Title: Comparative Analysis of Trace Soil Evidence by Infrared Microspectrometry

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Abstract

The objective of this experiment was to determine if soil particles could be matched to a known soil sample by infrared microspectroscopy. The infrared spectrum for soil samples collected at five different locations was collected. These samples were labeled as followed: Creek Side Golf (CSG), Mossy Oak Woods (MOW), Mossy Oak Yard (MOY), Sparrow Drive (SD), and Wilmington Beach (WB). These spectrums severed as a standard and were compared to two unknown soil samples randomly selected from the five locations. Based on the spectrum it was determine that unknown 1 came from the Sparrow Drive and unknown 2 came from Mossy Oak Woods. This proves that it is possible to determine the origin of trace soil particle. In order to determine if this holds true in all instances, a larger sample size and unknowns would need to be examined. The practical application for this experiment would be matching trace soil evidence found on a victim or suspect to a specific crime scene.

Introduction

Infrared (IR) spectrometry is the study of the interaction of infrared light with matter. The sample is measured by sending a beam of IR light through the sample and the energy absorb at each wavelength is recorded. This is used to determine the functional groups present in a sample. Functional groups in different environments will have different energy levels. The stronger the molecule's bond the further left on the spectrum it will appear. Similarly, the larger the mass of a molecule the further right on the spectrum it will appear. There are some complications in the spectra due to band shifts and overlapping. In short this means that the functional group does not appear where it should; instead it will be shifted

further up or down on the spectrum. In this experiment an infrared microspectrometer was used. This allows the operator to measure extremely small samples, along the scale of micrometers. The microspectrometer sends an infrared beam through the microscope and the beam is either transmitted or reflected off the sample. In transmittance mode the IR light is passed through the sample. In reflectance mode the IR light is bounced off the sample.

There are many known ways to compare soil samples. The typical ways are to find common characteristics such as color, texture, density, and mineral and organic composition. These methods require a large sample. This experiment was dealing with extremely small sample sizes. The method employed in this experiment would allow 2 or 3 specs of dirt to be matched to a known origin.

Experimental

Reagents

In this experiment liquid nitrogen was used to prevent the instrument from over heating. Distilled de-ionized water was used as the solvent for the soil samples.

Apparatus

A Perkin Elmer Spectrum Spotlight 150 FT-IR Microscope (Figure 1) was used to analyze the samples. A Dewar was used to transport the liquid nitrogen used in the instrument. Soil samples were collected and placed in Ziploc bags. A few soil particles were mixed with distilled de-ionized water in a test tube. A sonicator was used to dissolve solids into solution. A calcium fluoride window was used instead of a microscope slide

Figure 1: Perkin Elmer Spectrum Spotlight 150 FT-IR Microscope

to place the samples on. Once the solution was on the calcium fluoride window, placing window in a furnace evaporated off the water.

Instrumentation

To determine appropriate aperture settings a piece of Teflon was analyzed at different settings and the S/N ratios were compared. It was determined that a sample smaller than 30μm by 30μm produced a spectrum with to much noise to analyze. The aperture settings for the instrument were set to 100μm by 100μm. After finding the appropriate apertures, the specific light beam path was determined. For transmittance mode the sample must be thin enough for a majority of the light to pass through the sample and produce an appropriate spectrum. In reflectance mode the sample must have a smooth, flat surface. Reflectance mode is less preferred than transmittance mode because some of the IR light gets blocked by the mirrors, causing the spectrum to be noisy and possibly off scale. For this experiment it was determined that transmittance mode was preferred, since soil samples tend to not have smooth, flat surfaces.
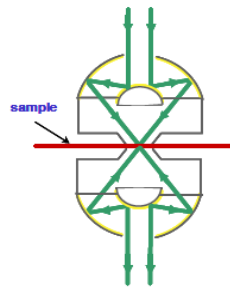
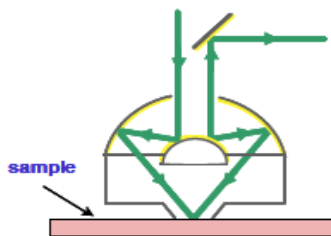Figure 2: FT-IR beam path in transmittance mode (Miller)

Figure 3: FT-IR beam path in transmittance mode (Miller)

Procedure

        The first step of this experiment was to collect soil samples from different locations. The next step was to determine appropriate aperture settings and sample size. This was done by examining a piece of Teflon under the IR microspectrometer. The appropriate aperture setting was determined to be 100μm by 100μm. Next the light beam path was determined. After some experimentation it was determined that transmittance mode was the best way to analyze a soil sample. This was largely due to the fact that soil samples don't have smooth, flat surfaces. The sample preparation for this experiment was simple. A few drops of distilled water was mixed in a test tube with a few particles of soil and sonicated. The sonicated solution was than placed on a calcium fluoride window and heated to evaporate the water. This was done for each sample and than a spectrum for each sample was collected. Two unknowns were than randomly selected from the soil samples. The unknowns were put through the same sample prep was the sample standards and spectrums were collected using transmittance mode. The unknown spectrums where compared to the standards to determine an origin. It was determined that unknown 1 came from Sparrow Drive and unknown 2 came from Mossy Oak Woods. This was in fact the original location for each of these samples and proved my hypothesis that it is possible to use infrared microspectroscopy to determine the original location of trace soil particles.

Results

Teflon S/N Ratio

The signal-to-noise ratio is used to determine the quality of an instrumental measurement. The higher the S/N Ration the better the spectra appears. This means that the peaks are more easily distinguishable from the background noise. The following table was collected from scanning a piece of Teflon in transmittance mode at different aperture settings.

