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Elemental Analysis and Forensic Comparison of Soils by Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ELEMENTAL ANALYSIS AND FORENSIC COMPARISON OF SOILS BY LASER-INDUCED
BREAKDOWN SPECTROSCOPY (LIBS) AND LASER ABLATION INDUCTIVELY COUPLED
PLASMA MASS SPECTROMETRY (LA-ICP-MS)

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Sarah Catherine Jantzi

2013

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Sarah Catherine Jantzi, and entitled Elemental Analysis and Forensic Comparison of Soils by Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: November 12, 2013

The dissertation of Sarah Catherine Jantzi is approved.

Dean Kenneth G. Furton
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University Graduate School

Florida International University, 2013

DEDICATION

To family, friends, music, love, chocolate, and sunshine. I couldn't have done this without you.

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forcing me to take time away from school to balance myself. Thanks to my body for putting up with the abuse that comes with doing a PhD at this age. I promise you'll be able to sleep soon ;)

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ABSTRACT OF THE DISSERTATION

ELEMENTAL ANALYSIS AND FORENSIC COMPARISON OF SOILS BY LASER-INDUCED
BREAKDOWN SPECTROSCOPY (LIBS) AND LASER ABLATION INDUCTIVELY COUPLED
PLASMA MASS SPECTROMETRY (LA-ICP-MS)

by

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Miami, Florida

Professor José R. Almirall, Major Professor

The elemental analysis of soil is useful in forensic and environmental sciences. Methods were developed and optimized for two laser-based multi-element analysis techniques: laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and laser-induced breakdown spectroscopy (LIBS). This work represents the first use of a 266 nm laser for forensic soil analysis by LIBS.

Sample preparation methods were developed and optimized for a variety of sample types, including pellets for large bulk soil specimens (470 mg) and sediment-laden filters (47 mg), and tape-mounting for small transfer evidence specimens (10 mg). Analytical performance for sediment filter pellets and tape-mounted soils was similar to that achieved with bulk pellets.

An inter-laboratory comparison exercise was designed to evaluate the performance of the LA-ICP-MS and LIBS methods, as well as for micro X-ray fluorescence (μ XRF), across multiple laboratories. Limits of detection (LODs) were 0.01-23 ppm for LA-ICP-MS, 0.25-574 ppm for LIBS, 16-4400 ppm for μ XRF, and well below the levels normally seen in soils. Good intra-laboratory precision (≤ 6 % relative standard deviation (RSD) for LA-ICP-MS; ≤ 8 % for μ XRF; ≤ 17 % for LIBS) and inter-laboratory precision (≤ 19 % for LA-ICP-MS; ≤ 25 % for μ XRF) were achieved for most elements, which is encouraging for a first inter-laboratory exercise. While LIBS generally has higher LODs and RSDs than LA-ICP-MS, both were capable of generating good quality multi-element data sufficient for discrimination purposes.

Multivariate methods using principal components analysis (PCA) and linear discriminant analysis (LDA) were developed for discriminations of soils from different sources. Specimens from different sites

that were indistinguishable by color alone were discriminated by elemental analysis. Correct classification rates of 94.5 % or better were achieved in a simulated forensic discrimination of three similar sites for both LIBS and LA-ICP-MS. Results for tape-mounted specimens were nearly identical to those achieved with pellets.

Methods were tested on soils from USA, Canada and Tanzania. Within-site heterogeneity was site-specific. Elemental differences were greatest for specimens separated by large distances, even within the same lithology. Elemental profiles can be used to discriminate soils from different locations and narrow down locations even when mineralogy is similar.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption
AES or OES	Atomic (or optical) emission spectroscopy
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
CI	Confidence interval
dc	Direct current
DNA	Deoxyribonucleic acid
EAWG	Elemental Analysis Working Group
EDS or EDX	Energy-dispersive X-ray spectroscopy (or detection)
FBI	Federal Bureau of Investigation
GLOWS	Global Water for Sustainability
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma MS
IRMS	Isotope ratio MS
IS	Internal standard
LA	Laser ablation
LA-ICP-MS	Laser ablation ICP-MS
LDA	Linear discriminant analysis
LIBS	Laser-induced breakdown spectroscopy
LOD	Limit of detection
MS	Mass spectrometry
m/z	Mass-to-charge ratio
NAA	Neutron activation analysis
NAZ	Normal analytical zone
Nd:YAG	Neodymium-doped yttrium aluminum garnet
NEIC	National Enforcement Investigations Center

NRC	National Research Council (of Canada)
PCA	Principal components analysis
PIXE	Particle-induced X-ray emission (spectroscopy)
PLS-DA	Partial least squares discriminant analysis
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
RCMP	Royal Canadian Mounted Police
RF	Radiofrequency
RM	Reference material
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SNR	Signal-to-noise ratio
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WDS or WDX	Wavelength-dispersive X-ray spectroscopy (or detection)
XRD	X-ray diffraction
XRF	X-Ray fluorescence spectrometry
μ XRF	Micro X-Ray fluorescence spectrometry
σ	Sigma, the standard deviation of a population

1. Introduction

1.1. Research Motivation

Soil evidence is encountered in many different crime scenarios, such as breaking and entering, stalking, theft, exotic plant smuggling, and murder. Although soil evidence is not often used in court, and is not considered a "silver bullet" in the way DNA evidence is, it is still quite useful during the course of an investigation. Soil evidence has use, for example, in eliminating or associating suspects, narrowing down a search region, finding a crime scene, ruling out alibi locations, and can be used to elicit a confession from a suspect who believes the weight of evidence is strong enough. In the United States, the forensic analysis of soil is becoming less and less common, as state and local laboratories down-size and focus on more high-yield (evidentially speaking) areas of expertise, such as DNA and toxicology. A forensic soil examiner requires extensive and specialized training in mineralogy, and many laboratories cannot justify this expense, given the relatively small (in comparison to DNA or drug evidence) number of cases requesting such an expertise. If methods existed that could be performed by a less specialized forensic examiner, they might be more readily employed. The methods described in this work may be used for determination of the elements present and for comparison of the elemental profiles from different locations. Although the main focus of this work is on forensic applications, these methods are equally applicable to a number of other disciplines, including agriculture, archaeology, earth and environmental sciences, and planetary geosciences.

1.2. Goals

It is expected that the trace, minor and major components of soil samples will contribute to the unique elemental profile of each location, and that these differences will be detectable by laser-based elemental analysis methods: laser-induced breakdown spectroscopy (LIBS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Such methods would provide useful associative and exculpatory evidence as well as provide investigative leads. The parallel use of LIBS and LA-ICP-MS, as well as μ XRF, would provide a means of validating the methods and to determine the relative utility and

significance of the results derived from each the analytical methods. Below is a brief summary of the goals of each chapter.

1.2.1. Development of Quantitative LIBS & LA-ICP-MS Analysis Methods

Chapter 4 will discuss method development for quantitative analysis of soils. Sample preparation methods were optimized for preparation of a soil pellet that can be used for LA-ICP-MS and LIBS. Sieving was explored. Quantitative elemental analysis methods were developed for LA-ICP-MS and LIBS. The sample set for the initial LIBS method development included ten USEPA NEIC contaminated soil samples, taken at various locations around an automotive battery manufacturing facility. Results obtained by LIBS were compared with results obtained by LA-ICP-MS and solution-based elemental analysis techniques. Following this, the sample preparation and analysis methods were re-optimized and tested on a set of soils taken from throughout Miami-Dade County, Florida, United States of America (USA).

1.2.2. Method Validation: Inter-Laboratory Comparison

Once the methods were developed and optimized, they needed to be tested and validated for their utility and robustness in the forensic community (Chapter 5). Any potential problems with the analysis needed to be identified and addressed. An inter-laboratory comparison exercise was designed and carried out for LA-ICP-MS, LIBS, and μ XRF. Identical specimens were sent to participants from local, state, and federal crime labs in the USA, Canada, and the Federal Republic of Germany (Germany). Participants analyzed the specimens according to the methods provided, using the equipment available to them in their laboratories, and reported their results. Data were compiled, possible issues with the methods were addressed, and the applicability of LIBS as a low-cost alternative was evaluated.

1.2.3. Development of Methods for Discrimination Based on Elemental Profiles

Chapter 6 will discuss the development and application of discrimination methods using the multi-element data generated using the above methods. The USEPA NEIC specimens were used to develop a preliminary LIBS discrimination method. Results obtained by LIBS were compared with results obtained by LA-ICP-MS. Following this, the methods were re-optimized and tested on a set of soils taken from throughout Miami-Dade County, Florida, USA in three studies. The heterogeneity of the soils was

examined on many levels. An element menu and multivariate statistical methods for discrimination were developed for LIBS and LA-ICP-MS. Discrimination results for LIBS were compared to those of LA-ICP-MS, and to discriminations using color, which is the most common currently used technique in forensic soil examinations.

1.2.4. Expanding the Sample Set: Different Soil Types & Different Geographic Regions

Miami-Dade County, and most of Florida, is underlain by limestone (calcium carbonate) bedrock, which is calcareous [1]. However, much of the United States contains other primary lithologies that contain different minerals, which may require adjustment of the sample preparation and analysis methods, including the element menu. Aluminosilicate, basaltic, and granitic lithologies account for most of the rest of the USA [2]. In eastern Canada and neighboring regions in the northeastern USA, glacial till is the dominant lithology. In Chapter 7, soil samples from regions underlain by these additional primary lithological types were tested to determine whether they are compatible with the methods developed in Chapter 4 and to test forensic discrimination potential for these regions.

