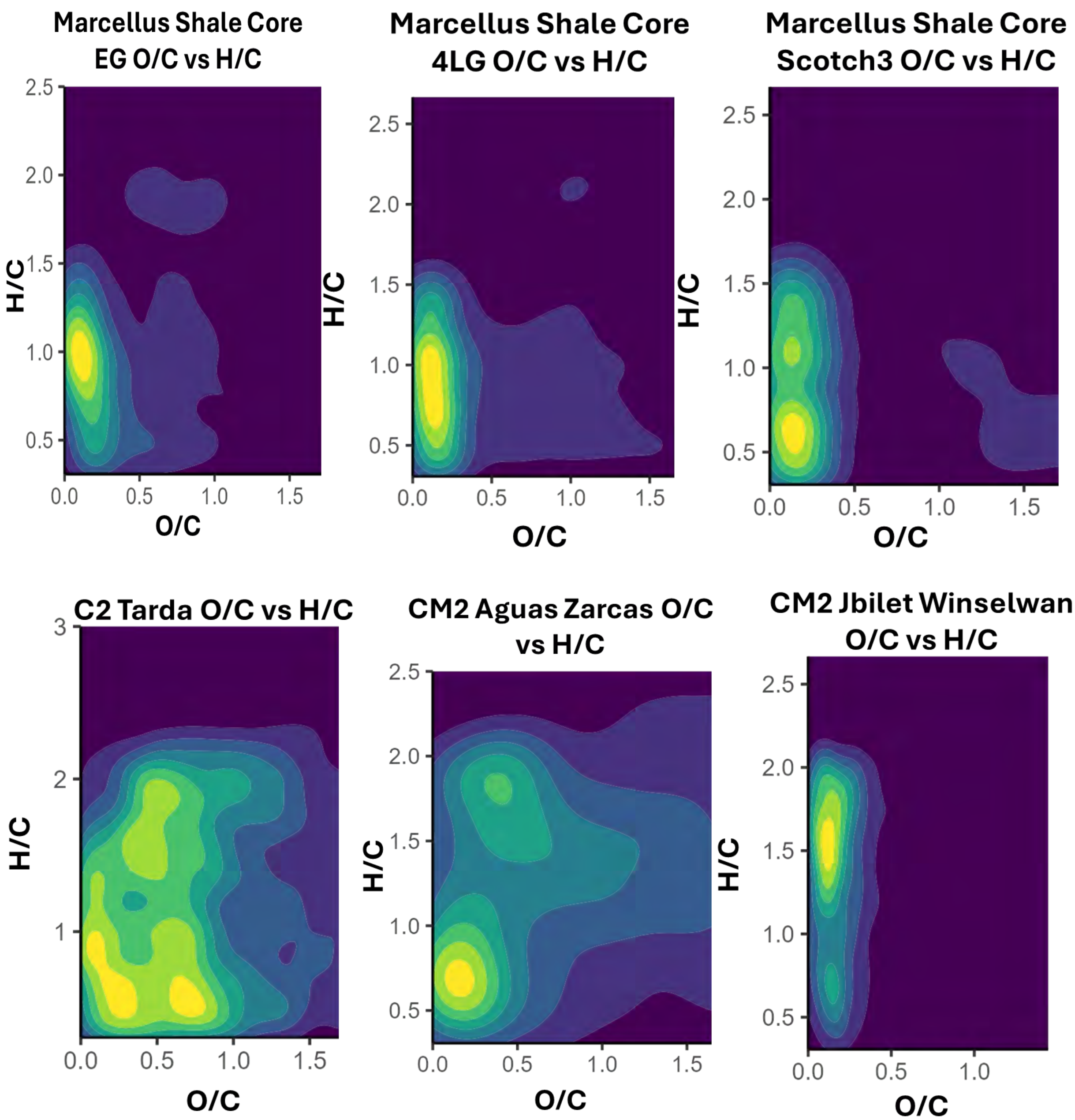


Figure 7. Heat maps laid over Van Krevelen plots for each sample. Ordered from least mature (left) to most mature (right).

3.3 Distribution

The data from each sample was processed and molecular formulas were assigned to each mass peak. Compared to the chondrites, the Marcellus Shale generally displays a higher concentration of higher mass peaks (500-1000 m/z), implying an increased presence of more complex organic material. A notable exception to this is Jbielt Winselwan, whose spectrum shows patterns more similar to the Marcellus Shale than the other chondrites. This may be due to Jbilet Winselwan's status as a find rather than a fall, meaning it was subjected to terrestrial conditions and contaminants for a longer duration than Tarda and Aguas Zarcas.

