

## **Frontier Analysis, Ltd**

### **TECHNICAL SERVICE RESPONSE NO.: UT053**

**Subject:** Analysis of Samples Related to a Bison Mutilation in Saskatchewan, Canada (Discovered March 13, 2008)

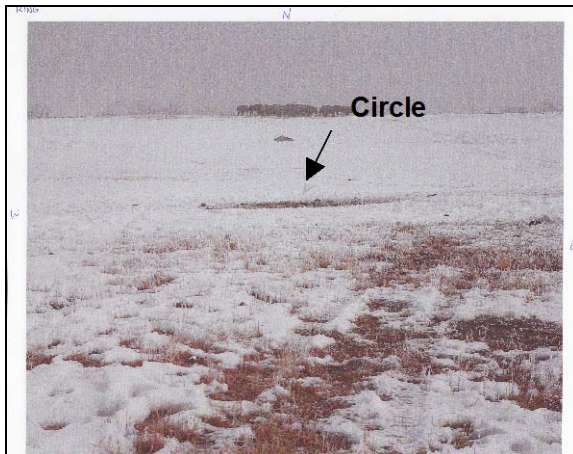
**Date:** August 7, 2008

**Requested By:** Barb Campbell  
Maidstone SK Canada

Nancy Talbott  
BLT Research

**Reported By:** P. A. Budinger  
Analytical Scientist

**Background/Objective:** A bison bull, last seen alive on March 10, 2008, was found mutilated on March 13, 2008 in a circle of melted snow. The animal had a cored rectum and was opened from his sex organs area. He was 12-13 years old and weighed 1780 pounds. He was in excellent health and had received all his inoculations. The herd included 14 other bulls. In another, nearby field were 200 bison cows and calves. The object is to determine anything anomalous in hair, hide and soil samples from the site. Following are photographs of the site.



The circle surrounding the mutilated bison.



A close-up of the circle.

Photographs by Barbara Campbell

**Conclusions:**

- The Bison hair sample contains 3-hydroxyanthranilic acid. The source of this compound is unknown. It cannot be related to a common substance introduced into the head by the farmer or from the environment of the bison. A literature search most commonly referred that it is a metabolic product of tryptophan.<sup>1</sup> Tryptophan was found in high levels in a mutilated cow in Utah. It was thought that the Utah animal had been sedated with tryptophan before euthanasia.<sup>2</sup> This analyst speculates that this may have been the case for this Bison.

- No anomalous materials are detected in the site soil samples. The usual soil components were detected: silicate mineral, inorganic carbonate, inorganic sulfate, humate material.

### **Procedure:**

Samples: The samples were obtained from the site on March 30, 2008, and submitted with the following information

- Hair Samples (Obtained March 20, 2008. Handled with latex gloves.)

S1 = Burned hair. Discovered 70 feet south of ring.

S2 = Unburned hair. Discovered in the ring. This sample is to be compared to the burnt hair.

Multiple infrared spectra were obtained from both samples. The burned hair was also extracted with chloroform, followed by acetone. Infrared spectra were then obtained from the chloroform soluble, acetone solubles, and the insolubles. The spectra were acquired on Thermo Scientific's Avatar 360 spectrometer using the Smart Harrick diamond SplitPea<sup>®</sup> sampling accessory.

- Soil Samples (Ground froze overnight again. Used axe to loosen (unfortunately). Scooped with plastic spoon. Used latex gloves.)

S1 = Where carcass was discovered.

S2 = Where carcass was discovered.

S3 = Within ring where ice was located.

S4 = Within ring where ice was located.

C1 = 100 feet south of ring. (Control for reference to the above.)