1.2.5. Small Specimens: Tape-Mounting for Forensic Transfer Evidence

In discussing the methods developed above with forensic examiners, a number of concerns were raised. One was that in cases involving transfer evidence, the soil specimens are quite small (on the order of tens of milligrams). Forensic examiners also prefer methods that are non-destructive, allowing for additional analyses by other confirmatory or complementary techniques, re-analysis by counter-experts, and archival storage. Sample preparation methods were developed for deposition of a thin layer of soil on adhesive tape. Analytical methods were re-optimized to accommodate these tape-mounted samples. Analytical performance and discrimination capabilities were compared with the pellet method (Chapter 8).

1.2.6. Environmental Application: Ruvu River Sediments

An environmental forensic application exists in which the source of a recent increase in turbidity of the Ruvu River in the United Republic of Tanzania (Tanzania) needed to be determined (Chapter 9). Sediments collected at various locations along the river were analyzed and their elemental profiles compared to the elemental profiles of soil samples collected upstream throughout the river basin from

various tributaries. The average amount of sediment collected was less than 100 mg, which was too small for the bulk pellet method developed in Chapter 4. In addition, the sediment adhered strongly to the filters and could not be quantitatively removed. Methods were developed for preparation of smaller pellets incorporating the filters. Analytical methods were re-optimized to accommodate these smaller, diluted filter sediment pellets. Analytical performance was compared with the bulk pellet method.

1.3. Significance

This work provides a suite of elemental analysis methods for soil that can be used by laboratories that do not have a dedicated soil examiner, but already possess, or are thinking of purchasing, LA-ICP-MS, LIBS or μ XRF instruments for other trace evidence (for example, glass, paint, metals, ink, and paper). This work also provides the first LIBS method for soil using a 266 nm laser. A 266 nm laser is important for forensic laboratories since the analysis of transparent materials, such as glass, requires a laser wavelength (and associated optics) in the UV range. This work explores a number of sample preparation strategies for use with various types and sizes of soil and sediment specimens. For example, this work provides innovative sample preparation techniques for specimens as small as 47 mg or less, which are common in trace evidence. These methods have been tested on various soil types from regions throughout USA, Canada, and the Tanzania, and by other laboratories in the USA, Canada, and Germany. Lastly, this work describes the application of data analysis techniques for forensic discrimination and provides recommendations for interpretation.

2. Background

2.1. Trace Evidence

Trace evidence consists of small amounts of material or small particles collected in a forensic investigation. Some examples include hair, fibers, glass or plastic fragments, pollen, paint chips, and soil. These materials are readily transferred between people and objects during the commission of a crime, as implied by Locard's exchange principle, which states that every contact leaves a trace [3]. These materials may adhere to items, such as shoes, clothing, or other objects (e.g., tires, blankets, or tools) used in connection with the crime, providing the possibility to associate (or exclude) a suspect to a victim, a suspect to a location, or a victim to a location. Such trace evidence is often referred to as "transfer evidence". Because of their small size, trace and transfer evidence often goes un-noticed by the perpetrator, making them a valuable source of information for the forensic examiner.

Provenancing is the determination of the source or origin of a specimen. For man-made products like glass, this may involve tracing a fragment found on the suspect back to the whole object (e.g., window pane) from which it came, or the manufacturing plant where it (and presumably many more like it) was made. For natural materials like soil, this may involve determining the neighborhood, city, county, or broader region (i.e., geographic provenancing).

Forensic comparisons involve the comparison of specific characteristics of a questioned specimen (evidence) to those of known specimens (taken from a known source). Discrimination (exclusion) occurs when the specimens can be distinguished on the basis of sufficient differences in these characteristics. Association occurs when a sufficient degree of similarity exists. What is deemed "sufficient" depends on the nature of the evidence and the number and type of methods of comparison used (see Section 2.6.1).

Analysis of trace evidence can provide information about characteristics, such as color, texture, size, morphology, refractive index, magnetism, and chemical composition (including elemental and isotopic composition). The more characteristics measured, the greater the informing power or discriminating power of the analysis. For example, high calcium content of a soil specimen would suggest that it came from a region with a calcareous lithology, of which there are many in the United States (see Figure 34). However, measurement of additional non-correlated characteristics can further narrow down the possibilities. For

example, high strontium content would eliminate most of the calcareous regions in the central states, leaving areas in southern Florida and southwestern Washington [4].

2.2. Soil

2.2.1. General Characteristics

Soil is ubiquitous, and yet often ignored. It is a complex, highly variable mixture of organic, inorganic, and often anthropogenic (man-made) material lying on top of bedrock. The organic material can include humus, pollen, plant material, animal material, bacteria, fungi, etc., each of whose relative abundances, depend on climate, land use, ecology, and other environmental conditions. Anthropogenic material may include glass, plastic, and metal fragments, pollution, fertilizer, pesticides, etc., each of whose relative abundance can be linked to population, land use, nearby industry, and wind patterns. Inorganic material consists of various minerals of various grain sizes, each of whose composition and relative abundance depends on formation conditions, geological age, weathering, and transport patterns. Major (matrix) elements include O, Si, Al, C, Fe, Ca, Na, and Mg. Minor elements include K, Ti, H, P, N, Ba, Sr and many others, depending on the location [5]. Trace elements can be useful as well, if a sufficiently sensitive technique is used.

Variations in overall soil composition can be seen spatially (over distance and at different depths), and can be affected in the short term by changes in season and human activity. The main source of elemental variation in soils is the mineral content, but organic and anthropogenic material may also provide a significant contribution. The mineral composition is probably the most stable over time.

2.2.2. Lithology

There are many different soil types defined by the local geology (lithology, or underlying bedrock) and an infinite number of blends, which are not immediately obvious without prior analysis. They can be classified according to the formation process as being igneous, sedimentary, or metamorphic, and then further sub-classified on the basis of other characteristics, such as chemical composition.

Igneous rocks are formed by cooled, solidified magma or lava, which is melted rock from the mantle or crust. If formed above the earth's surface they are called volcanic or extrusive, for example basalts and

andesite. Plutonic (intrusive) rocks are formed below the surface, and include granites, diorite and quartz. The composition of the rock depends on the particular conditions (temperature, depth, magma source) of the cooling magma [6]. Magma is rich in silicon, oxygen, aluminum, sodium, potassium, calcium, iron, and magnesium, which combine to form the silicate minerals that account for over ninety percent of all igneous rocks [6]. Felsic rocks like granites and rhyolites are high in felsic minerals (quartz, or silica, and alkali feldspar), and usually light in color and density [6]. Mafic rocks like basalts and gabbro are low in silica, high in mafic minerals (high in iron and magnesium), dark, and dense [6]. Granitic and basaltic rocks have distinct chemical compositions; therefore the soils above them should also be chemically distinguishable.

Sedimentary rocks are formed from deposits that solidify under specific conditions of temperature, moisture and pressure. They can be accumulations of weathered and eroded material from other rocks (such as gravel, sand, clay, shale), animal remains (such as limestone or peat), or material precipitated in water (such as oolite or gypsum) [6]. Chemically speaking, sedimentary rocks can be divided into two main categories. Calcareous rocks are composed mostly of CaCO_3 , such as chalk and limestone, but can also be MgCO_3 and $\text{CaMg}(\text{CO}_3)_2$ (dolomite). Argillaceous rocks are composed of aluminosilicates, such as clay, shale, and slate.

Metamorphic rocks are igneous and sedimentary rocks that have undergone a change in crystallinity [6]. For example, limestone can recrystallize into marble; shale can be foliated into slate. The original chemical composition is largely retained.

Approximately 10 000 to 20 000 years ago, during the Pleistocene, most of Canada and the northern USA were covered by glaciers [6,7]. In eastern Canada, movement was in an overall southerly direction, abrading the underlying bedrock as it moved. This abraded sediment, ranging from boulders to clay-sized grains, was entrained by the glaciers, mixed with other sediments, and deposited as “till” along the way [7]. Because of non-linear transport and mixing, the soils in these regions are not necessarily representative of the lithologies beneath them, or even directly north of them. Besides glaciers, air and liquid water serve to change the landscape as well. Finer particles can be transported by the wind. Labile minerals, such as sulphides, can be oxidized by the air. Soluble minerals, such as carbonates, can be dissolved by water [7].

2.2.3. Particle Size

Minerals occur in a variety of sizes and shapes (“texture”). Particle sizes of a mineral can be reduced by actions such as glacial processes and weathering to an optimum particle size for a mineral (“terminal mode”), determined by hardness, cleavage, and its condition before the action [8]. It is widely held that particle size affects the metal and trace elemental content of a soil specimen [9]. Chromites (Cr), scheelites (W) cassiterites (Sn), and cinnabars (Hg) are found in sand-sized fractions (between 0.062 and 2 mm). Gold, platinum group elements, and transition metals are often more prevalent in the finer fractions (< 0.06 mm) [7]. As a result of the higher surface area and high exchange capacities of particles in the clay fractions (< 0.004 mm), accumulation of elements is common there [7,9].

2.2.4. Forensic applications

There are a variety of cases in which the forensic analysis of soil would yield important information, each with its own set of requirements and limitations. The most common may involve transfer of small amounts of soil from a crime scene to the suspect simply by walking around. Such transfer evidence is prone to loss with movement over time, especially of the larger, heavier particles, and it can be difficult to recover and analyze such small samples. More complex cases may involve the relocation of a victim’s body from the primary crime scene to a secondary location or burial site. Soil from the primary site may be distinguishable from that of the secondary site and help to determine its location. While these may or may not be larger specimens, the potential for mixing with other soils presents a problem. Exotic plants or animals illegally imported may be accompanied by soil from the location of origin that could be used to distinguish it from local soils and point to possible sources. This may also be applicable for victims of human trafficking.

Environmental forensics is a separate field, in which the analysis of soil can be used to determine the source and extent of pollution, and to monitor remediation efforts. Provenancing of river sediments can be important for a number of reasons, including sourcing a contaminant or finding the source of a sudden increase in turbidity. Soils taken at locations upstream are compared to filtered river sediments, which present additional challenges for sample preparation and analysis [10]. Illegal imports and environmental forensic cases may provide large amounts of sample, but known specimens may be difficult to obtain.