Table 1: Aperture values with their S/N ratio, used to determine appropriate settings

| Aperture	Signal (A)	Noise (A)	Stand Dev=(Npp/5)	S/N=Signal/Stand Dev
800	0.0856	0.0037	0.0007	115.7
700	0.0189	0.0007	0.0001	135.0
600	0.0355	0.0012	0.0002	147.9
500	0.0307	0.0025	0.0005	61.40
400	0.0283	0.0007	0.0001	202.1
300	0.0309	0.0005	0.0001	309.0
200	0.0301	0.0011	0.0002	136.8
100	0.0267	0.0014	0.0003	95.36
90	0.0330	0.0030	0.0006	55.00
80	0.0245	0.0054	0.0011	22.69
70	0.0292	0.0078	0.0016	18.72
60	0.0302	0.0082	0.0016	18.41
50	0.0379	0.0151	0.0030	12.55
40	0.0176	0.0302	0.0060	2.914
30	Too noisy to distinguish peaks			

Creek Side Golf
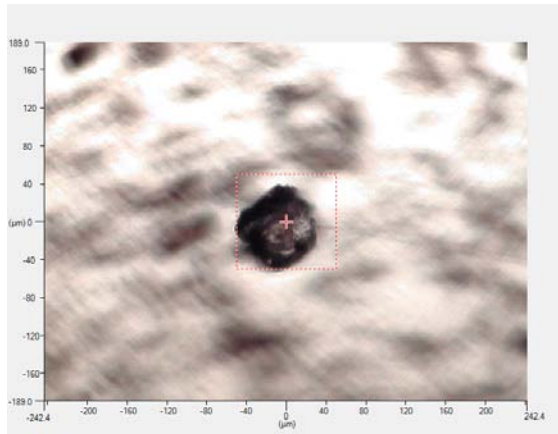
Figure 4: Picture of an area scanned by FT-IR Microspectrometer for the Creek Side Golf Sample
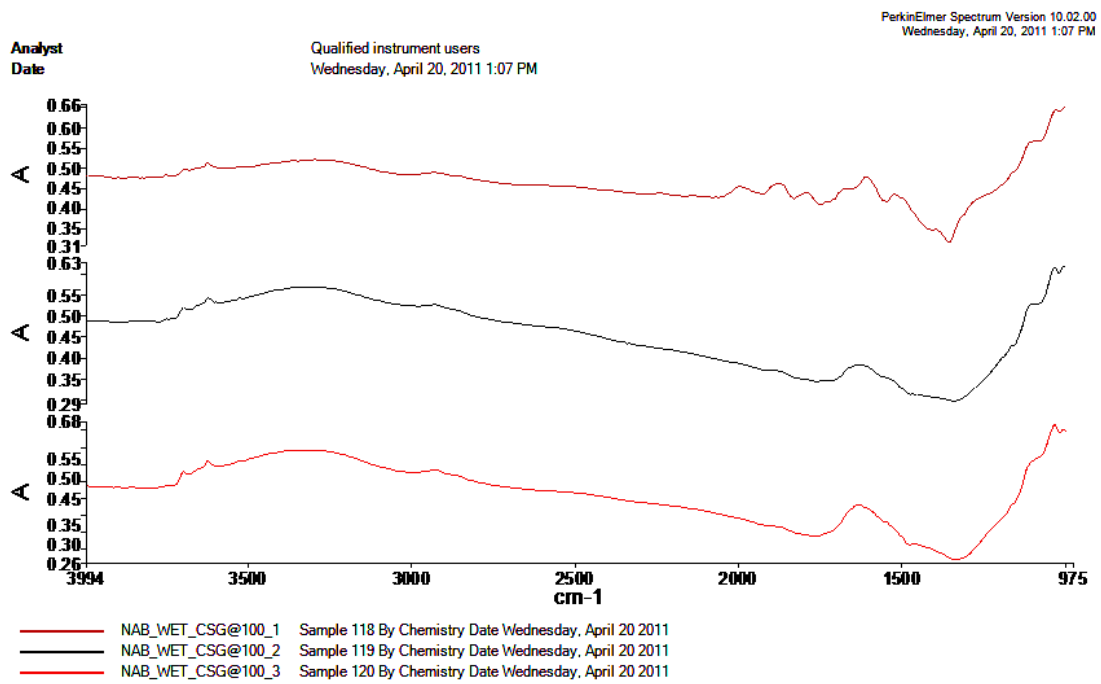

Figure 5: Spectra collected from Creek Side Golf Sample
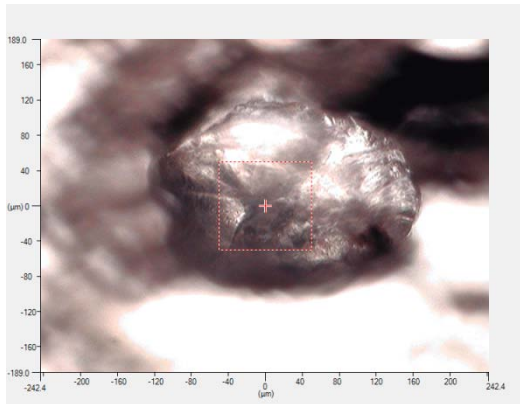
Mossy Oak Wood