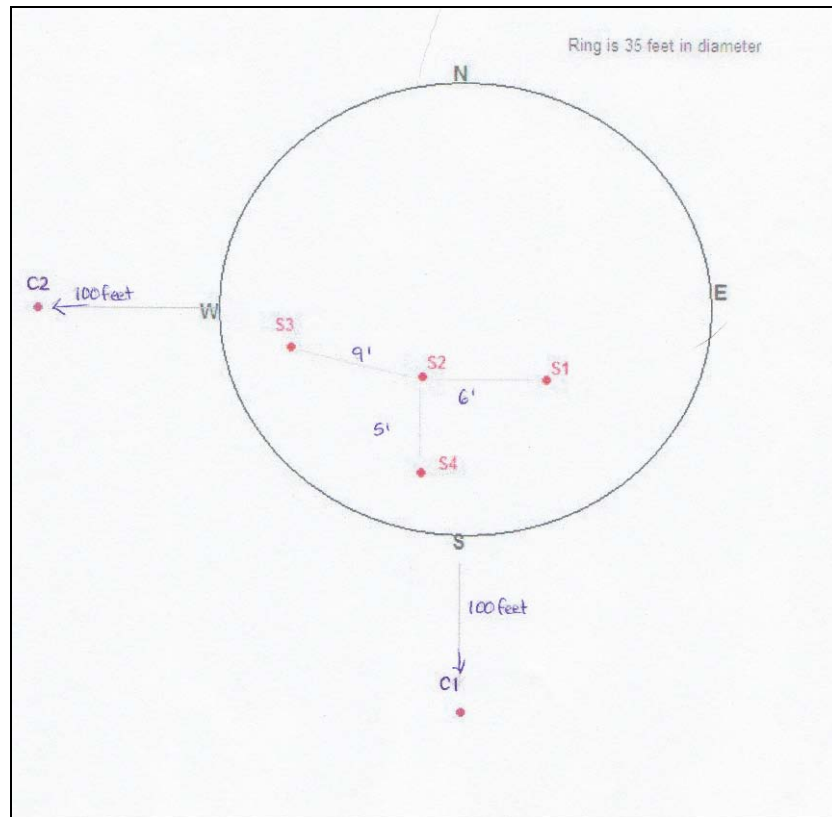
C2 = 100 feet west of ring. (Control for reference to the above.)

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<sup>1</sup> [http://en.wikipedia.org/dict/3-hydroxyanthranilic\\_acid](http://en.wikipedia.org/dict/3-hydroxyanthranilic_acid); <http://www.drugs.com/dict/3-hydroxyanthranilic-acidhtml?printable=1>; <http://cancerweb.ncl.ac.uk/cgi-bin/omd?3-hydroxyanthranilic+acid>; and many other references.

<sup>2</sup> Frontier Analysis T. S. T. No.: UT023, "Analysis of Samples from a Cow Mutilated in the Fall of 2001 (Logan, Utah)".

The following drawing of the site shows the locations where the samples were obtained.



By Barbara Campbell

Infrared spectra were obtained from all the 'as received' samples. In addition, the samples were quantitatively extracted with distilled water. Also, selected samples were extracted with chloroform. Infrared spectra were then acquired from the extracts.

• Hide Samples (Obtained with sterilized scalpels. Handled with latex gloves.)

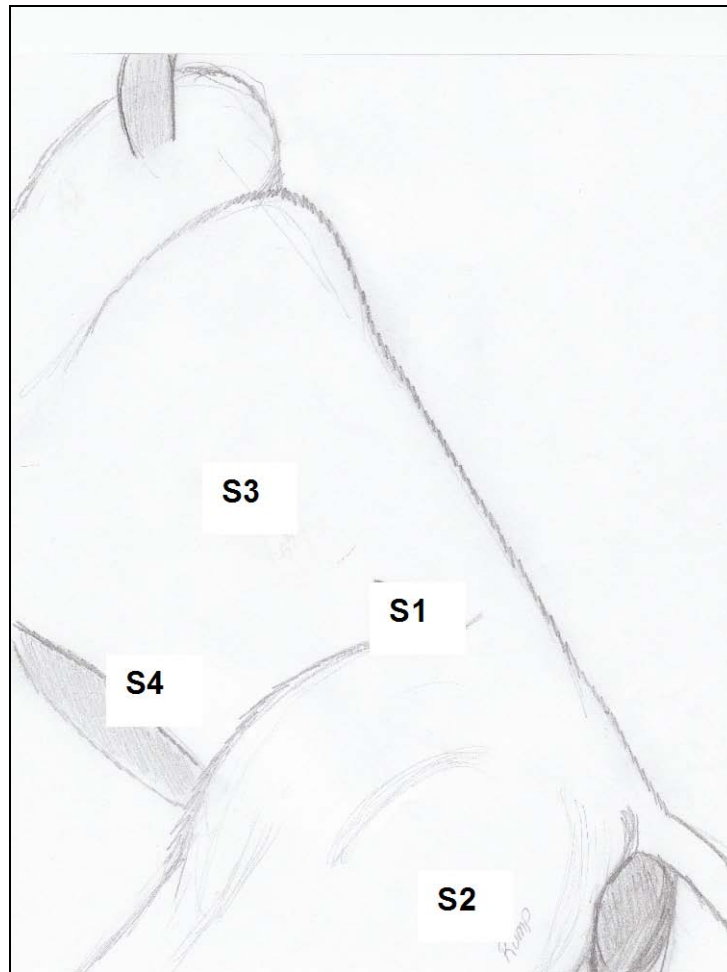
S1 = Section where hair is missing, includes section with hair.

S2 = Section with possible burn. Strange track mark.

S3 = Section where hair looks cut. Still some strands in bare area.