The variable nature of soil from one location to the next makes it an ideal forensic matrix, providing the differences upon which discriminations and provenancing can be made. However, this means that collection of representative known specimens throughout the area of interest is crucial, as does careful sample preparation, analysis, and interpretation in order to account for the natural variation within a location.

2.2.5. Current Methods of Forensic Soil Examination

Specimens encountered by forensic examiners are often very small. This problem is exacerbated by the need to split evidence into replicate samples, analyze them by multiple techniques, and to retain some for archival purposes. Ideal analysis techniques should consume as little sample as possible. More sensitive techniques require less sample [11].

Ideally, “standard” methods of analysis should be used, when available (e.g., American Society for Testing and Materials (ASTM) standard test methods and guidelines) since these often have established figures of merit and error rates. When no standard methods exist, published methods should be used, and confirmed with other, independent, techniques to improve the confidence in the results. Ultimately, the methods must be accepted by the scientific community before they can be accepted by the courts in a criminal or civil legal proceeding [12].

When a questioned or known specimen is received it is subject to a visual examination, in which characteristics such as color, morphology, texture and structure are recorded. These are non-destructive, but somewhat subjective determinations, and it is not uncommon for soils from the same area to have different colour and morphology [3,13,14]. Color analysis is done by comparing the color of each soil specimen to a standard set of color charts, most commonly the Munsell Color Scale. The Munsell Soil Color Book contains pages of color chips in a range of soil colors. Each page contains chips of a particular hue (color), and chips vary vertically in value (lightness), and horizontally in chroma (saturation or color intensity) (see Appendix 1). For example, a Munsell designation of 10YR 5/2 is a yellow-red soil, more yellow than red (10YR) of intermediate lightness (5) and not much color saturation (2). Specimens having similar Munsell designations are then subject to side-by-side comparisons to determine their similarity [15]. In many cases, if specimens are discriminated on the basis of color, the analysis stops there.

Further analysis depends on the equipment and expertise available, and may include particle size distribution, density, organic content, nitrogen content, and pH [3,14,16]. Each of these provides information on only a single variable, and some are destructive. Separation into different particle size or density fractions further reduces sample size. Mineralogy is considered the gold standard, but it requires extensive specialized training and a painstaking grain-by-grain analysis. The polarized light microscope required may already be available in the laboratory, but X-ray diffraction (XRD) is not commonly available in forensic laboratories. Particles such as pollen grains, shells, or man-made debris can also be very useful in making comparisons.

Elemental analysis can be performed in laboratories that have access to the required instrumentation. Elemental analysis requires some training, but provides simultaneous information on a number of variables (elements), and therefore provides high discrimination power. It is also less subjective and can be used for many other types of trace evidence (for example, glass, paint, ink, paper, metals, and gunshot residue).

2.3. Sampling and Sample Preparation

Because of the heterogeneity within an area, small changes in location can result in differences in elemental profile. Sampling and sample preparation protocols must take this into account so that the known specimens taken are representative of the location in question and that the part of the sample that is actually subjected to the analysis is representative of the entire specimen.

2.3.1. Sample Collection

Known specimens should be taken as soon as possible, as near as possible to the location where the transfer occurred, and at a similar depth [17]. If the exact location is unknown, a database may be used if one exists. Even if no formal database exists, researchers studying agriculture, ecology, or geology may already have data on the area of interest (for example, [4,18-21]). Either way, these data can really only be used to narrow down the search location, and known specimens should be collected in those areas and analyzed to verify the data provided. If no data are available, a number of known specimens may be taken at various locations to characterize the area and get a picture of the spatial variation. Unfortunately there is no clear consensus among scientists as to how many samples must be taken from a given location and how

far apart, as it depends on the technique, elements of interest and their concentrations, the degree of heterogeneity of the area and how similar it is to other relevant sites [22,23].

When analysis, especially at trace levels, is being performed, contamination is an important issue. All specimens should be collected, handled and stored using metal-free equipment. Other precautions may be necessary depending on the matrix and analytical method employed. As with all forensic evidence, specimen collection requires very detailed documentation and handling procedures in the form of sketches, descriptions, photos, logs, labels, seals and chain of custody forms.

2.3.2. Sample Preparation for Soils

Various sample preparation procedures can be employed depending on the size and nature of the specimen, and the analysis to be performed. Manipulation of the specimen should be kept to a minimum, as each step in the sample preparation process has the potential for contamination or loss. Reagents should be trace metal grade or better so that they do not contribute anything to the elemental profile. The first step is to take a representative sub-sample of the specimen; therefore the specimen should be well-mixed. For transfer evidence, in which the questioned specimen consists of fine particles, known specimens should be sieved to a similar particle size fraction, as described in Section 2.3.2.1 below. For bulk questioned specimens, sieving is not necessary if comparing to bulk known specimens. For analysis of color, sieving is recommended, but no further preparation is required.

For elemental analysis, addition of internal standard may be necessary if one is not already present (see Section 2.3.4), and milling or grinding may be required to homogenize and reduce particle size (see Section 2.3.2.2). For ICP methods (ICP-OES or ICP-MS), milled specimens are usually leached or digested for introduction as solutions, but this requires strong acids, high temperatures, and long times, and is often incomplete. Lithium metaborate fusions have been used, to create glass disks containing internal standard or spikes, and the flux (lithium metaborate and other salts) in approximately 1:1 ratio with sample, which are then digested and run as solutions. Preparation requires extremely high temperatures, which causes the loss of volatile elements. The addition of Li, F, and B, along with any trace impurities in the flux, means that these elements cannot be measured [24,25].

For analysis by laser-based methods (LA-ICP-MS or LIBS), a sample in powder form must be fixed so that it can withstand the laser pulses. Fusion glasses can be used, but in addition to the above issues, upon cooling the lighter elements rise to the surface and the heavier elements settle, resulting in a heterogeneous disk [25]. Pelletizing is becoming common, and is described in Section 2.3.2.2. Very small specimens present additional challenges, and will be discussed in Section 8.

2.3.2.1. Sieving of Soil Specimens

Transfer specimens are the result of transfer from the crime scene or other relevant location to an object or person. The size of the particles transferred and retained depends on soil humidity, porosity of the clothing or object, elapsed time between the transfer event and collection, and any movement or activity during that time. Known specimens are “bulk”, representing the full range of particle sizes of the location of interest. In order for a fair and accurate comparison, any known specimens should be of similar particle size distribution to the questioned specimens. Sieving is recommended for forensic transfer cases for the following reasons:

- Size fractionation and preferential loss often occurs with transfer. Smaller particles are more likely to be transferred and, of those, the larger particles are more likely to be lost post-transfer through normal wear or movement [9,19,26,27].
- The “nugget effect”: for small sample sizes, inclusion of one or a few large particles of a particular mineralogy could skew the elemental profile of a sample. Sieving would eliminate such “nuggets” and improve precision [26].
- Trace elements are often found in higher abundance in the finer fractions [26].
- Smaller particle size leads to more efficient analysis (e.g., digestion or laser ablation), resulting in increased accuracy and precision [24].

Disposable plastic mesh is favored over metal sieves to avoid contamination. The choice of particle size is a compromise between ease of sieving, yield, and analytical results. Pye et al. suggest using the less than 150 μm fraction as it is sufficiently fine to address the above points, but not so fine that sieving becomes difficult or the resulting sample too small for analysis [9,26]. Sieved samples may not be

representative of the bulk soil, so sieved samples should only be compared to other sieved samples of the same size fraction. Sieving is an extra step that reduces the amount of available sample, adds to the preparation time and can be a source of contamination or loss. Not all transfer samples experience size fractionation, and not all forensically encountered soil samples result from transfer, therefore it is not always necessary or recommended.

2.3.2.2. Milling & Pelletizing

Pellets can be made by pressing powdered samples. They are reusable across multiple techniques and easy to store. Studies have shown that smaller particles pressed into a pellet produce a more robust and homogeneous pellet that releases smaller, more uniform particles during ablation, and a binder is not required [24]. As mentioned earlier, soil is inherently heterogeneous. Particle size reduction and homogenization of the soil, and any internal standard added, can be achieved by grinding or milling. Arroyo et al. found that the smallest and most uniform particle sizes were obtained using a high speed ball mixer mill, compared to a planetary ball mill or a mortar and pestle [24]. The milling or grinding parts in contact with the sample may cause some contamination as a result of carryover from a previous sample, or material from the parts themselves, especially when hard minerals are milled. Agate parts can be used for harder minerals, but are extremely expensive. Tungsten carbide and stainless steel are the most commonly-used materials, with tungsten carbide imparting less contamination than the stainless steel.

2.3.3. Reference Materials and Calibration Standards

Reference materials (RMs) are standards in which the concentration of particular analytes are measured repeatedly by multiple analytical techniques, and given a concentration with a confidence interval, certified by a company or standards agency. Depending on the agency, they may also be referred to as Certified Reference Materials (CRMs) or Standard Reference Materials (SRMs). Reference materials can be used to confirm the accuracy of a calibration scheme, or can themselves be used as calibrators. If measured on a daily basis, and/or at the beginning and end of an analysis day, RMs can be used to monitor daily method performance, reproducibility, and instrumental drift. In addition, if the RMs are known to

have come from different locations than the questioned specimens to be analyzed, they may also serve as negative controls; they should be easily distinguishable from one another and from the specimens.

Ideally, an RM should be matrix-matched, meaning that it has the same major elements and minerals present as the specimens, since they can have an effect on the analysis. Since soil is so variable, perfectly matrix-matched standards are not usually available. A number of soil and sediment RMs are available from a number of sources, but a multi-standard calibration set is not currently available. Calibration standards can be made in-house to meet the requirements of the analysis. A suitable matrix must be chosen, and elements of interest added at concentration ranges that bracket the concentrations found in the specimens. The standards must be homogenized. Calibration standards and RMs should be processed and analyzed in the same fashion as the specimens.