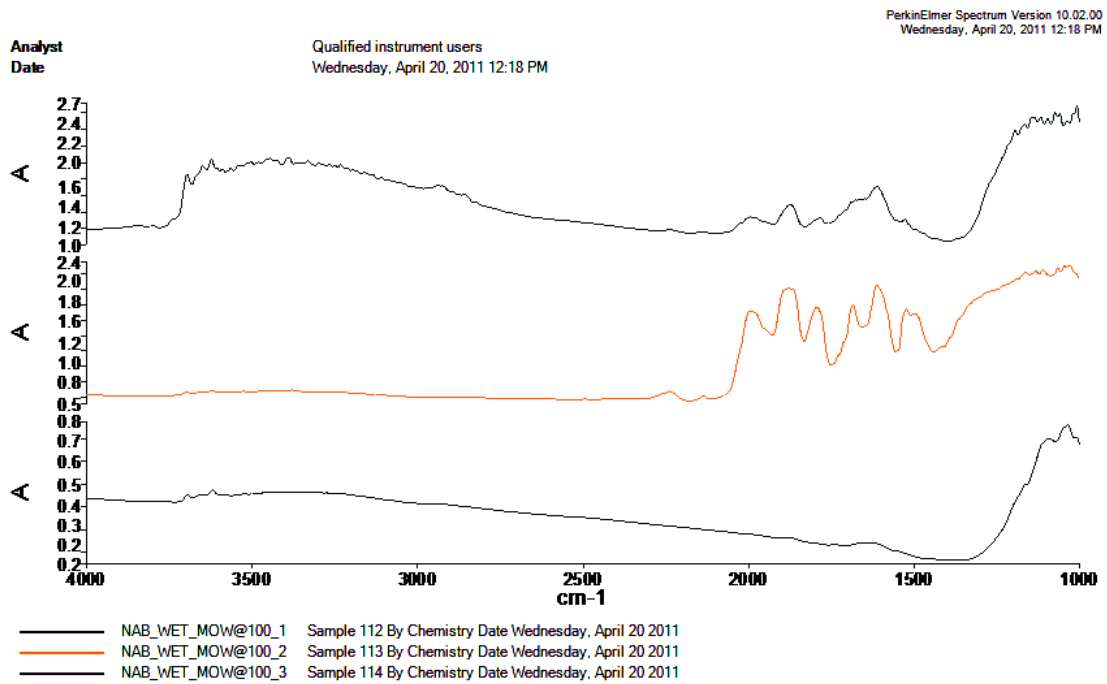Figure 6: Picture of an area scanned by FT-IR Microspectrometer for the Mossy Oak Wood Sample

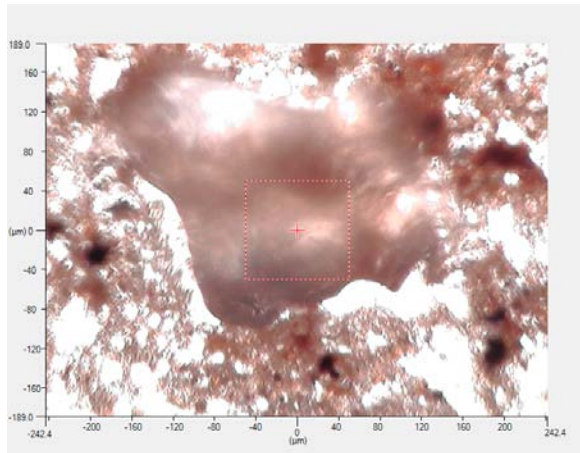Figure 7: Spectra collected from Mossy Oak Wood Sample

Mossy Oak Yard

Figure 8: Picture of an area scanned by FT-IR Microspectrometer for the Mossy Oak Yard Sample
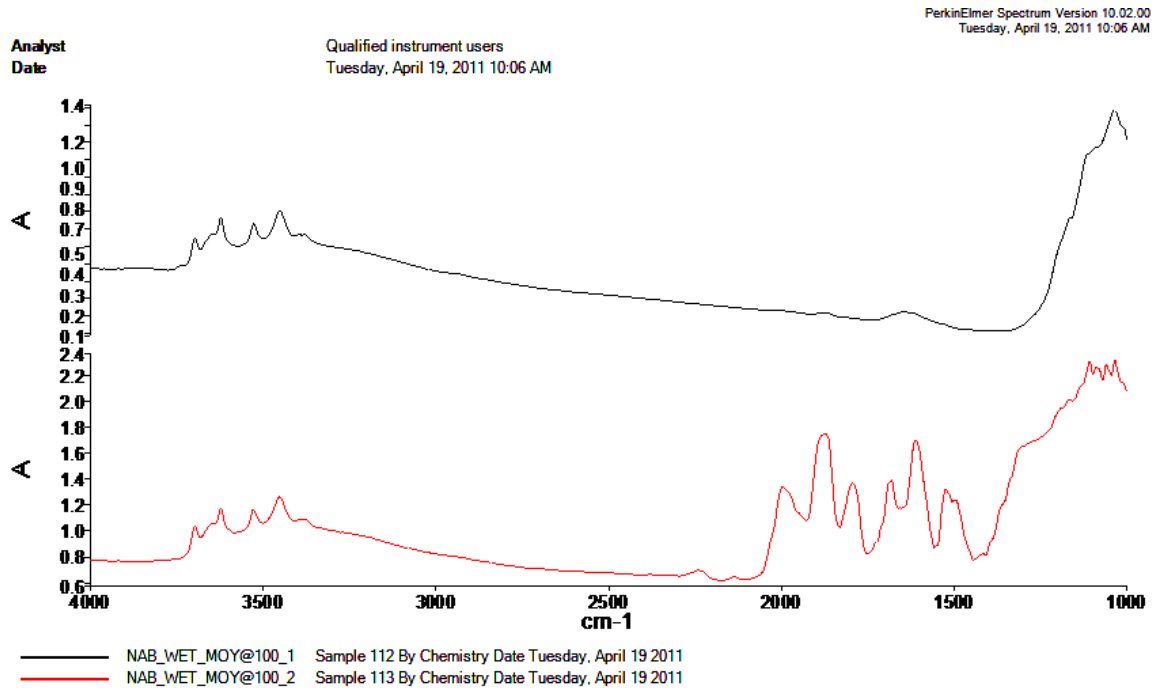