S4 = Normal section of hide. Includes an edge of where stomach area was excised.

The hide samples had suffered severe degradation by the time they were received by this laboratory. Therefore, they were not analyzed. However, the following drawing of the bison shows the areas sampled.



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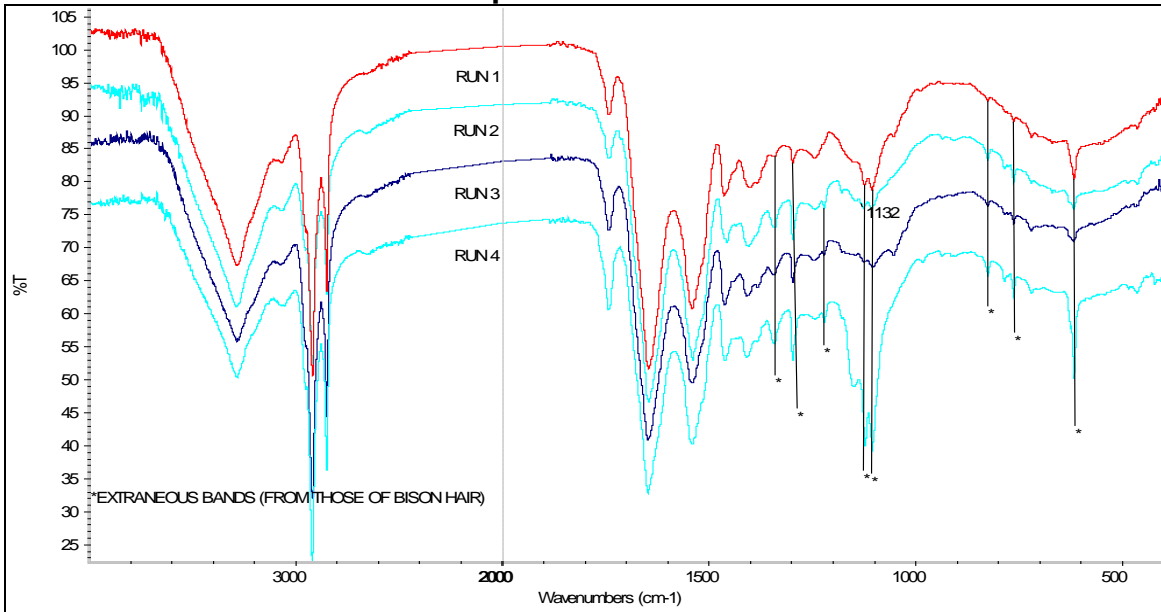
### **Results:**

The results of the individual tests done on the samples follow. These results are summarized in the conclusions section on the page three of this report.

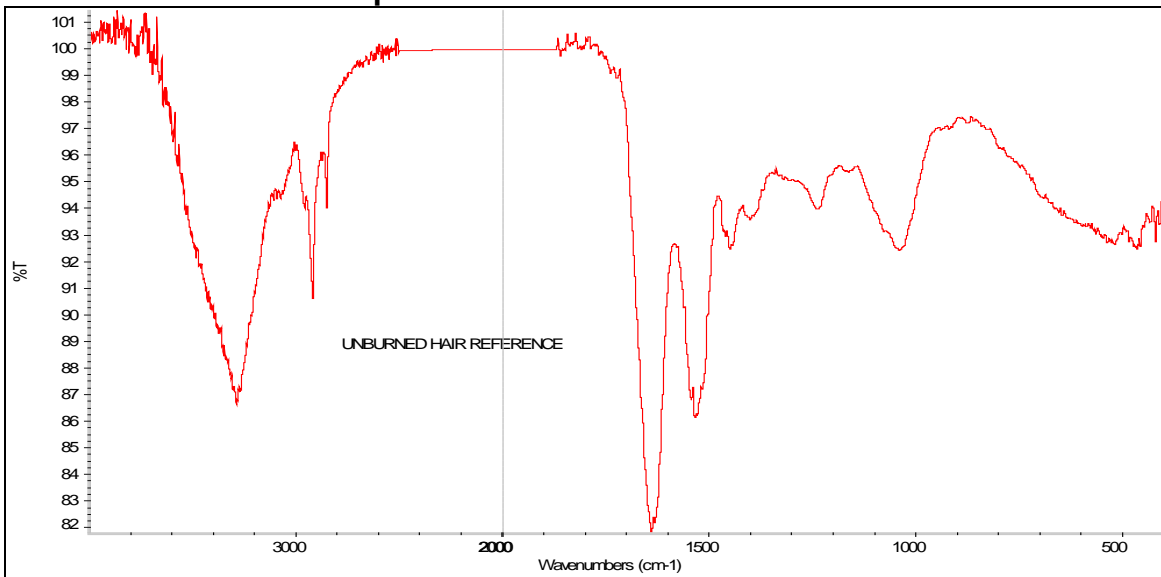
### **Analysis of Burnt Hair from the Mutilated Bison**

**Infrared Analysis of the 'As Received' Hair:** The infrared spectra obtained from the burned hair were compared to a spectrum from the unburned hair that served as a reference. The burned hair spectra expectedly show prominent absorption bands typical of hair, but in addition, sharp extraneous bands are displayed. This shows foreign material is on the burned hair. The spectra of the burned hair followed by an unburned hair reference for comparison is displayed below. (The burned spectra additional bands are labeled.)