2.3.4. Internal standards

To account for sample loss, instrumental fluctuations, and drift, an internal standard is often employed. This is especially important in laser-based elemental analysis techniques (LIBS and LA-ICP-MS), since the laser power fluctuates from one pulse to the next, resulting in fluctuations in the amount of mass removed. In ICP-MS, the sample interface becomes dirty over time, resulting in a reduction of signal intensity over time. The data can be normalized to an internal standard. This is done by dividing the signal of an analyte of interest by the signal obtained from the internal standard in the same spectrum. There are two strategies for internal standardization in elemental analysis:

- Choose an element that is already present in the specimen in a known and fixed amount. For example, matrix elements, silicon in float glass samples, or carbon in paper or other plant-based samples have been used. Soil, however, is often too variable for this strategy to be effective unless the same soil type is analyzed routinely.
- Choose an element that is absent, or present in very low amounts, in the specimen, then add it to the specimen in a known and fixed amount. The ideal element is one that exhibits similar characteristics to the analyte(s) of interest. For example, it should have a similar ionization potential and/or mass-to-charge ratio (m/z). It should be added at a concentration similar to the expected concentration of the

analyte(s) of interest or a concentration that would produce a measured signal of similar intensity.

Some elements that have been used include Ge, Y, Sc, Lu, and In.

Other normalization strategies that do not involve selection of an element are discussed in Section 2.5. If an internal standard is to be added, it should be done as early as possible in the sample preparation process.

2.4. Elemental Analysis

Elemental analysis provides information on which elements are present in a specimen and in what quantities. This “elemental profile” can be used to provide a chemical characterization of the material. Questioned and known specimens can be discriminated on the basis of differences in the elemental profile, or associated if no differences are found. If there are no known samples to compare to, a database can be used to narrow the search for the origin of a material [28]. There are many analytical techniques capable of providing such elemental information, each with advantages and disadvantages, which are discussed briefly below.

In analytical chemistry, it is widely held that as more replicate measurements performed, the mean of these measurements approaches the true value and becomes more accurate. In addition, in order to discriminate between two specimens, the within-specimen variation must be smaller than the between-specimen variation. The within-specimen variation can be determined by making replicate measurements of the specimen. This provides a picture of the homogeneity or heterogeneity of the specimen and the precision of the measurement. A compromise must be made between the speed of analysis and number of replicate measurements required to provide accurate and precise results. During method development and optimization, accuracy and precision are evaluated in order to determine the minimum number of replicates required.

Since specimens must be split into replicate samples for analysis by multiple techniques and to retain some for archival purposes, the analysis techniques chosen should consume as little sample as possible. The more sensitive the technique, the less sample is required [11], which is an advantage for forensic analysis. The technique chosen must be capable of providing a detectable signal representative of the concentration of the analyte(s). The major (matrix) elements are the most abundant, usually more than 1 % by weight;

minor elements are usually present between 0.1 and 1 %; trace elements are those with concentrations below 0.1 % [5]. When the possible sources are quite far apart (for example, in a case involving multiple states or countries, or where the source is unknown) major elements may be sufficient for discrimination, because of large differences in parent rock lithology, and a less sensitive technique could be used. However, in most forensic investigations, possible sources are in the same city or county. Since specimens from nearby sources often share the same lithology, the discrimination will be based on minor and trace elements, and a more sensitive technique should be used [29].

Some of the more prominent techniques used for the elemental analysis of soils in various disciplines include inductively coupled plasma mass spectrometry (ICP-MS), laser ablation ICP-MS (LA-ICP-MS), ICP-optical emission spectroscopy (ICP-OES), LA-ICP-OES, X-ray fluorescence (XRF), scanning electron microscopy energy-dispersive X-ray spectroscopy (SEM/EDX), neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), particle-induced X-ray emission (PIXE), isotope ratio mass spectrometry (IRMS), and laser-induced breakdown spectroscopy (LIBS).

2.4.1. Advantages and Disadvantages of Various Techniques

Non-destructive techniques include XRF, PIXE, and SEM/EDX (if sample coating is not required). Quasi-non-destructive micro-sampling techniques include LA-ICP-MS, LA-ICP-OES, LIBS, and NAA, where small quantities of sample (on the order of micrograms) are removed, leaving the rest of the sample intact. Solution-based ICP-MS and ICP-OES are considered destructive techniques, requiring harsh, time-consuming and often incomplete dissolution and digestion of the sample prior to analysis [24]. NAA, LA-ICP-MS, LA-ICP-OES, LIBS, XRF, SEM/EDX, and PIXE are capable of analyzing samples in solid form so that chemical digestion steps are not required. AAS is both destructive and requires a separate sample and analysis for each element. Multi-element techniques (EDX, PIXE, XRF, LIBS, NAA, and the ICP techniques) are ideal in that information on a variety of elements can be obtained in a single analysis.

For forensic laboratories, which have limited resources, affordability and accessibility are also important factors. The cost per analysis includes the price of the instrument, maintenance, consumables (i.e., gas and oil), electricity, waste disposal, and operator training and salary. NAA, for example, requires a nuclear reactor and as a result is usually only found in universities and large national laboratories. The cost

per analysis is quite high and includes disposal of spent reactor fuel [30], which is also a safety concern. SEM/EDX, XRF, PIXE and ICP-MS are much more common but still quite expensive. Lower cost options include LIBS and ICP-OES. Field-portable instruments are available for LIBS and XRF [14,31], however the analytical performance (especially spectral resolution and sensitivity) are usually lacking when compared to their laboratory-based counterparts so they are best reserved for screening purposes [32].

Emission spectroscopic techniques (EDX, PIXE, XRF, ICP-OES and LIBS) produce spectra containing information on all elements without the requirement for prior selection of the elements of interest (“element menu”), providing a richness of information. If an interesting element is discovered, the data can be re-examined without re-running the samples [22]. IRMS, ICP-MS and LA-ICP-MS provide isotopic information [33]. ICP-MS has excellent sensitivity, with LODs in the ppb-ppt range [34]. ICP-OES, LA-ICP-MS and graphite furnace AAS are not far behind with LODs in the ppb range. LIBS and flame AAS have good LODs, in the 10 ppm range. The least sensitive are XRF and SEM/EDX, with LODs in the 100 and 1000 ppm range, respectively, precluding their use for most trace elements. LIBS and ICP-OES have good sensitivity in the low atomic mass range, where XRF and ICP-MS are subject to low sensitivity and interferences [32,35].

While solution-based methods are less susceptible to the effects of sample heterogeneity because of the relatively large sample mass required, this size requirement can be a problem in forensic analysis where samples obtained are often very small and split to allow for analysis by multiple techniques or set aside for future re-analysis. To combat heterogeneity issues in laser-based techniques, the beam can be scanned or rastered across a larger area (samples are often spun during acquisition in an XRF) or a depth profiling mode can be used if surface oxidation or other surface issues are suspected. SEM has the added advantage of high-resolution imaging capabilities, while the LA methods, LIBS, μ XRF and EDX are capable of elemental mapping, with spatial resolution determined by the analytical beam (spot) size. Other factors to consider include ease of operation, cost of maintenance, training and skill level required, analysis time and ease of acceptance in court [14].

Specific examples of the use of these techniques for forensic analysis of soil are discussed Section 2.6.4. The techniques chosen for this work were limited to sufficiently sensitive elemental analysis

techniques already available in some forensic laboratories (LA-ICP-MS and μ XRF), and to LIBS which has garnered interest for its potential applicability as a cost-effective alternative.

2.4.2. LA-ICP-MS

2.4.2.1. ICP-MS

Inductively coupled plasma mass spectrometry is an analytical technique that uses an inductively coupled plasma (ICP) to atomize and ionize samples and a mass spectrometer (MS) and detector to separate the positive ions of each isotope and measure their amounts. Samples must be introduced in the form of an aerosol in order for the process to proceed reliably and efficiently. For soils the introduction method of choice is LA (see Section 2.4.2.3).

The ICP consists of a torch, a radiofrequency (RF) coil, and a power supply. The torch is made of quartz and contains three concentric tubes: the outer tube, middle tube, and sample injector. The plasma gas (usually argon) flows tangentially around the outside of the middle tube before reaching the plasma, which helps to keep the outer tube from melting and results in a toroid-shaped plasma. The auxiliary gas (usually argon) is used to keep the plasma from getting too close and melting the injector tip, and flows between the middle tube and the sample injector. The sample aerosol is introduced into the plasma through the sample injector. The RF coil (load coil) is made of copper tubing wrapped in a coil around the outer tube of the torch. The coil is held grounded and alternating current RF power is applied whose frequency is controlled by a RF generator. This imparts an electromagnetic field to the torch. The plasma is initiated with a few seed electrons supplied by a spark. These electrons are accelerated by the RF field and collide with argon atoms in the torch, causing the release of additional electrons, entraining a chain reaction, and thus a plasma that is sustained as long as the RF continues (i.e., RF energy is inductively coupled to the plasma). Plasma temperatures are high ($\sim 10\,000$ K) on the outside and cooler ($5000 - 7000$ K) in the center where the sample is introduced. As the sample leaves the injector, it encounters the pre-heating zone, where any solvent is dried off. It enters the plasma in the initial radiation zone, where atomization occurs, and the atoms proceed to the normal analytical zone (NAZ), where they are ionized. Argon is an ideal gas because its first ionization potential (15.8 eV) is greater than the first ionization potential of most elements, and

therefore high enough to ionize, but low enough not to exceed the second ionization potential for most elements, so that doubly-charged ions are kept to a minimum [36-38].