Across all six samples, the molecular formulas vary, but are consistent with the majority of assigned formulas being a part of the CHNO group (blue area in Figure 8) with around 50-75% of all formulas in this group. The primary differences lie in the minority groups, notably the CHO (dark green) and CHOS groups (light green). The CHO group displays an increased presence in the Scotch3 and Jbilet Winselwan samples, at around 50-100% more assigned formulas compared to the other four samples. The CHOS group is seen much more in the Marcellus Shale samples compared to the chondrites, where it is almost non-existent.

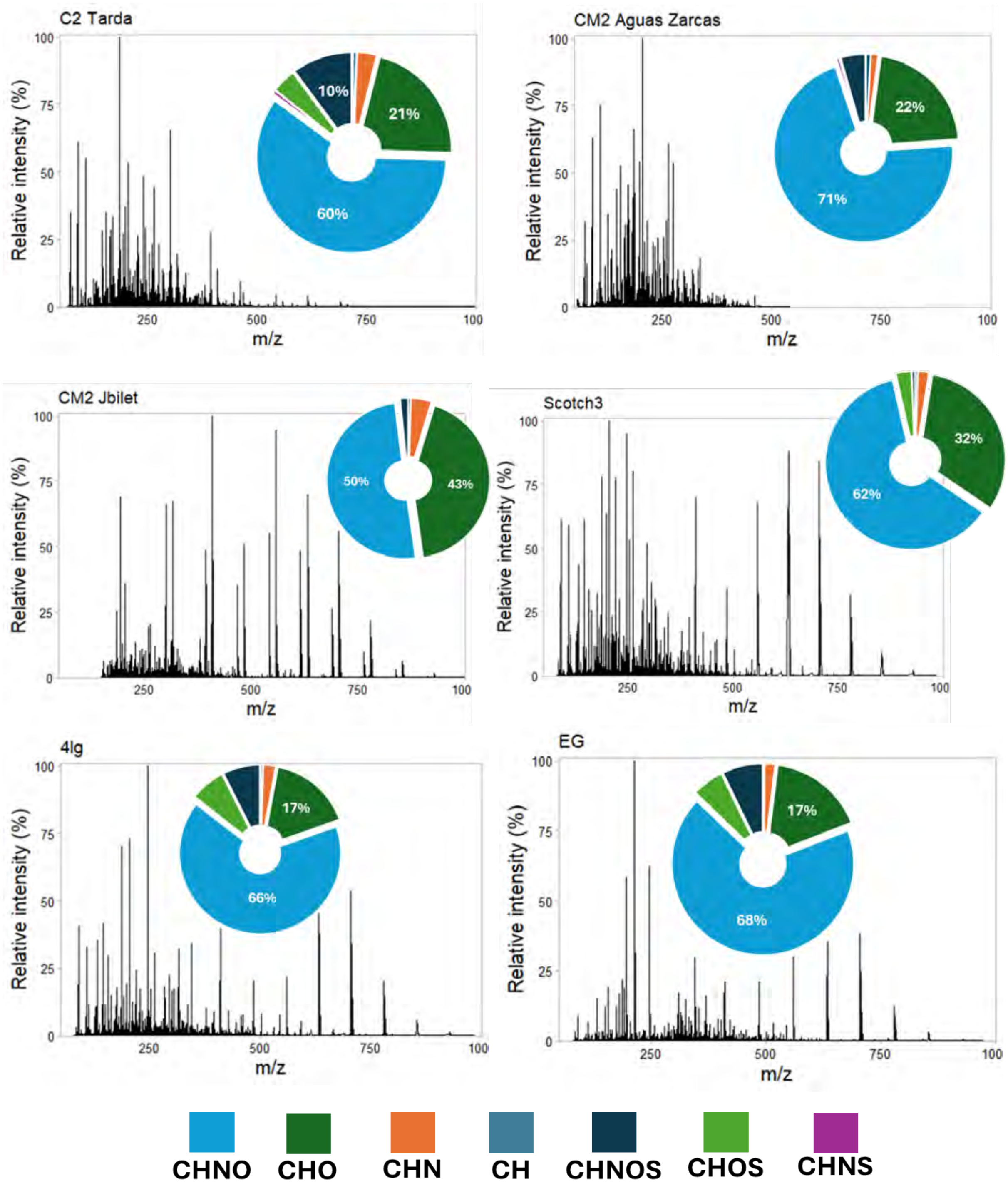


Figure 8. Mass spectra and donut plots of each sample. M/Z=0-100 is absent from Jbilet Winselwan's spectrum due to an error during data collection.