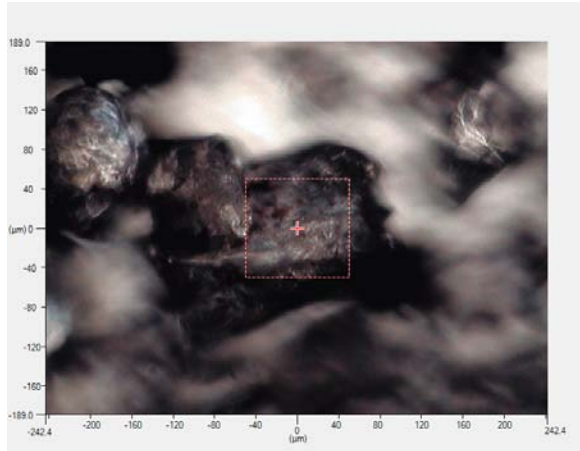Figure 9: Spectra collected from Mossy Oak Yard sample

Sparrow Drive

Figure 10: Picture of an area scanned by the FT-IR Microspectrometer for the Sparrow Drive sample
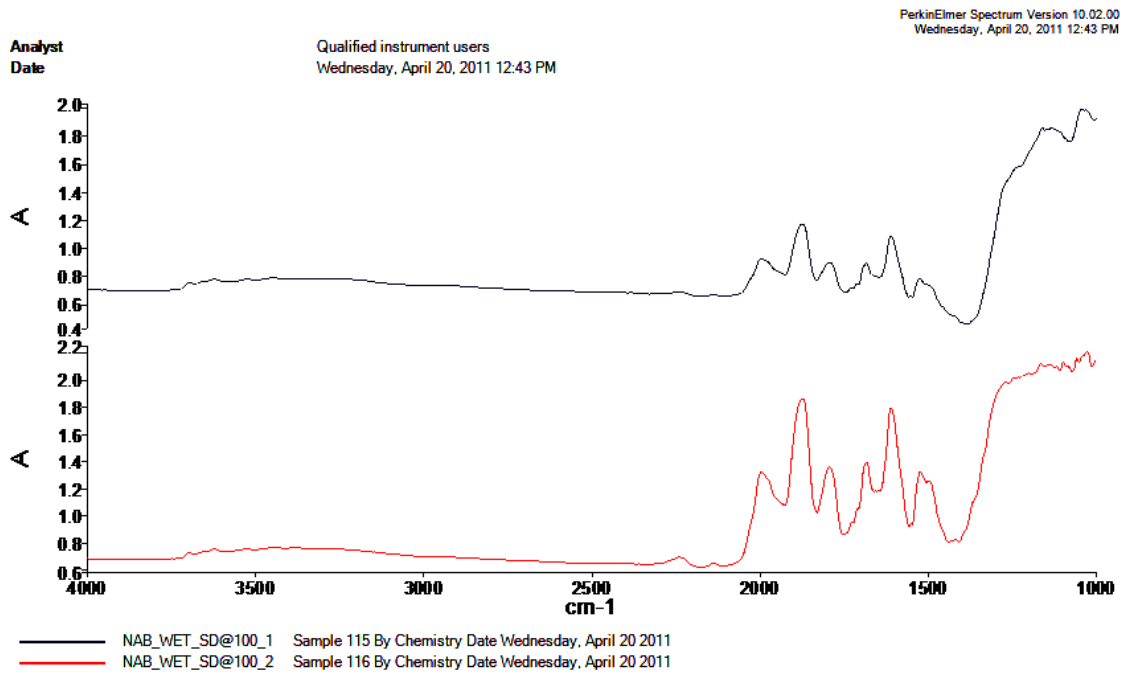

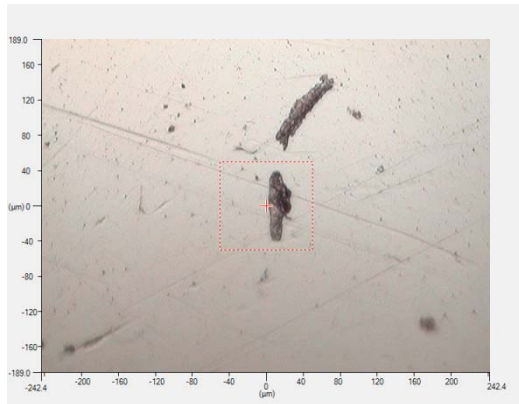**Figure 11: Spectra collected from Sparrow Drive sample**