Ions formed in the NAZ at atmospheric pressure (760 Torr) must be directed into the mass spectrometer, which is under vacuum. This is done via the interface, which serves to reduce the pressure step-wise, and filter out unwanted material (photons, particles and neutral species) to prevent them from reaching the MS and detector. First is the sampler cone, which is a wide, flat cone with a small (1 mm) orifice placed in the NAZ that allows a narrow beam of ions to pass through it and expand as they enter the low vacuum (1 - 2 Torr) region on the other side. The skimmer cone with a smaller orifice (< 0.5 mm) is placed behind it to allow an even smaller beam of ions from the center of the ion beam through to a high vacuum region (10^{-3} - 10^{-6} Torr). Behind the skimmer cone is a photon stop, which is a small metal disk used to physically prevent photons and neutral species from continuing through to the detector, thereby reducing background signal and improving SNR (and therefore LOD) for trace elements. Positive ions are directed safely around the photon stop and collimated into a beam by an ion lens system (ion optics) and directed into the MS, which is held at 10^{-6} Torr. The velocity of the ions is set in the plasma such that all ions have the same velocity. Therefore each isotope has a different kinetic energy, with heavier ions having higher energies than lighter ones. The ion lens voltages required for maximum transport efficiency of each mass differ, so no one voltage is ideal if multiple elements are being measured. Therefore the voltages are scanned in concert with the mass spectrometer in order to apply the optimal lens voltage for the isotope being analyzed [36-38].

There are various types of mass analyzers, each with advantages and limitations. The most stable, cost-effective, and easy to operate is the quadrupole mass filter, a work-horse for applications that can be performed at unit resolution. The quadrupole is made up of parallel rods, through the center of which the ion beam is directed. Filtration occurs by applying both a dc potential and a RF alternating current potential to the rods in pairs. The two pairs have opposite polarities, making them oscillate out of phase, and imparting a spiral path on the ion beam. At a given applied potential, only ions with a specific mass-to-charge ratio (m/z) will make it to the exit; all others will be destabilized and collide with the rods. The dc potential is scanned to allow ions from each m/z through, resulting in a mass spectrum (intensity versus

m/z) as ions at each m/z are counted at the detector. A complete scan (sweep) can be achieved in less than 0.1 s, so that multiple scans can be collected, which enhances the signal-to-noise ratio (SNR). This is also important for analyzing transient signals from the LA system. Resolution (the width of a peak at 10% of its height) is approximately 1 amu. Most quadrupole mass spectrometers employ a discrete dynode electron multiplier as the detector, with an amplification of up to 10^6 fold [36-38].

2.4.2.2. ICP-MS Optimization

Oxides and doubly charged species can be formed in the ICP, which are undesirable. Elements with low ionization potentials, such as the alkaline earths and some rare earth and transition elements, can form doubly charged ions. The result is a reduction in signal at the expected m/z accompanied by a peak at half the expected m/z (since $z = 2$, instead of 1). If the isotope has an even mass, this may cause an interference with another isotope with that m/z . Oxides are the result of combination of elements with oxygen in the plasma from the sample, solvent, or contaminated gas supply. In LA-ICP-MS there is no solution, so the oxides are minimal, but conditions should still be optimized to reduce their formation [37]. Oxides cause a reduction in signal at the expected m/z and a peak at the m/z of the oxide, which may interfere with another isotope. Formation of doubly-charged species and oxides can be reduced by adjusting the sample introduction flow rates. Low flow rates favor doubly charged species formation, while high flow rates favor oxide formation, therefore an intermediate flow rate must be used to minimize both [38].

As samples are analyzed, the sampling interface and lenses experience a buildup of material. This results in instrumental drift over time. Standards are therefore analyzed at the beginning and end of the analysis day, and a correction is performed. The sampling interface must be cleaned periodically, which results in some down time, as the plasma must first be extinguished. Laser ablation introduces much more material into the ICP-MS and therefore this cleaning must be performed more frequently than with solution. The vacuum system must be shut off in order to clean the autolens and to change the vacuum pump oil.

Peak hopping mode is useful when analyzing a specific set of isotopes. Instead of scanning through the entire range of ion lens voltages, only specific ranges for the isotopes of interest are scanned (i.e., ‘hopping’ from one isotope peak to the next). This means that more sweeps can be performed in the same

amount of time, resulting in improved SNR for the same analysis time. This is especially useful when using LA sample introduction. The drawback of peak-hopping mode is that the isotopes for analysis must be selected in advance and if data for additional isotopes are required, re-analysis is necessary.

The discrete dynode electron multiplier used in this work is dual-stage: a digital (pulse) detection mode is used for low intensity signals (up to 2×10^6 cps) and an analog detection mode for high intensity signals. These detectors need to be cross-calibrated to ensure linearity across the entire dynamic range, particularly at the cross-over point (where the detection switches from one mode to the other). For matrices with small variations in elemental concentrations, cross-calibration is not crucial, as each isotope will likely only be measured by one of the detectors and will never cross over to the other one, but this is a dangerous assumption for questioned specimens of unknown composition, particularly for materials like soil having widely varying concentrations of many elements. Quantitation problems can occur if isotopes in the calibrator are detected by one detector mode and isotopes in the specimens by the other detector mode, or if the internal standard is affected (see Figure 17). This is also important when forensic comparisons are to be made between specimens. Therefore the dual detector must be cross-calibrated for all isotopes of interest before analysis begins.

2.4.2.3. Laser Ablation (LA)

Classically liquid samples are introduced via a nebulizer, which provides a homogeneous and uniform flow of sample aerosol to the ICP. Other advantages include easy addition of spikes and internal standard, and that the solutions can be concentrated or diluted to an ideal concentration range. Some of the drawbacks of solution mode include a limit to the amount of dissolved solids, isobaric interferences from the solution (polyatomic species such as oxide and halide formation from combination of the analytes with the water and acids in the solutions) and that the solutions are consumed and cannot be re-analyzed. For soils, solution mode is not ideal for additional reasons. Because many minerals are quite difficult to dissolve, long and harsh digestions using strong acids (often hydrofluoric acid) at high temperatures are required. These digestions are often incomplete so sometimes leachates are used instead of digestates, but the degree of leaching is highly affected by the conditions such as temperature and particle size. The ideal

sample introduction method is a “total” one, in which the sample introduced into the plasma is totally representative of the specimen, with minimal sample preparation [36-38].

Laser ablation (LA) is the most popular way to achieve this. It is capable of vaporizing almost any solid material without significant oxide or halide formation. This is achieved by tightly focusing a high energy laser beam onto the specimen surface, resulting in vaporization and removal of small particles. The laser, optics, and physical processes are similar to the LIBS process (see Section 2.4.3 for more detail), albeit at a lower energy. It should be noted that some minerals are more likely to couple with the laser and so are more easily analyzed, while others, particularly the clear, colorless minerals, may produce a challenge. In order for the LA (or LIBS) technique to be useful, fractionation must be minimized. In other words, the ablated material must be representative of the specimen; not just the most easily volatilized materials. Because the temperatures in a LIBS plasma are so high and the ablation occurs as a small explosion, all species get vaporized and ablated into the plasma, regardless of their individual thermal properties [39,40]. In LA, however, lower power lasers are used. The Nd:YAG (neodymium-doped yttrium aluminum garnet) laser’s fundamental wavelength of 1064 nm is not used because of poor laser-to-sample coupling and fractionation [24,41], as well as increased damage to the sample [42,43]. Lasers in the UV range provide much better coupling for most matrices, resulting in smaller particles and less fractionation, allowing for greater applicability.

There are two commonly used laser types: Nd:YAG and excimer (halogen halide gas-filled, for example, ArF). Lower wavelength excimer lasers offer the smallest beams and particles and least fractionation, but are more expensive, have more complicated optics, and require frequent gas changes and a highly skilled operator [36], so are reserved for specific applications. In general, Nd:YAG lasers are less expensive, easier to operate, require less maintenance, and can be Q-switched, for increased energy and thus better coupling, and so are used in the majority of LA systems for most applications.

Most LA systems are equipped with a camera or ocular for focusing the laser to the appropriate location on the sample. Ablation takes place in a small, closed cell and the aerosol is swept into the ICP by a carrier gas. The use of He, rather than argon, in the ablation cell promotes the generation of smaller particles, and in the ICP, its high thermal conductivity reduces the formation of aggregates [44]. Analysis

of the aerosol from a single laser pulse is possible, but SNR and precision is improved by using a series of rapid pulses and doing multiple MS sweeps over this time. Ablation can be done in spot or depth profile mode, where the laser drills down at a single location, or in a line or raster mode, where the laser ablates the surface in a line or raster pattern. The choice depends on the size and nature of the sample, and the type of information desired. The spot size can also be selected, keeping in mind that smaller spots cause less damage and provide higher spatial resolution, while larger spots provide a more representative measurement of non-homogeneous specimens and higher intensity signals because of the increased mass ablated. Other parameters such as energy, repetition rate (number of pulses per second), ablation time, speed of raster or depth profile, can be optimized.

2.4.3. LIBS

LIBS is a relatively simple and versatile spectroscopic technique that uses the in situ atomic emission of the sample in a local microplasma. Samples can be solids, liquids, gases or aerosols, and can be analyzed in vacuum, at atmospheric pressure, in air or bathed in other gases [39]. Because the particles ablated in LIBS are directly measured in the plasma, there is no need for an ablation cell or a carrier gas to sweep the particles away. No vacuum pumps are required, there is no sample interface to clean, and therefore no drift in sensitivity. As with other atomic emission spectroscopic techniques, atoms are excited by an input of energy and allowed to relax by emitting energy in the form of light. The wavelength of the light emitted is a quantum mechanical characteristic of the atom, and can be used to identify which elements are present. Since the emission peaks of atoms are narrow, they are referred to as emission lines. The intensity of an emission line can be related to the amount of that atom present.