3.4 Structure

In order to better understand the differences in molecular structure between the three terrestrial samples, the Double Bond Equivalence of each sample was used. Double bond equivalence measures the number of double bonds and rings in a molecular formula (Dong et al., 2023). This study uses the equation that also accounts for the impact of oxygen on the structure rather than the standard DBE equation. This equation uses the molecular formula, specifically the number of carbon, hydrogen, nitrogen, and oxygen atoms in the formula to give insight into the types of bonds in the structure as well as the structure itself. Rings are a common structure in certain complex organic molecules where the atoms form a loop. This allows for an approximation of the structure of the identified organic molecules.

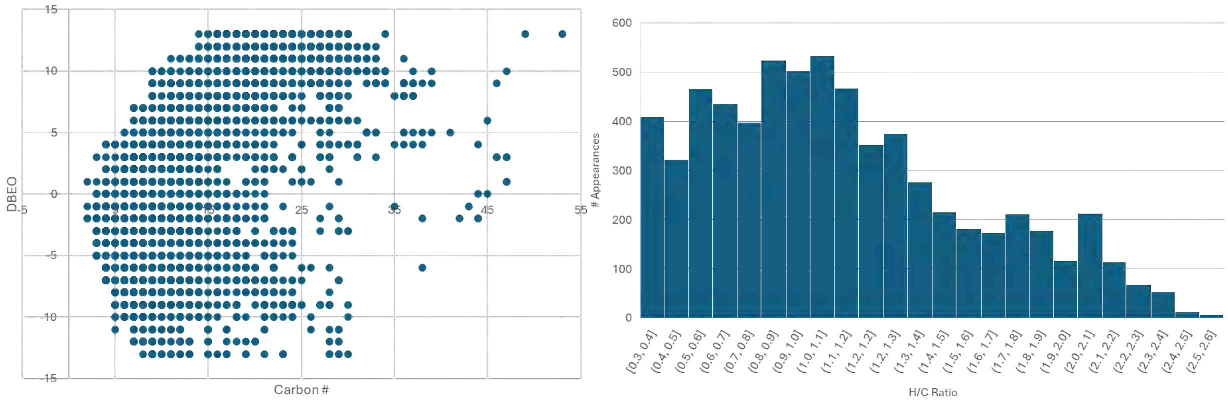
The Double Bond Equivalence accounting for oxygen (DBEO) was plotted against carbon number for each sample (Figure 9). Generally, the chondrite samples tend to show a preference for lower carbon numbers compared to the Marcellus Shale samples, which have an increased prevalence of high (35-55) carbon numbers.

In order to determine if there is a statistical difference between the Marcellus Shale and carbonaceous chondrite samples, the Mann-Whitney U Test was performed. This is a non-parametric test designed for use with two samples and is the non-parametric equivalent of a t-test. A critical p value of 0.05 was used, and the calculated p value was 0.00. This indicates a significant difference between the terrestrial and extraterrestrial samples.

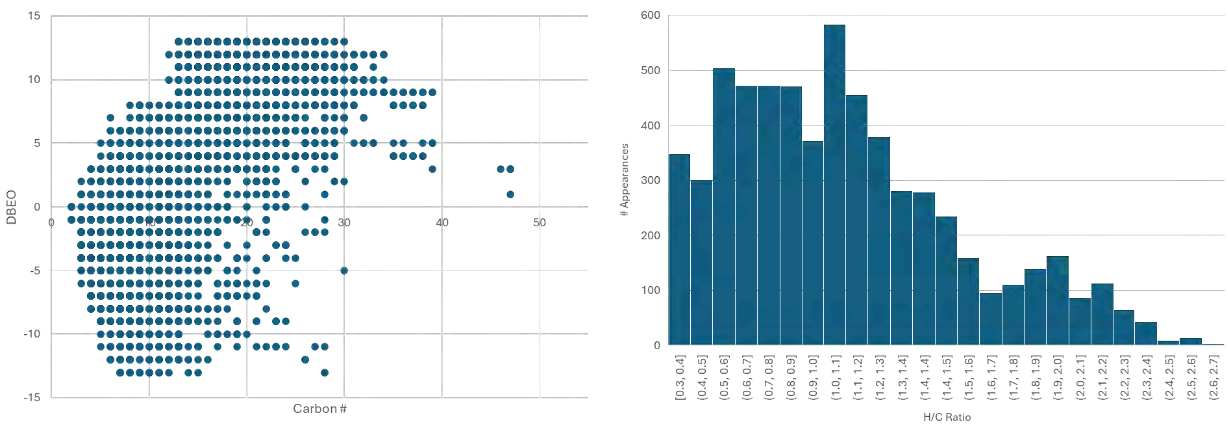
The same test was used to test the difference among the extraterrestrial samples. They were differentiated by their classification as CM2 and C2-ung. A critical p value of 0.05 was used, and the calculated p value was 3.41×10^{-56}. As the calculated p value is lower than the critical p, this indicates a significant difference between the extraterrestrial samples based on classification.