Wilmington Beach

Figure 12: Picture of an area scanned by the FT-IR Microspectrometer for the Wilmington Beach sample
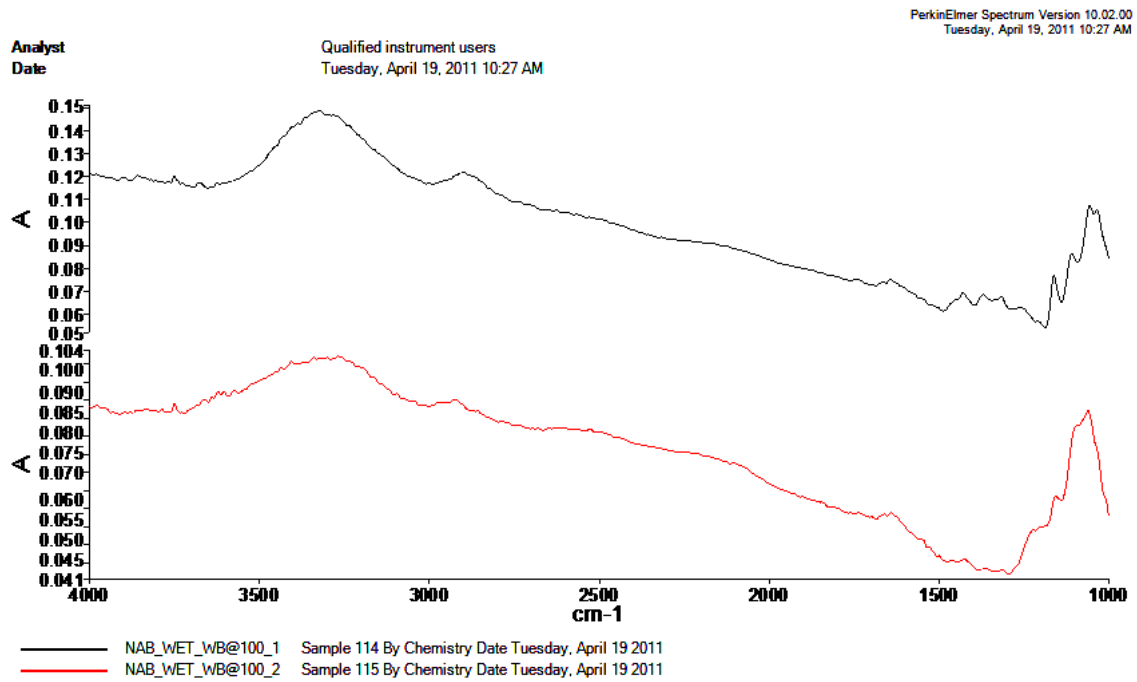

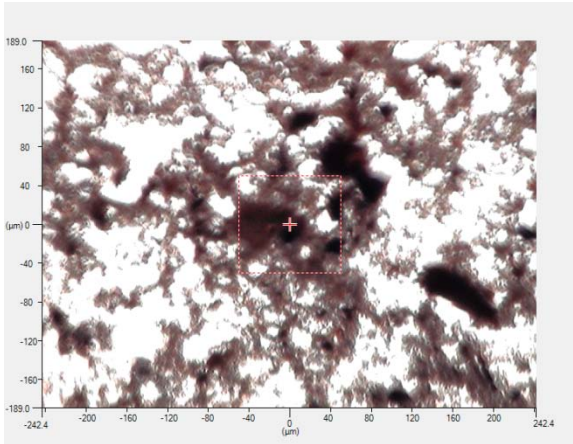Figure 13: Spectra collected from Wilmington Beach sample

Unknown 1

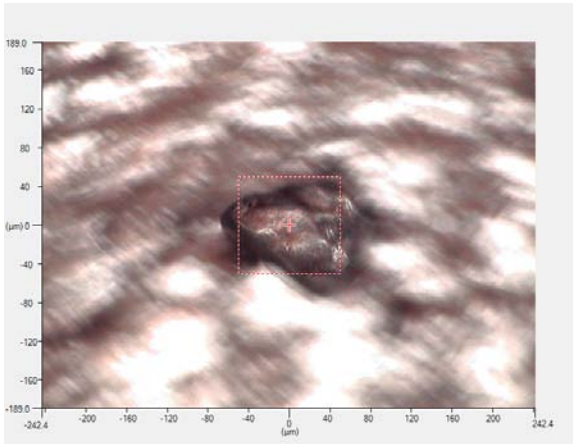Figure 14: Picture of area scanned for Unknown 1 A