In general, the pulsed laser beam is expanded, collimated and refocused to a tight spot on the sample (see Figure 1). When it interacts with the sample, the incoming radiant energy causes the formation of a shockwave at the surface. This causes bonds to break, heating leading to vaporization, and a small plasma of excited state gaseous atoms and ions. The electrons in the excited state species relax by emitting photons with wavelengths characteristic of the elements present. The light from the plasma emission is collected and analyzed by a spectrometer and a detector, resulting in an emission spectrum such as the one shown in

Figure 2. The choice of components has a very large effect on the quality of the data produced, in terms of accuracy/resolution, sensitivity, and precision/reproducibility.

Figure 1. Schematic of a LIBS apparatus [45].

Figure 2. LIBS spectra of two different soil specimens with different elemental profiles. Solid line: CS1 site, sub-plot 3, sample D, replicate 1; dashed line: FIU1 site, sub-plot 3, sample F, replicate 1. The upper image is the full spectrum. An expanded view of the region in the box is shown below it, with annotations for some emission lines of interest.

2.4.3.1. LIBS Optimization

The choice of laser is important, as it must provide enough power to generate a visible plasma (i.e., surpass the breakdown threshold), a wavelength that couples well with the specimen matrix, good shot-to-shot reproducibility, short controlled pulses, thermal stability, reasonable cost, and easy alignment and operation [38]. As discussed in section 2.4.2.3 above, Nd:YAG lasers meet most of these requirements when operated in Q-switched mode, except that they require a cooling system. An Nd:YAG laser is made up of a flashlamp above a rod of lasing medium in a resonant cavity, with a mirror on either end. The flashlamp is a broadband light source that acts as a pump, or source of photons. The lasing medium is an yttrium aluminum garnet (YAG) crystal doped with neodymium ions, which absorb some of the light in such a way that the upper energy level is more populated than the lower (a population inversion). As light

is reflected back and forth through the rod, photons of a certain wavelength (1064 nm) will experience an amplification by causing some of the Nd ions to relax and emit additional photons, which depletes the population inversion. When closed, the Q-switch prevents this from happening by blocking one of the mirrors, which allows the population inversion to really build up. When the Q-switch is opened, the photons are able to resonate back and forth through the cavity, allowing a large amount of amplification to occur rapidly, which then exits the laser as a high power pulse. The pulse lasts only a few nanoseconds (until the population inversion is depleted) and then the Q-switch is closed again to allow the population inversion to build up again for the next pulse [39]. The timing of the flashlamp and Q-switch, as well as the pulse repetition rate, can be controlled externally by a delay generator.

The Nd:YAG fundamental wavelength is 1064 nm, which is suitable for many specimen types, but the frequency can be double, tripled, quadrupled, or quintupled to achieve a more suitable wavelength (532, 355, 266, or 213 nm, respectively), though some energy is sacrificed in the process. While most LA systems use the UV wavelengths (266 and 213 nm), most LIBS systems still use the fundamental 1064 nm wavelength because of its lower cost for higher energy output. However, the same drawbacks to longer wavelength lasers apply to LIBS (see Section 2.4.2.3), particularly for optically challenging materials like glasses and certain minerals. Another difference between LIBS and LA is the laser power. Because the goal of the microplasma in LA is only to remove small particles, and not for spectroscopy, a much lower laser power can be used for LA than in LIBS.

The emitted laser light is a collimated beam of only one wavelength. Irradiance is the power per unit area (i.e., power density), usually reported in W/cm^2 at the sample surface. Similarly, fluence is the energy per unit area (J/cm^2). In order for dielectric breakdown to occur, the irradiance must be above the breakdown threshold. The actual irradiance required depends on the atmosphere and pulse wavelength and duration. In general it is on the order of $10^8 - 10^{10} \text{ W}/\text{cm}^2$ for solid samples, but for a 266 nm Nd:YAG laser with a pulse duration of 4 ns, it is on the order of $10^{11} \text{ W}/\text{cm}^2$ [39]. In order to achieve the required irradiance, the laser light is focused through a series of lenses to a very small spot on the surface of the specimen [39]. A single lens could be used, but a multi-lens Galilean telescope system (see Figure 1) can be used to create a much tighter focus, resulting in a smaller spot, and therefore higher irradiance.

As the laser pulse first reaches the sample, a few free electrons are accelerated by interaction with the electric field of the photons, and then collide with the photons and neutral species. This causes a local increase in temperature, which vaporizes the surface of the sample and begins the atomization and ablation process [39]. It also causes further ionization, which in turn produces more electrons, and a chain reaction of ionization, resulting in a plasma that is sustained for the duration of the laser pulse [40]. Once the breakdown event occurs, the plasma expands rapidly and generates a shockwave, which acts as an explosion to mechanically ablate more material. Temperatures of 10 000 K can be achieved, which allows the ions and atoms in the plasma to become excited to higher energy levels. After the laser pulse stops, the plasma begins to decay and cool.

White continuum (not quantized) light, caused by bremsstrahlung radiation (radiation released during collisions of free electrons) and recombination radiation (radiation released during capture of free electrons by an ion), is emitted in the early stages of the plasma and decays as the plasma cools. Emission of light from ionized atoms begins around the same time, but decays more slowly. Then as ions recombine with electrons, emission of light from neutral atoms occurs. The continuum emission can swamp the detector, resulting in high background and poor SNR for the quantized atomic emission lines of interest. However, since the continuum decays more quickly than the atomic emission, it can be filtered out temporally by delaying the collection of the emission until the continuum has decayed sufficiently to allow adequate detection of the emission lines [39,40]. Typically this occurs at approximately 1 μ s after the laser pulse [40]. Because of the low ionization potential of argon, plasmas formed in argon decay more slowly than those in air, resulting in a longer emission time.

The light from the plasma is directed through a series of lenses to a spectrometer, where it is separated into its component wavelengths. The choice of spectrometer is crucial for matrices with complex spectra, such as soil and steel, while less important for matrices with simpler spectra such as glass and plant material. The spectral resolution is the most important factor, so that emission lines do not overlap to form broad peaks. With some types of spectrometers, such as the Czerny-Turner, a high resolution is only achievable for a narrow spectral window, which must be chosen in advance. If the emission lines of interest occur in different regions of the spectrum, the analysis must be repeated for each spectral region, which

adds time and reduces the precision of multi-element data. Echelle spectrometers provide good resolution over the entire spectrum (typically 200 – 900 nm) [46].

A sensitive and reliable detector should be used to obtain good precision and SNR. The timing of the detector can be controlled externally through a delay generator, or internally when a gated detector is used. The delay time (“gate delay” in a gated detector) is the delay between the opening of the Q-switch and the start of the signal acquisition by the detector. The integration time (“gate width” in a gated detector) is the duration of the signal acquisition. These two parameters can be adjusted to achieve a low background and high intensity and precision in the emission lines of interest. If ionic lines alone are desired, the delay and integration times should be kept short. For neutral lines, the acquisition should be later. For general LIBS applications, both are of interest, so the delay time is short (typically 1 – 2 μ s) and the integration time is long (typically 5 μ s – 1 ms). Note that emission lines are reported with their ionization states in roman numerals (I for neutral, II for ionic (first ionization), III for ionic (second ionization), and so on).

In comparison to other laser types, the Nd:YAG has good shot-to-shot reproducibility, however there are still minor fluctuations that can affect the intensity of the emission lines. In addition, if the specimen is heterogeneous at the scale of the laser spot size, the spectrum may differ from one shot to the next. Both of these can pose problems when comparing one spectrum to another (for example, when comparing questioned and known specimens). Both can be solved by averaging or accumulating the spectra from multiple laser pulses, resulting in increased accuracy and precision [40].

In order for quantitative analysis to be successful, the plasma must be optically thin, meaning that there is no appreciable re-absorption of the emitted light. If re-absorption (also known as “self-absorption”) occurs, it can lead to peaks that appear saturated or split and result in non-linear calibration curves that appear to level off [40]. If only a few emission lines are affected, weaker emission lines of the same element can be chosen. If the effects are widespread, the amount of material in the plasma should be reduced.

2.4.4. μ XRF

In XRF, high energy X-rays strike the surface of the specimen in an evacuated chamber, releasing an electron from an inner atomic shell. The inner-most electrons are held in the K shell. Further out are three L

shells, then five M shells for the heavier metals, and so on. An electron from another more outer shell fills the vacancy, resulting in a release of energy corresponding to the electron binding energy difference between the two levels. This release can be in the form of an X-ray photon or an Auger electron from a further outer shell. XRF is concerned with the X-rays emitted, which have specific energies characteristic of the elements present in the sample.

Using an EDX detector, a spectrum is generated showing the intensity versus energy, with the intensity of each line being proportional to the number of X-rays counted at that particular energy. The X-ray lines are superimposed on a continuum background from bremsstrahlung radiation, which reduces the SNR of XRF. The EDX detector can reliably detect X-rays in the 1 – 40 keV range corresponding to K lines from sodium to neodymium, and L lines from neodymium to uranium. Lighter elements are problematic because of the presence of the detector window. Artifacts may be seen in the spectra as a result of various properties of the detector.

Resolution is not as good as for atomic emission techniques, so interferences may occur, but the spectra are simpler. For each element a series of lines are seen, corresponding to the various allowed transitions (e.g., the $K_{\alpha 2}$ line results from a transition from the second L shell to the K shell), providing a sort of “fingerprint” pattern that can be used to identify the element [47].

Micro XRF (μ XRF) employs a small spot size (X-ray beam diameter), usually on the order of 1 mm or less, which can be used for elemental mapping. Most instruments with capillary optics, 35kV excitation energy, and EDX detection will provide adequate performance [48].