In addition to this, a statistical test known as the Kruskal-Wallis test was performed to determine whether there is any significant difference among the terrestrial samples. This is a non-parametric test designed to be used with three or more samples and is an extension of the Mann-Whitney U Test. A critical value of 0.05 was used, and the calculated value was 0.00 (rounded to two decimal places), indicating a significant difference among the Marcellus Shale samples. This is to be expected, as organic compounds are expected to differ significantly between thermal maturities in the Marcellus Shale.

Marcellus Shale Core EG



Marcellus Shale Core 4lg



Marcellus Shale Core Scotch3

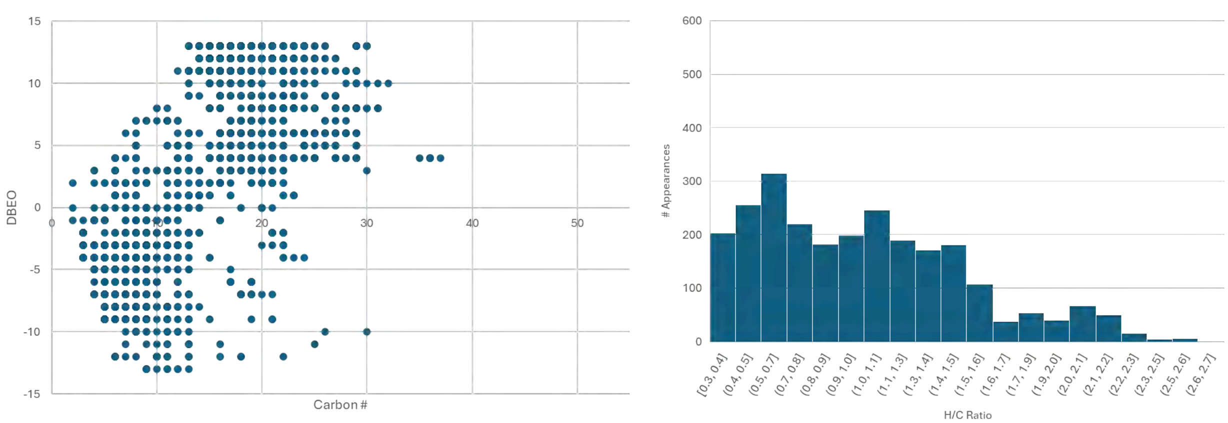


Figure 9. DBEO vs carbon number plots (left) and appearance count vs H/C ratio histograms (right) for each sample.

4. Discussion

Generally speaking, the data collected and analyzed throughout this study reflect the expectations set during the proposal stage relatively well. The most notable exception to this is CM2 Jbilet Winselwan. Especially in its mass spectrum, Jbilet showed patterns associated more strongly with biotic samples rather than abiotic samples, the most obvious being the abundance of high mass peaks, which looks more like the Marcellus Shale spectra rather than the other chondrites. This is likely due to Jbilet's status as a find rather than a fall, meaning it has had more exposure to terrestrial conditions and contaminants than the other chondrites used.

These results could be helpful in current and future life-detection missions such as ExoMars. The data collected from these missions may be able to be filtered more thoroughly when searching for potential signs of life with the results of this study. These results can also be applied to past life-detection missions to help inform the analysis of the already-collected data.