Figure 15: Picture of area scanned for Unknown 1 B
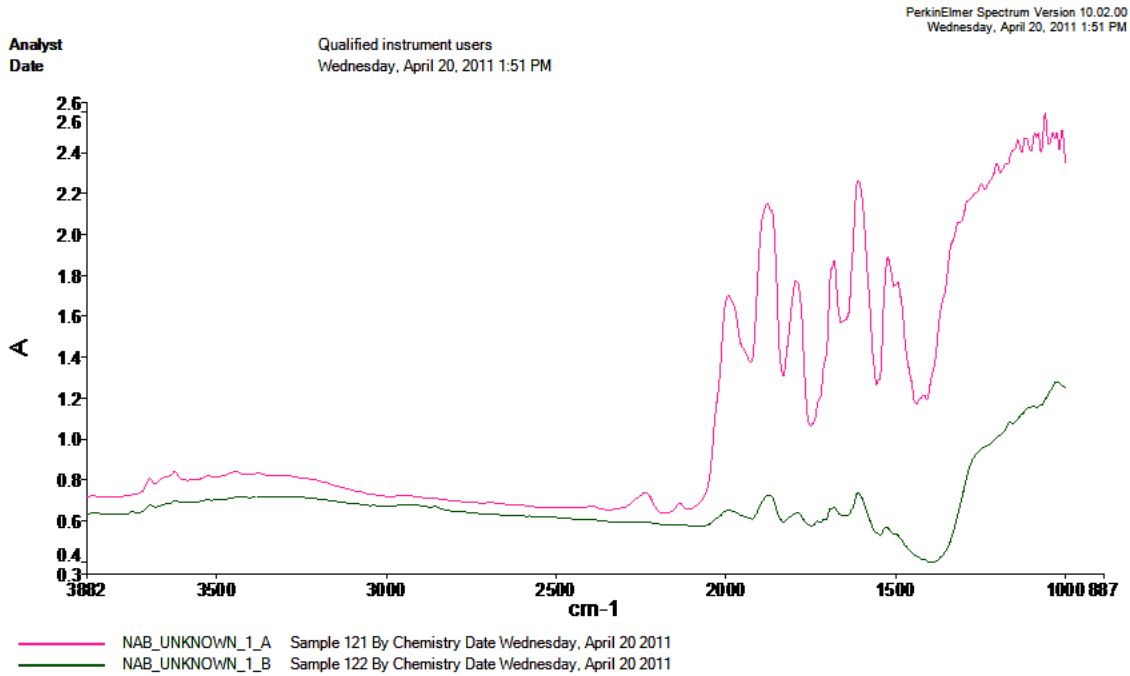
Figure 16: Spectra collected from Unknown 1
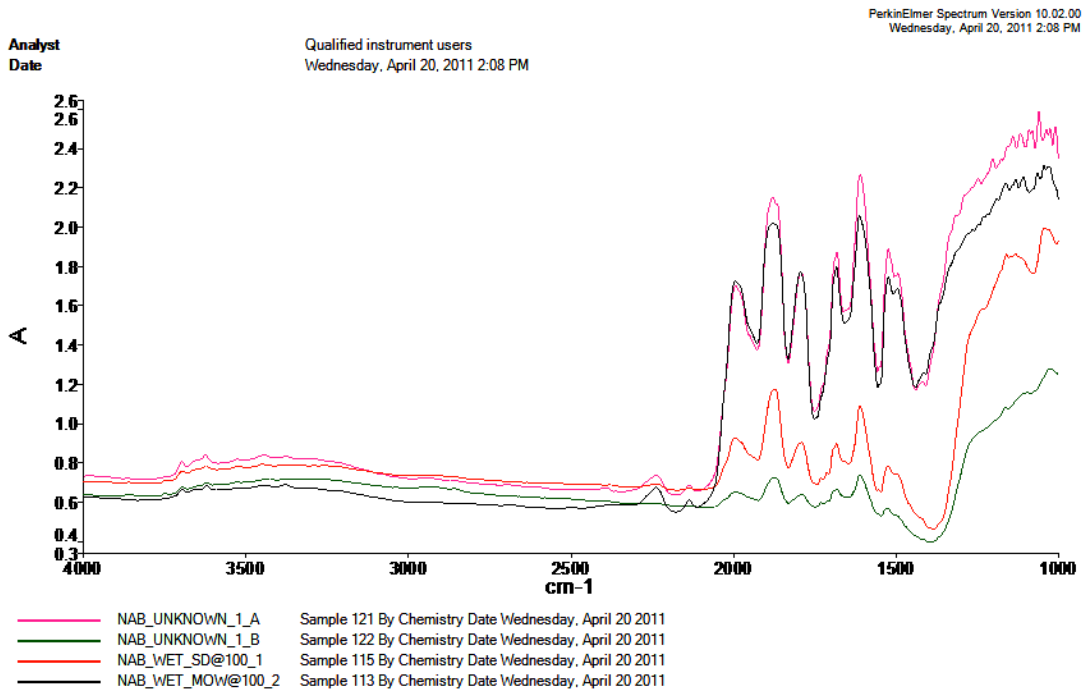
Figure 17: Spectra collected from Unknown 1 compared to most similar spectra (MOW and SD samples)

Table 2: Noticeable Peaks from Unknown 1 A and B and comparative spectra

Unknown 1 A	MOW 2	SD 2	Unknown 1 B	SD 1
3696	3696	3696	3695	3696
3624	3621	3623	3623	3623
3444	3378	3451		3528
2236	2238	2239	3318	3450
2136	2136	2136	2922	2238
1993	1994	1996		2136
1879	1878	1873	1995	1995
1794	1794	1794		1881
1681	1683	1682	1874	1874
1610	1612	1611	1788	1791
1523	1523	1523	1683	1683
1493	1494	1494	1611	1611
1416		1416	1526	1525
				1159
				1132
			1027	1043

Unknown 2
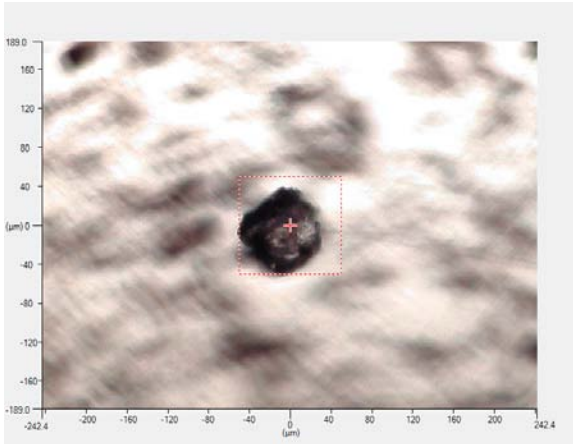
Figure 18: Picture of area scanned for Unknown 2 A
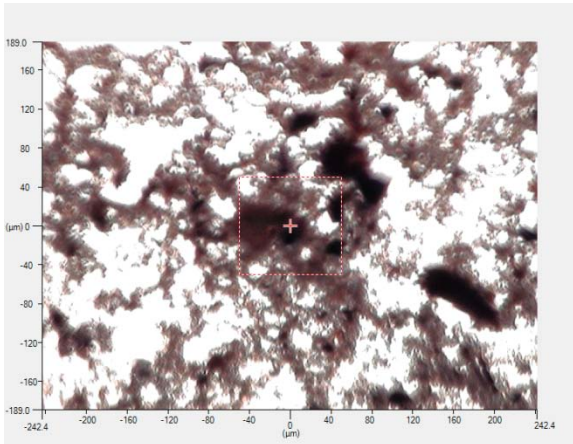
Figure 19: Picture of area scanned for Unknown 2 B