### Infrared Spectra of Burned Hair

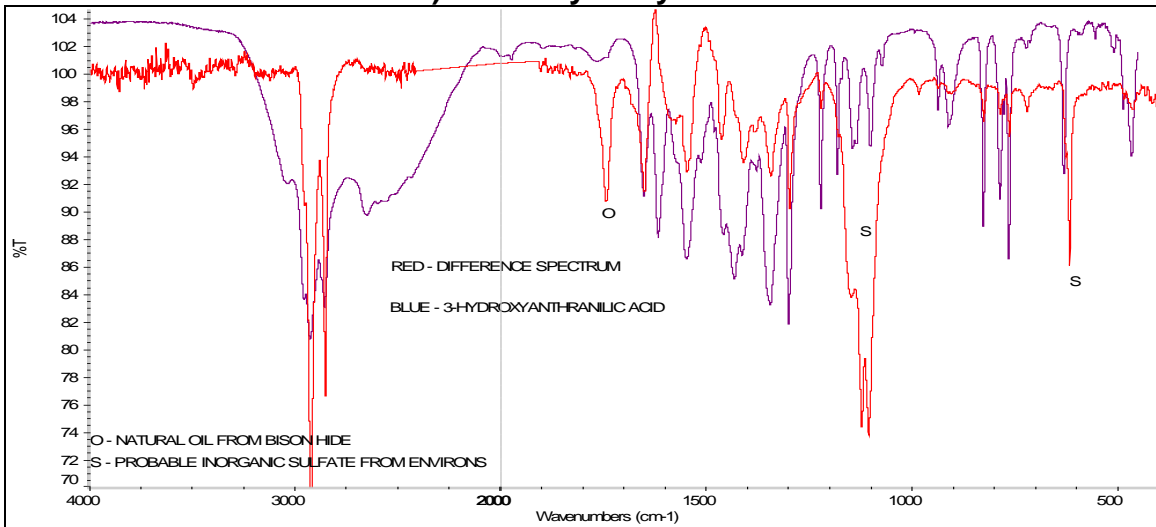


### Infrared spectrum of Unburned Hair Reference



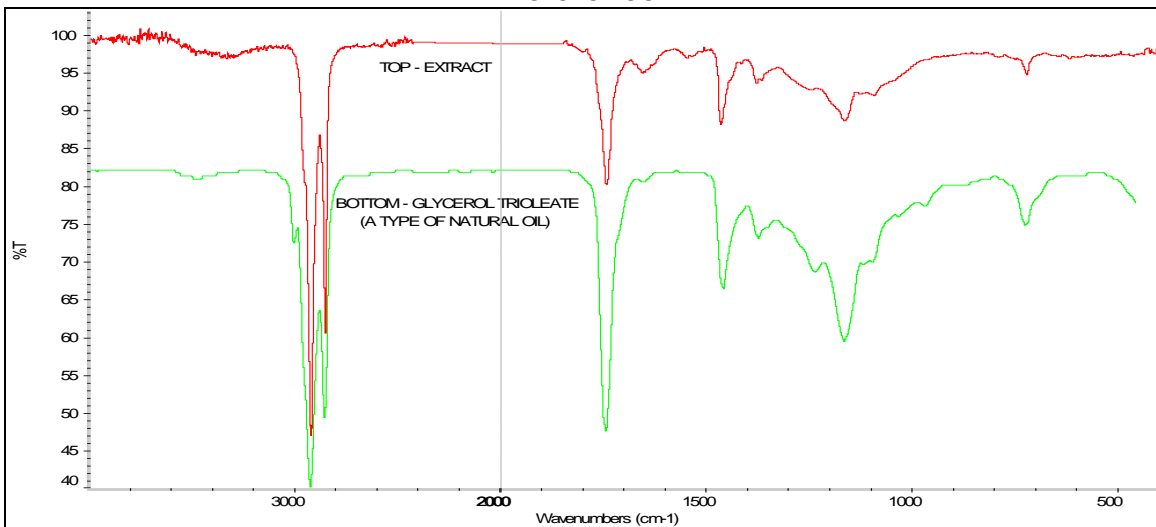
A difference spectrum was generated between spectra of the burned hair versus that of the unburned hair. This effectively nulled out the interfering absorption from the hair and enhanced and revealed additional bands from the foreign material. This computer procedure permitted a specific identification of the material as 3-hydroxyanthranilic acid. Also noted is some natural oil from the bison's hide and probable inorganic sulfate mineral from the environment. Following are the difference spectrum and a reference of 3-hydroxyanthranilic acid for comparison.