There were a few slight limitations to this study, the most prominent being time. The two-semester period given for this study was enough to complete the study as designed, but prohibited more in-depth comparisons and analyses between different subgroups within the abiotic and biotic umbrellas. Being able to explore these subgroups more may have given a more comprehensive analysis of the distinctions between abiotic and biotic organic material as a whole.

Taking into account the results of this study as detailed above, I can make a few conclusions about the two hypotheses being tested. Based on the molecular diversity, distribution, and structure, there is a significant difference between the biotic and abiotic members. Additionally, there is a significant difference between the C2 chondrite and the CM2 chondrites in those same categories. This leads to both null hypotheses being rejected.

5. Conclusion

The results of this study indicate a statistically significant difference between the samples when grouped into abiotic and biotic endmembers as well as among the abiotic endmembers in three categories: diversity, distribution, and structure. These data and methods can be applied to past, present, and future life-detection missions when attempting to differentiate organic matter of abiotic origin from that of biotic origin.

Future work may include a more in-depth exploration of the subgroups within the abiotic and biotic categories. Comparison of the remaining classifications of carbonaceous chondrites could give a better insight into the variability of organic material found in abiotic sources. Similarly, comparing every thermal maturity zone of the Marcellus Shale as opposed to the three studied during this project would also better categorize the types of biotic organic material. With more comprehensive data on each end member, a more complete analysis of the differences between abiotic and biotic organic material could be conducted, potentially aiding in the search for extraterrestrial life.

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Appendix A: Geologic Context of the Marcellus Shale

The Marcellus Formation is a black oil shale member located in the northeastern United States, in the Appalachian Basin (Figure 10). It is Devonian in age and consists of five primary maturity zones: immature, oil-bearing, wet gas, dry gas, and overmature. The three samples used in this study were collected from the oil-bearing, wet gas, and dry gas zones.

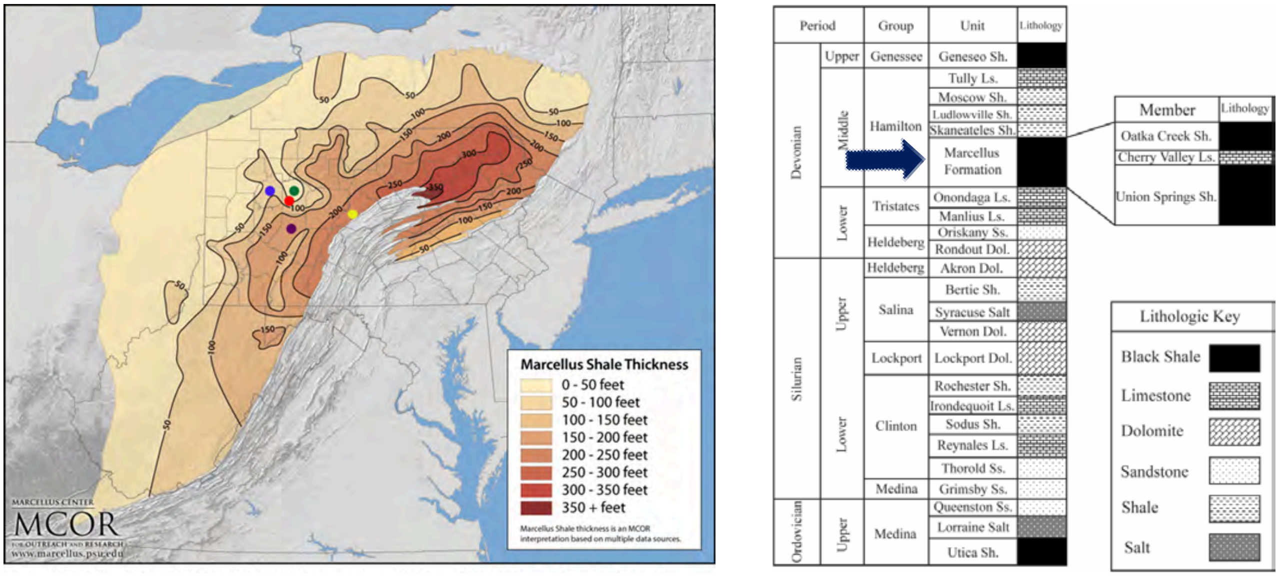

Figure 10: Thickness map (Kaufman & Zhu, 2014) and stratigraphic column (Blocho et al., 2021) concerning the Marcellus Formation.