2.5. Signal Processing and Data Analysis

Subtraction of background signals and normalization are important for both qualitative and quantitative analysis. In LIBS, background emissions, as well as the emission lines themselves, are affected by plasma conditions, which are in turn affected by the matrix. Background-subtraction is especially important in LIBS since atomic emission lines are superimposed on a broad continuum background.

As discussed in Section 2.3.4, an internal standard may be added if none is naturally present. Normalization is done by calculating the ratio of background-subtracted signal from the peak of interest over that of the internal standard. Other normalization methods exist. One that is used in XRF for glass

analysis involves calculating the ratio of the background-subtracted signal of a peak of interest over that of the same element in a RM spectrum [48]. Another involves normalizing the background-subtracted LIBS intensities to the sum of the plasma light collected over the whole spectrum.

The absence of a signal (or one below the LOD, i.e., a non-detect) for an element in a particular specimen may also be important information if a measurable signal is detected in other specimen(s). In quantitative analysis, measurements should be accurate, especially if a database is to be used, but for the purposes of direct comparisons between a known sample set(s) and questioned samples in a single case, precision of the measurement is more important. Calibration curves are normally employed to determine the specimen concentrations from the signal intensity. Using these, one can visually determine the linear dynamic range. Concentrations above or below this range cannot be reliably quantified. This is particularly important in LIBS analysis, where self-absorption is common. In LA-ICP-MS, however, the linear dynamic range is quite large, covering several orders of magnitude. In this case, a single-point calibration scheme can be employed after validation for the matrix of interest [24].

The conversion of signals to concentrations requires prior knowledge of the element menu in order to include the elements of interest in the calibration standards. In addition, using linear regression imparts some error. Since concentrations are not explicitly required for discrimination purposes, LOD-corrected normalized background-subtracted intensities can be used instead for LIBS and XRF; however care should be taken to monitor self-absorption for emission lines not calibrated. Ratios of the normalized intensity of one emission line over the normalized intensity of another emission line provide enhanced discrimination power [45]. For example, in Figure 2 the Mg I 517.3 nm peak has a greater intensity in the CS1 specimen than in the FIU1 specimen. The Ba II 493.4 nm peak on the other hand has a lower intensity in the CS1 specimen than in the FIU1 specimen. Similarly Fe I 495.8 and Mg I 518.4 provide opposite responses. Considered separately, these differences provide some level of discrimination, but when the ratio of Fe I 495.8/Mg I 518.4 or Ba II 493.4/Mg I 517.3 or is used, the difference becomes much more important. For both LIBS and XRF, when quantitative results are not available, ratios of normalized signal intensities may be used to make the data more comparable, and to enhance to differences and similarities. This strategy has been applied to glass, paint and soil analysis [45,49,50].

2.6. Statistical Analysis & Interpretation

It is important to apply appropriate statistical methods to the data, and to apply them correctly. The choice of statistical method depends on the nature of the question to be answered, the analytical technique(s) used and the nature and size of the data itself. Unfortunately, many statistical techniques are applied incorrectly by scientists with little statistical training [51]. Despite the assumption by many (including the jury) that “numbers do not lie”, data can be interpreted and misinterpreted (whether intentionally or not) in many ways depending on the desired outcome [52].

For a given case, even within the same county or region, different sets of elements may provide the key to distinguishing different sources. For this reason it is useful to monitor as many elements as possible, even if the actual discrimination is made using a smaller element menu [22]. To avoid biasing the results, known specimens from all possible source locations should be characterized and the most discriminating and reliable elements determined before the questioned specimens are compared.

2.6.1. Match Criteria

If two specimens differ significantly (i.e., beyond the natural variation or heterogeneity of the source) in multiple variables (e.g., each individual element, or other characteristics such as color), they can usually be excluded as having come from different sources. The conclusion is less clear when the specimens can only be excluded by one variable. For homogeneous matrices like glass, a significant difference in only one element is often considered an exclusion, but for heterogeneous samples like soil, it may not be. If no significant differences are found, it is tempting to consider it a “match”; however this is not strictly correct. A match implies that the two specimens originated from the same source, to the exclusion of all others. While it may be true in some cases, there is no way to prove it without analyzing all other sources, which is impractical and impossible. The more independent variables analyzed, the better the discriminating power and the greater the confidence in the conclusion [50,53]. The confidence and significance of the results should be clearly stated in the final report and when giving expert testimony.

To be able to confidently state that one specimen can be distinguished (or discriminated) from another, the within-sample variance (usually measured by making replicate analyses of the same specimen) must be much smaller than the between-sample variance [54]. In effect, if all replicates of each specimen

were plotted (for example using a bivariate plot, scatter plot or a multivariate plot such as principal components analysis (PCA) scores plot or linear discriminant analysis (LDA) canonical plot), replicates from the same specimen would overlap, while there would be no overlap among replicates from truly different specimens [50]. The measurement variance from the method itself should be very small and the majority contribution should come from specimen heterogeneity.

For glass, a number of match criteria have been tested in order to assess the Type I and Type II errors for LA-ICP-MS analysis [55,56]:

- n sigma interval around one of the two glasses to be compared
- n sigma interval around both of the two glasses to be compared
- modified n sigma criterion with fixed relative standard deviations
- Student's t-test for each of the 18 elements (with and without Bonferroni correction)
- modified t-test with fixed relative standard deviations
- ANOVA with Tukey's post-hoc correction followed by t-test at the 95 % confidence level

The authors of these studies concluded that either a four sigma match criterion with or without fixed relative standard deviations or a modified t-test with fixed relative standard deviations resulted in the best simultaneous reduction in both error types. Curran has also published a series of papers discussing the statistical treatment of elemental glass data, including three sigma, range overlap, Hotelling's T^2 , permutation testing and Bayesian analysis [57-60].

The "match criteria" used to define differences between samples are matrix- and analytical technique-dependent. Man-made matrices, such as glass and paint layers, tend to be fairly homogeneous and therefore can have more rigorous match criteria. Soil specimens, on the other hand, are quite heterogeneous, even within a single source, and are made up of many different particles. The question then becomes how similar the samples need to be in order to be considered to have originated from the same source, and what level of confidence this conclusion holds [23]. This depends on the nature of the technique used, and more importantly, on the variation in the region and whether samples from different sources can have the same

elemental profile. It is important to characterize the site of interest and nearby or geologically similar sites in order to get a broader picture of the natural variation to better answer this question [23].

2.6.2. Univariate Statistics (Significance Testing for Pair-wise Comparisons)

In forensic examinations, there are often only a few questioned specimens and a few known specimens to compare. The examiner must determine if the questioned specimens can be associated with one another, and more importantly, with any or all of the known specimens. This is done by performing pairwise comparisons, in which each specimen is compared to all other specimens. The number of possible pairs is equal to $n(n-1)/2$, where n is the total number of specimens. Significance testing (also known as hypothesis testing) is used to compare the means of two populations (i.e., specimens) for a particular characteristic of the specimens (for example, the concentration of strontium). A null hypothesis is defined (for example, there is no difference between the two specimens other than random variation), as well as an alternative hypothesis (for example, that there is a difference, beyond random variation, in the two specimens). Assuming that the data are normally distributed, a p -value is calculated and compared to a pre-determined significance level α , defined as 1- the confidence level (for example, α is 0.05 for the 95 % confidence level). If the p -value is less than α , the observed result is unlikely (i.e., a one in 20 chance at the 95 % confidence level) under the null hypothesis, and therefore the null hypothesis may be rejected. The process is repeated for each element. Type I errors occur when the null hypothesis, that there is no difference between the specimens, is incorrectly rejected (i.e., detecting a difference when, in fact, there is no difference). Type II errors occur when the null hypothesis is incorrectly accepted (i.e., true differences are not detected) [61].

2.6.2.1. Student's t -test

The simplest significance test is the Student's t -test, which has a number of variations. Here the applicable one is the 2-tailed comparison of two experimental means, in which the null hypothesis is that there is no difference between the means of the two specimens (i.e., that the difference between the two means is zero). Assuming that they have similar standard deviations (homoscedastic), the test statistic, t , can be calculated to test whether the difference in means differs significantly from zero [61]. This t -statistic

is compared to a 2-tailed critical value that is determined using the confidence level and the degrees of freedom. The p-value is the probability that $|t|$ is greater than the critical value. Another variation is a heteroscedastic test that is used when it cannot be assumed that the two specimens have similar standard deviations.

2.6.2.2. Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) is used when there is more than one pair of means to be compared. The null hypothesis is the same as above: that there is no difference between the means of each population (i.e., specimen) other than random variation. The basis of this type of significance test is to compare the variation within populations to the variation between populations in order to determine if the null hypothesis should be rejected. The sum of the square of the residuals is calculated, which represent the random error. The sum of the square of the treatments (in this case the specimens) is also calculated, which represents the actual differences in the populations. If the treatment means are similar, then most of the variation was a result of random error, there is no difference in populations, and the null hypothesis cannot be rejected [61].

2.6.2.3. Tukey's Honestly Significant Difference Test

One drawback with ANOVA and the Student's t-test is that the confidence intervals and p-values generated do not take into account the fact that multiple comparisons have been performed. Therefore they are too inclusive, and prone to false inclusions (type II errors, in this case false positives). Therefore, Tukey's honestly significant difference (HSD) test should be applied post-hoc for a more accurate estimate of the p-values and confidence intervals (i.e., family-wise confidence intervals) [62].