### Infrared Spectra of Difference (Bison Burned Hair Surface Versus Unburned Hair Surface) and 3-Hydroxyanthranilic Acid Reference

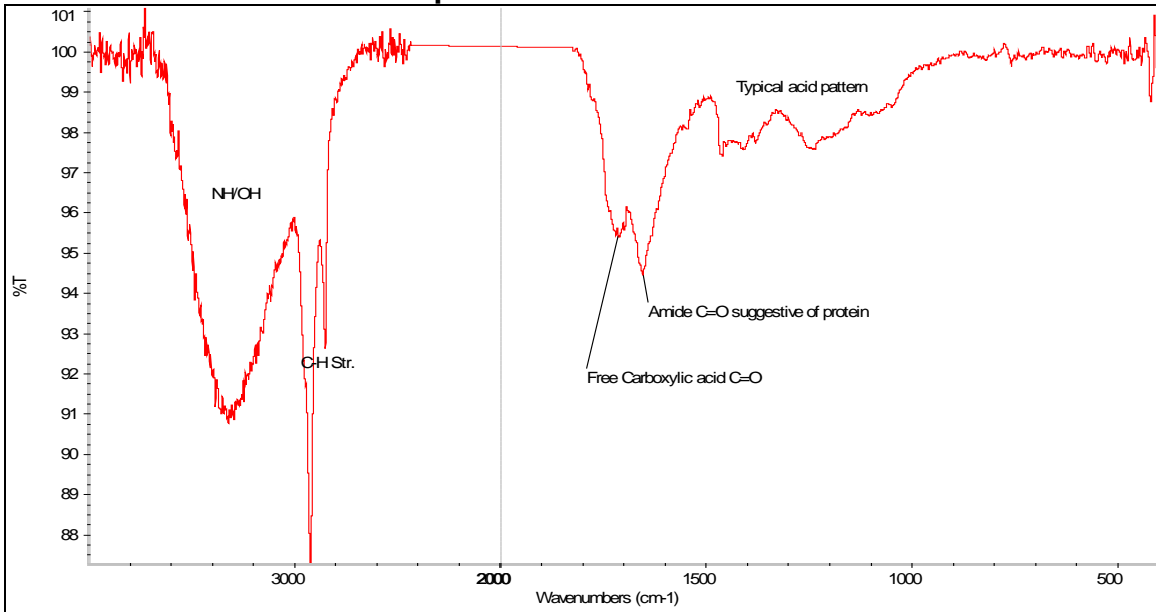


**Analysis of Solvent Extracts:** All solvent extracts from the burned hair are in very small amounts. Infrared spectra of the solvent extracts do not detect unusual components. A spectrum of the chloroform extract shows only a natural oil (glycerol ester) from the animal. A spectrum of the acetone extract, which was done on the chloroform extracted material, shows a natural long chain carboxylic acid and possibly some soluble protein. And finally, the spectrum of the chloroform/acetone insolubles expectedly shows mostly hair and a small amount of inorganic sulfate mineral. Following are the labeled spectra along with a reference of a glycerol ester for comparison.