Appendix B: Additional Images of the samples

Images were taken of all samples in varying stages of preparation (Figure 11). CM2 Jbilet Winselwan and C2 Tarda were photographed prior to being powdered. CM2 Aguas Zarcas and the three Marcellus Shale samples were photographed after being prepared and mounted on the sample plate.

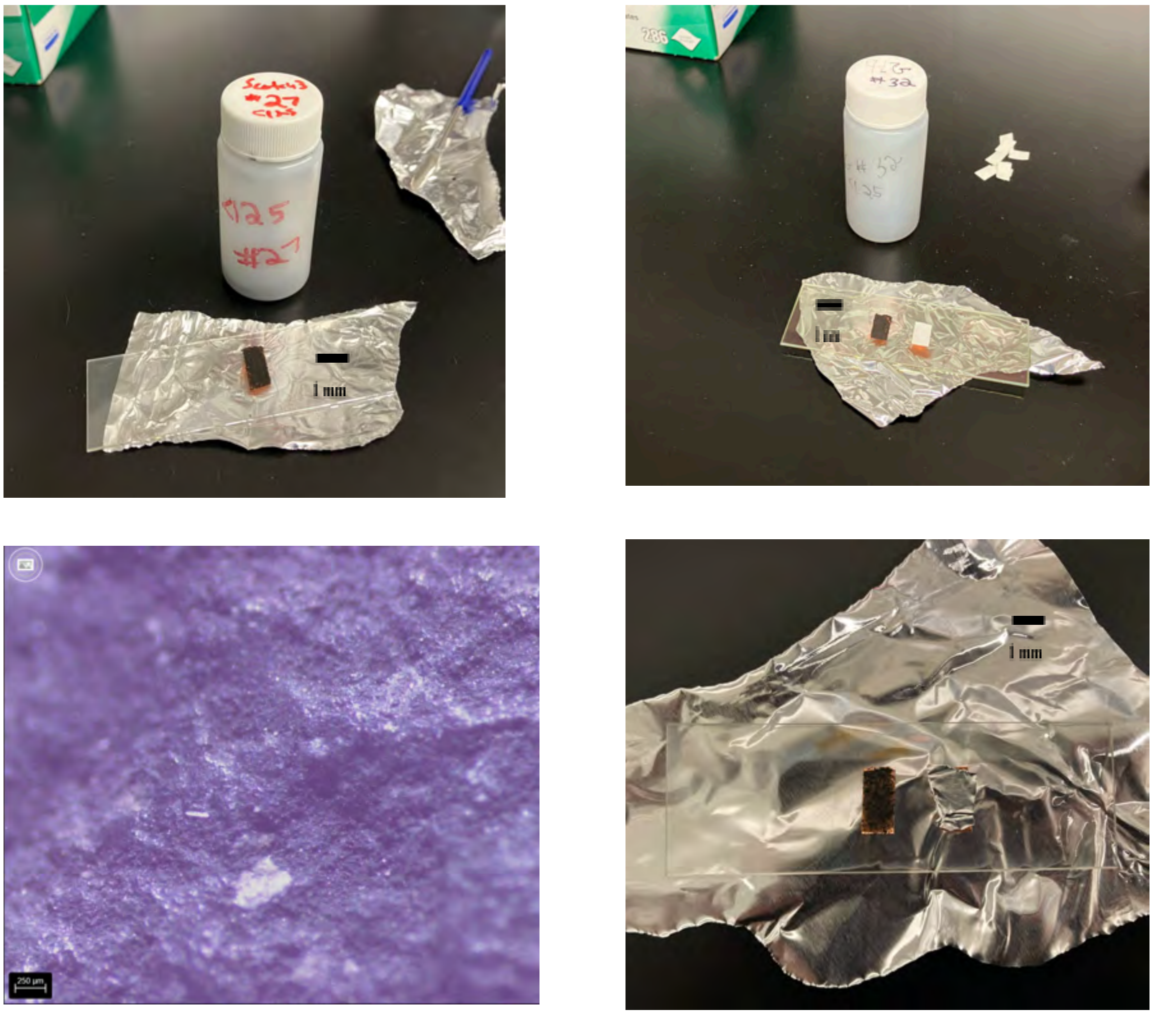

Figure 11. Photographs of the samples that were excluded from the main text. Marcellus cores Scotch3 (top left), 4lg (top right), C2 Tarda (bottom left), and CM2 Aguas Zarcas (bottom right).