Analyst   Qualified instrument users
Date   Wednesday, April 27, 2011 12:01 PM

—— NAB_UNKNOWN_2_A	Sample 123 By Chemistry Date Wednesday, April 20 2011
—— NAB_UNKNOWN_2_B	Sample 124 By Chemistry Date Wednesday, April 20 2011
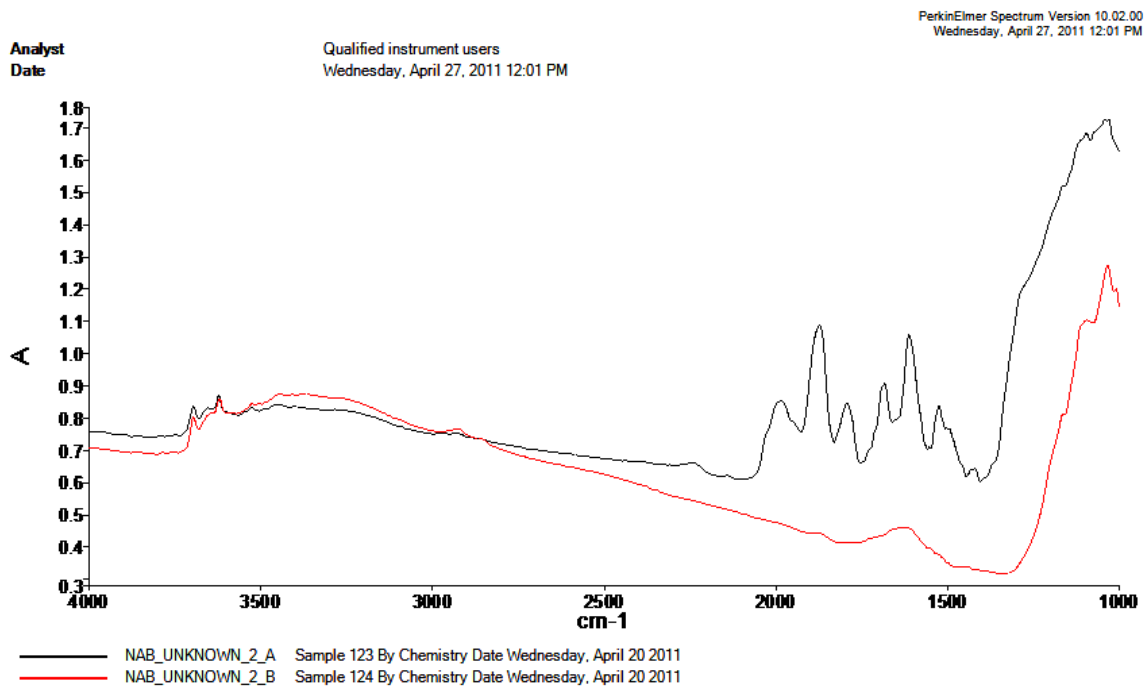
Figure 20: Spectra collected from Unknown 2