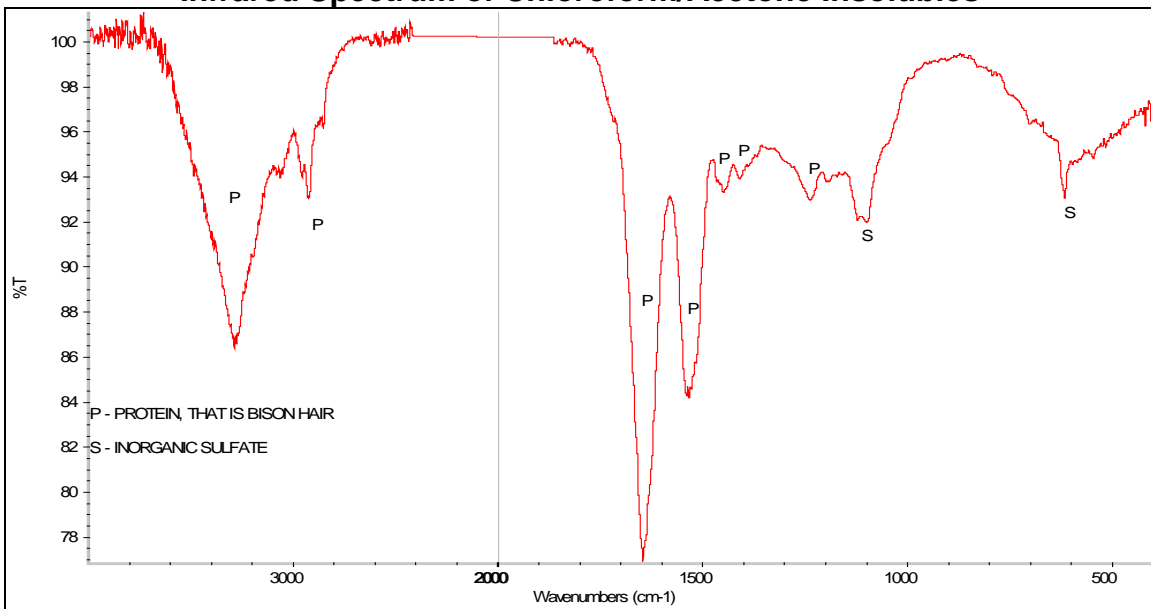
### Infrared Spectra of Chloroform Extract from the Hair and Glycerol Trioleate Reference



### Infrared Spectrum of Acetone Solubles



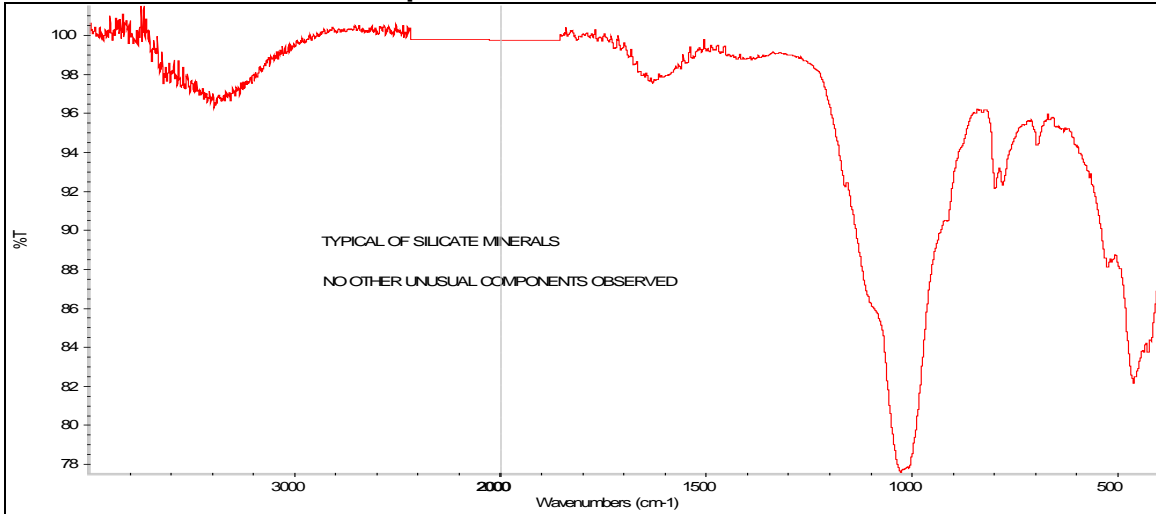
### Infrared Spectrum of Chloroform/Acetone Insolubles



### Analysis of Soils from the Mutilation Site

**Infrared Analysis of the 'As Received' Soils:** Infrared analysis of the S4 site soil shows nothing unusual, only typical soil minerals are detected. Because this is a site sample and did not reveal any additional information, the others from the site were not examined.