Analyst   Qualified instrument users
Date   Wednesday, April 27, 2011 12:03 PM

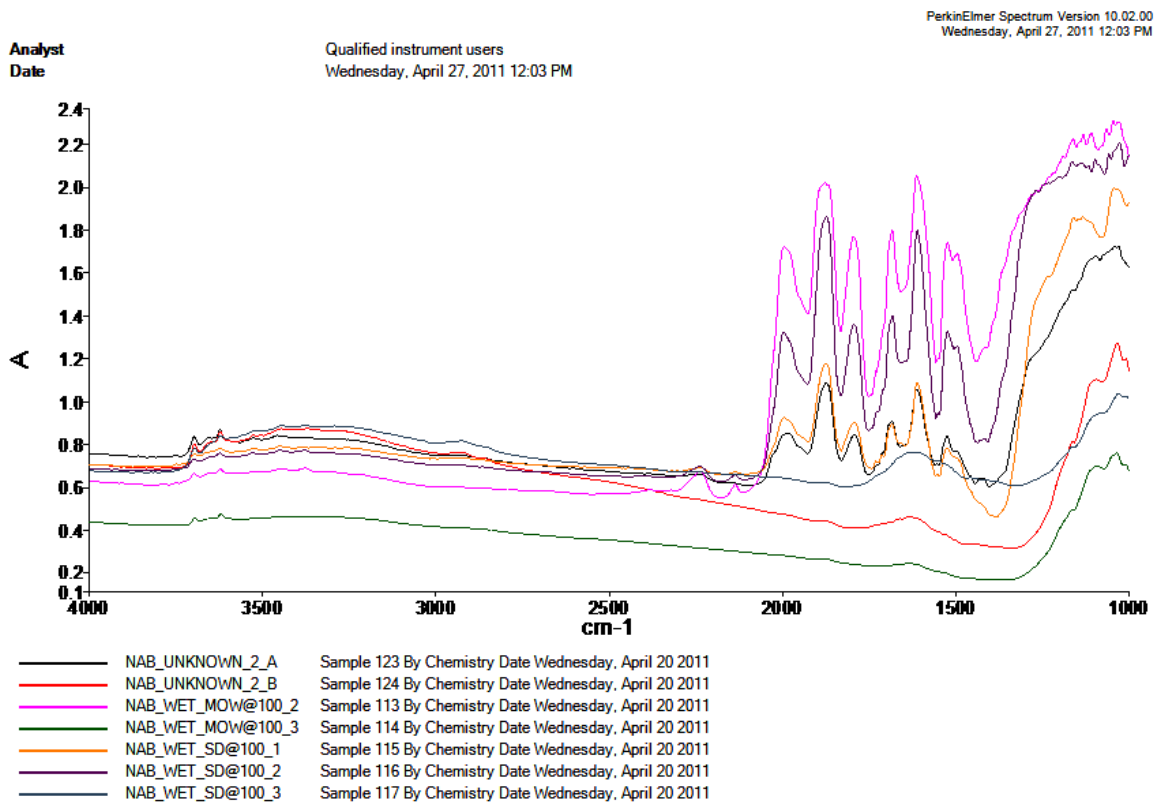|	
—— NAB_UNKNOWN_2_A	Sample 123 By Chemistry Date Wednesday, April 20 2011
—— NAB_UNKNOWN_2_B	Sample 124 By Chemistry Date Wednesday, April 20 2011
—— NAB_WET_MOW@100_2	Sample 113 By Chemistry Date Wednesday, April 20 2011
—— NAB_WET_MOW@100_3	Sample 114 By Chemistry Date Wednesday, April 20 2011
—— NAB_WET_SD@100_1	Sample 115 By Chemistry Date Wednesday, April 20 2011
—— NAB_WET_SD@100_2	Sample 116 By Chemistry Date Wednesday, April 20 2011
—— NAB_WET_SD@100_3	Sample 117 By Chemistry Date Wednesday, April 20 2011

Figure 21: Spectra collected from Unknown 2 compared to most similar spectra (MOW and SD samples)

Table 3: Noticeable Peaks from Unknown 2 A and B and comparative spectra

Unknown 2 A	SD 1	MOW 2	Unknown 2 B	MOW 3
3696	3696	3696	3696	3895
3622	3623	3621	3620	3619
3526	3528		3370	3445
3456	3450	3378	1636	1628
	2238	2238	1094	1093
	2136	2136	1033	1035
1995	1995	1994		
	1881			
1873	1874	1878		
1793	1791	1794		
1682	1683	1683		
1612	1611	1612		
1524	1525	1523		
1421		1494		
	1159			
	1132			
	1043			

Discussion

In this experiment Teflon was used to determine the appropriate aperture settings and beam path mode. It was determined that setting the apertures below 30μm yielded a spectrum that was too noisy to determine the peaks. This meant that the sample had to be larger than 30μm by 30μm. In the experiment, trace amounts of a sample were analyzed. These soil particles were extremely small. In order for the experiment to work, the soil particles needed to cover a larger area. Another problem discovered was that the soil samples were too thick to be analyzed with transmittance mode and too ridged to use reflectance mode. This meant that the particles needed to be thinner and have a smoother surface. By dissolving the soil particles in water and than recrystallizing them on a calcium fluoride window the samples would be thinner, smoother, and cover a larger surface area. The particles were sonicated in order to get them into solution. This solution was than placed on a CaF window and heated to evaporate the water. Because of the complexity and inconsistency of soil, the samples were scanned in different spots to collect multiple spectra for the same sample.

In this experiment the infrared spectra from soil samples collected at 5 different locations were used to compare to 2 unknowns. The Wilmington Beach sample is extremely different than the other 4 samples. This is because the beach sample has a small amount of organic compounds and consists mainly of glass. It had 1 broad peak in the 3300 cm^{-1} region followed by a smaller broad peak in the 2900 cm^{-1} region and than a gagged peak in the 1100 cm^{-1} region. Creek Side Golf was also different than the other samples. This sample was collected near water and would have different organic matter than soil collected farther away from a creek. It had 2 peaks on the front end of a broad peak. These two peaks appeared at 3700 and 3600 cm^{-1}. A smaller peak at 2900 cm-1 and another peak around 1600 cm-1 followed the broad peak. The other 3 samples were extremely similar. Mossy Oak Yard had a series of peaks in the 3400-3700 cm^{-1} regions. The Sparrow Drive sample and the Mossy Oak Woods sample were the most similar. These 2 samples had approximately 10 peaks that either matched up or where within 2 cm^{-1} of each other. These 5 soil samples were than compared to the unknowns to determine the original source of the unknown samples. Both unknowns were narrowed down to either the Sparrow Drive sample or the Mossy Oak Woods sample.

Conclusion

For this experiment it was possible to match the unknowns to their original sample standard. Unknown 1 was determined to be the Sparrow Drive sample and unknown 2 was determined to be the Mossy Oak Woods sample. This was the origin of these two samples.

Future Research

More research needs to be done in order to determine if this process will work in every instance. A larger sample size would determine if this particular method would improve current soil comparison techniques.

References

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5. Smith, B.C. *Fundamentals of Fourier Transform Infrared Spectroscopy.* NY, CRC Press, Inc, (1996).

6. Cox, R.J.; Peterson, H.L.; Young, J.; Cusik, C.;Espinoza, E.O. "The Forensic Analysis of Soil Organic by FTIR." *Forensic Science International*. 108 p 107-116 (1999).