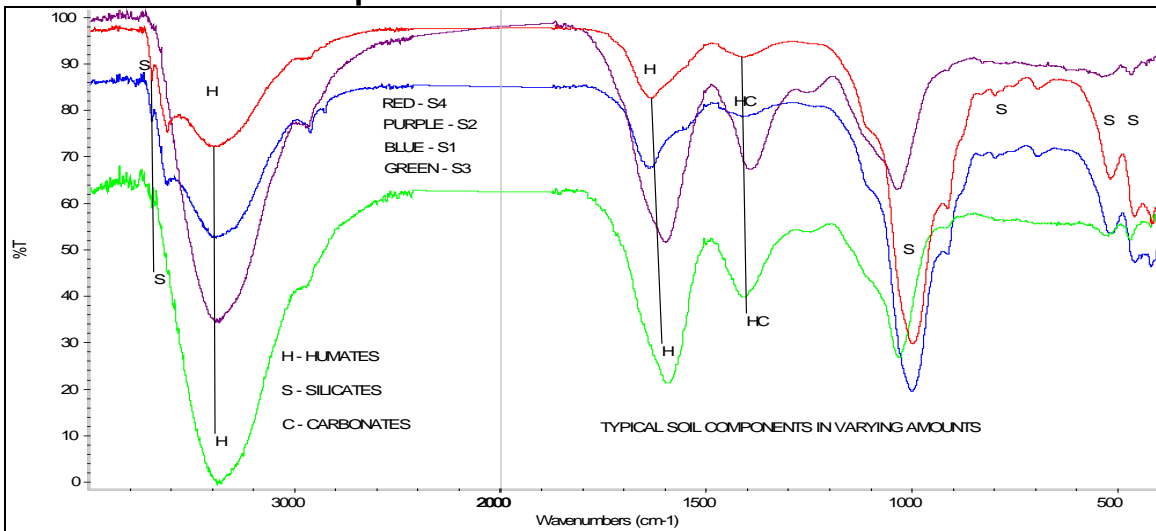
### Infrared Spectrum of 'As Received' S4 Soil



**Analysis of Water Extracts:** All soils (site and controls) were extracted with water to determine whether any unusual materials were present. Very small amounts (less than 1 wt.%) of materials were soluble.

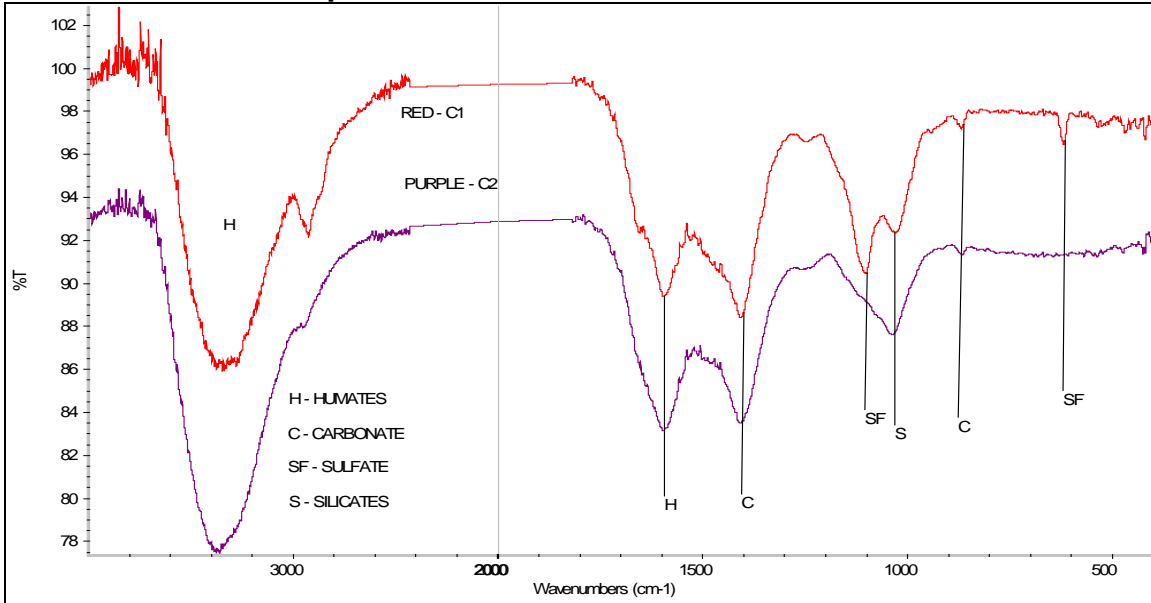
Infrared analysis of the extracts from the site shows typical components common to soil. These include humates, inorganic carbonates, and fine silicate mineral that could not filtered out. Band ratios show they are unevenly distributed. These compare to the materials found in the extracts from the control samples. The control samples contain the above mentioned humates, inorganic carbonate and silicate fines. In addition, some inorganic sulfate is present in one sample (C1). Following are the spectra of the site soil extracts followed by the control soil extracts.

### Infrared Spectra of Water Extracts from Site Soils



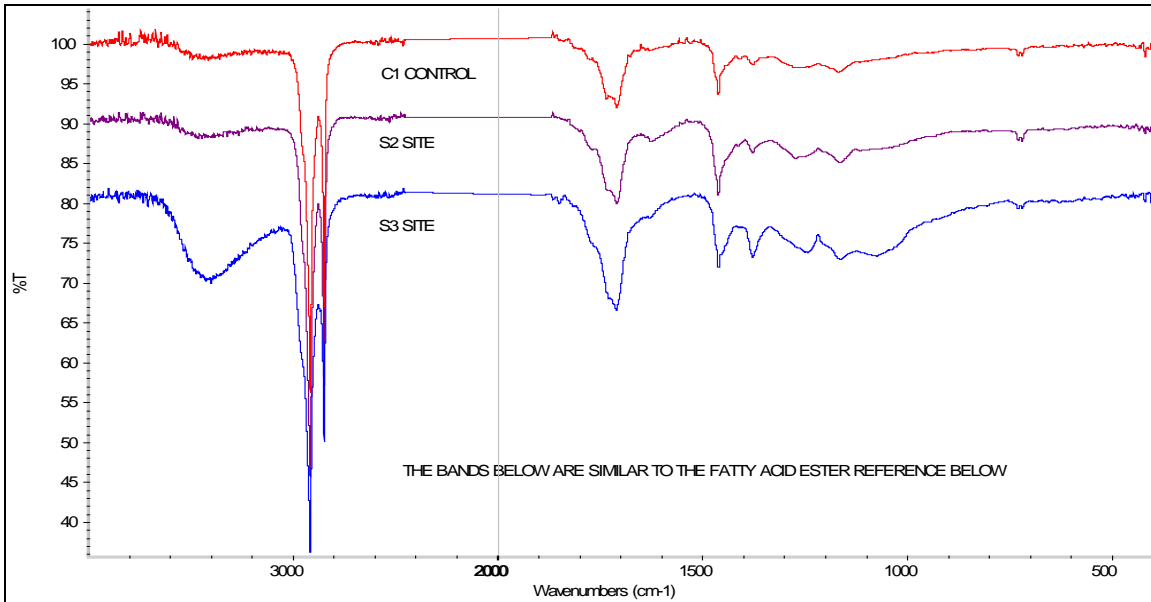


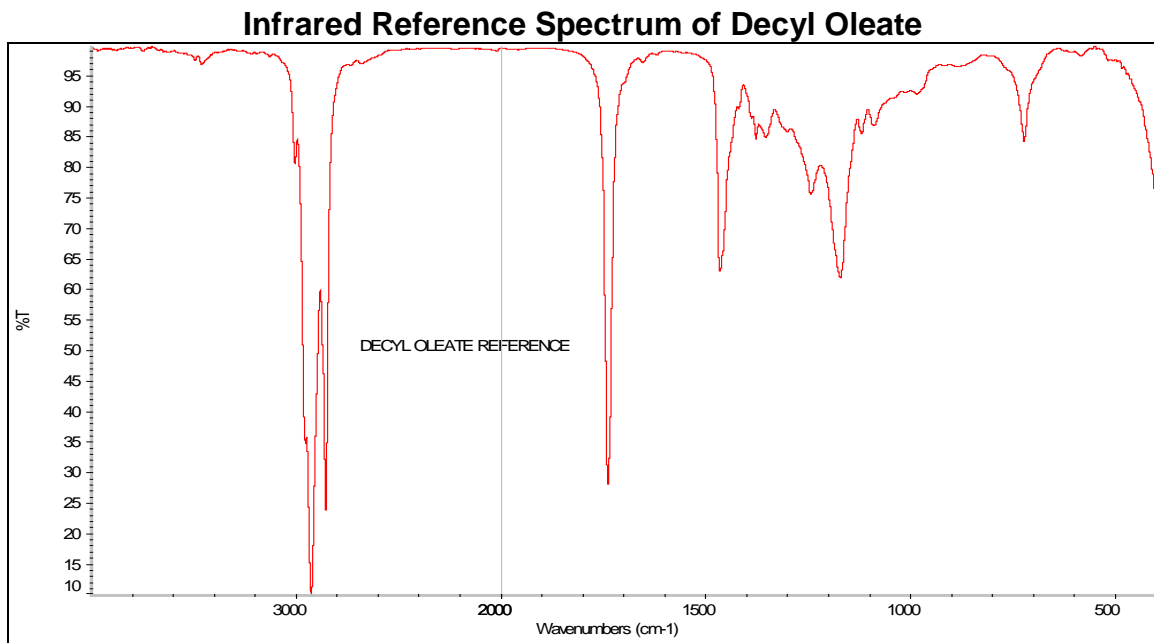
### Infrared Spectra of Water Extracts from Control Soils



**Analysis of Chloroform Extracts:** The S2 site, S3 site and the C1 control soils were extracted with chloroform. The extracts were in very small amounts. Infrared spectra of all the samples are similar and show primarily a long chain fatty acid ester, which are natural materials commonly found in soils. Nothing unusual was detected. The spectra of the extracts followed by a reference of a fatty acid ester are displayed below.

### Infrared Spectra of Chloroform Extracts from S2 and S3 Site Soils and C1 Control Soil





FILE: UT053

Phyllis A. Budinger