2.6.3. Multivariate Statistics

Elemental analysis provides data for a large number of elements. Each element can be considered a variable. Univariate comparisons overlook the power of a multivariate comparison, and can become quite tedious when repeated for each element or variable. Since each element contributes to the elemental profile and discrimination, it is useful to visualize the data for multiple elements on multiple specimens simultaneously. A bivariate plot (biplot) can be used to compare two variables, where the x-axis is the

intensity of one element and the y-axis is the intensity of another element line (see Figure 26a). A third dimension may be added on the z-axis (see Figure 26b and c), but unfortunately only three spatial dimensions can be visualized. Multivariate techniques can handle the data from all variables (elements) simultaneously. Data reduction (more correctly, variable reduction) is performed so that multivariate data can be represented in two or three dimensions, making multivariate visualization possible. In addition, modeling can also be performed, in which data from a training set (i.e., known specimens) can be used to build a model. The questioned sample is then fed into the model and a prediction returned. There are a number of multivariate techniques available, depending on the type of data available and the type of questions to be answered [61].

Hotelling's T^2 is a multivariate version of the Student's *t*-test where each pair of samples is compared using all variables (elements) simultaneously, rather than one-at-a-time [58]. A major drawback is that the number of observations (the total number of replicate measurements for all specimens) must be greater than the number of variables. For cases where there is only one questioned specimen and a couple of known specimens, the element menu is limited [55]. A multivariate ANOVA (MANOVA) exists, but it is extremely restrictive and rarely used. Pearson's product moment correlation is greatly affected by the most abundant elements (matrix elements). These do not vary much for soils on the scale of a typical local forensic case since they originate from the same lithology [50]. Spearman's rank correlation is a non-parametric technique that ranks the variables (elements) in order of abundance. For specimens from the same lithology, the differences between each of the matrix elements and some minor elements can be so great that their rankings do not change even if the concentrations vary, while trace metal rankings would change considerably with small changes in concentration [50].

In general, multivariate statistical techniques have one major drawback: the number of degrees of freedom is limited by the number of variables. Therefore, a large number of observations (i.e., replicate measurements) must be made in order for the result to have meaning (because of the underlying mathematical equations). Some exceptions are linear correlation analysis and partial least squares discriminant analysis (PLS-DA), which can handle a large number of variables relative to the number of observations. Both are supervised classification methods, in which a training set of known specimens is

used to develop a model to be used to classify questioned specimens. Some researchers, especially in the LIBS community, have begun comparing complete spectra (with each wavelength along the x-axis representing a variable) rather than comparing individual elements. Since there are thousands of variables in each spectrum, this requires more computational power than the other methods described below, and is prone to over-fitting because of the noise in the spectra [63]. While PLS-DA may seem tempting, in that the whole spectrum can be put in, with minimal data processing (or spectroscopic expertise) required, it is not recommended because of its potential for over-fitting, especially in a forensic setting where the number of known specimens available to train the model are usually quite limited.

2.6.3.1. Principal Components Analysis (PCA)

Principal components analysis (PCA) is probably the most widely used variable reduction and multivariate visualization technique. Principal components analysis is performed by creating a set of linear combinations (principal components, PCs) of the variables with the aim of maximizing the amount of variation expressed in each one. The first PC represents the most variation, with each subsequent PC representing less and less of the variation. The first few PCs therefore represent the majority of the useful variation, while the last few are arguably just fitting the noise and can be ignored. Not all variables (elements) contribute equally or in the same direction. Each PC is orthogonal to all others, and can be considered as an axis or dimension. In a PCA scores plot, data is plotted as projections (or scores) onto these axes (usually the first two or three PCs are plotted) [61].

PCA has some advantages over other multivariate techniques. It does not require input data to be normally distributed. In addition, it is an unsupervised technique, meaning that prior knowledge of the identity of the specimens is not required. This makes it an ideal exploratory tool, useful for identifying natural groupings in the data, so it is often performed before classification models are employed. It can be used in predictive modeling, and is particularly useful when the questioned specimen does not belong to any of the known groups.

2.6.3.2. Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis (LDA) is a supervised method of classifying unknown samples into groups by creating a linear combination of the variables (in this case a linear discriminant function) on a set of training data of known groups. In order to predict which group a questioned sample belongs to, the distance from each point in the dataset to the multivariate mean (centroid) of each group is measured, and the closest group is chosen. This could be used in a case where a questioned sample from a suspect must be classified as belonging to the crime scene location, an alibi or other location(s) or neither. A canonical plot, which resembles a PCA plot, is produced by plotting projections (scores) onto the canonical axes. In order to estimate the number of misclassifications, a leave-one-out (also known as jackknife) cross-validation is often performed [61].

A classification model is only as good as the training data used to construct it. The training set should include all classes that would be reasonably expected to be encountered (i.e., crime scene, alibi location, and other pertinent locations, if applicable), and enough specimens and replicate measurements to give a representative picture of each class. When a large number of known specimens are available, methods such as linear discriminant analysis (LDA) may be used to build models that can be used to classify questioned specimens [22]. However, if the questioned specimen does not come from one of the known classes (locations), it will still be incorrectly assigned to the closest class. This is a danger of relying solely on the numbers alone. With a quick look at the canonical plots, it should be obvious to the naked eye whether or not the questioned sample really fits in with the known specimens of the predicted class. If not, the possibility of an additional location should be considered.

2.6.4. Forensic Soil Elemental Analysis and Discrimination in the Literature

This section describes examples of the use of various elemental analysis techniques for soil analysis in the context of forensic discrimination. Most soil analysis by LIBS has been for environmental, military and space exploration applications [22]. Only Martin, Labbe, Andre et al. [64], Martin, Wullschleger, Garten et al. [65], Martin, Wullschleger, Garten, & Palumbo [66], Martin, Labbe, Andre et al. [67] have published work on the forensic analysis of soil by LIBS. However, Martin et al determined the total carbon content rather than using the elemental profile.

Wilson, Davidson, & Cresser [68] used ICP-OES and SEM/WDX for archeological discrimination of soil types (bracken, dung, lime mortar, peat and turf). They found that site-to-site variation was a result of anthropogenic activity more so than geological composition so it should be possible to differentiate between different locations within a particular soil type, which would provide much more forensic impact than classification simply on the basis of soil type.

Rawlins and Cave [29] used samples from a high spatial resolution soil geochemical survey (one sample per 2 km²) in eastern England to try to discriminate samples within a geological soil type by XRF (using both EDX and WDX detectors). Large bulk samples were taken and sieved and a large sub-sample of each was ground and pressed into a pellet before analysis of 19 elements. Analysis of variance (ANOVA) was used to compare pairs of samples for each element individually (a univariate approach). Mahalanobis distance (a multivariate approach) was also used to take the correlation between variables into account. Discrimination was successful in over 95 % of samples from each of six soil types. The most discriminating elements were determined to be Mn, Mg, Rb, Zr, Sr, Ni and Zn. The least discriminating elements were U and Mo. Hiraoka [18] created a training set of over 110 Japanese sites, each containing ten soil samples that were sieved, ground and pressed into pellets. The XRF data were normalized to a Japanese RM, Mahalanobis distances were calculated, and PCA was used to provide a visual discrimination. Fourteen blind samples were collected by police to test the method, ten of which were correctly identified.

A database was created of over 500 specimens from coastal dune sediments in England and Wales. Data on particle size of bulk samples and ICP-OES for major oxides and 20 elements (Ba, Co, Cr, Cu, Li, Ni, Cs, Cr, V, Zn, Zr, Pb, Y, La, Ce, Nd, Sm, Eu, Dy and Yb) by lithium metaborate flux of sieved samples were included in this database [53]. In a murder investigation, Pearson's correlation matrix was used to determine the degree of correlation of the database samples with sand found on a glove in order to determine the most likely source location. They collected additional known specimens at the most likely location, however the body had been moved and no useful information was found there. Results may have been skewed by the presence of dirt on the gloves from previous use expand on this.

Jarvis, Wilson, & James [23] used ICP-OES (Al, Fe, Mg, Ca, Na, K, Ti, P, Mn, Ba, Co, Cr, Sc, Sr, V, Zn and Zr) and ICP-MS (Ce, Cs, Dy, Er, Eu, Gd, Hf, Ho, La, Lu, Nb, Nd, Pr, Rb, Sm, Ta, Th, U, Y and Yb) after lithium metaborate flux to examine and compare instrumental variation and within-sample repeatability. Sieved samples of only 0.05 g were used in order to mimic transfer samples obtained in forensic casework.

Pye and Blott [50] also used ICP-OES and ICP-MS on lithium metaborate fluxes of sieved samples with an expanded suite of 49 elements and major oxides. Five questioned specimens taken from a suspect's boots were compared to nine known specimens taken from the crime scene. It was determined, on the basis of the concentration of each element and ratios of selected elements and oxides as well as Pearson's product moment correlation and Spearman's rank correlation, that the samples from the boot were not from the same source as the known specimens [50].

Elemental analysis with multivariate statistical analysis has been applied in forensic science, but more robust methods are required.

3. Materials & Methods

The materials used throughout this work and final methods are described here. Method development steps that led to these final methods are described in their respective Sections, along with specific specimen and sample descriptions and any method modifications made to accommodate the needs of particular studies.

3.1. Preparation of Calibration Standards, Reference Materials, and Specimen Samples

All specimens were dried and labeled with a unique identifier upon receipt, before storing or weighing out sub-samples. “Soil specimens” in this work were bulk surface samples, collected to a depth of 5 cm or less. “Sediment filters” consisted of river sediments collected onto pre-weighed cellulose nitrate (47 mm diameter and 0.45 μm pore size, Whatman Ltd., Piscataway, NJ, USA) filters. Filters with adhered sediment were dried and weighed to determine the mass of sediment.

3.1.1. Reference Materials and Calibration Standards

In-house calibration standards were created using clean silica sand (US EPA NEIC, Denver, CO, USA) as a blank matrix since it was relatively low in trace elements. Various concentrations of elements of interest were added in the form of single element ICP (CPI International, Santa Rosa, CA, USA) and ICP-MS (Ricca Chemical Company, Pocomoke City, MD, USA) standard solutions.

The following RMs were used: PACS-2 (“Marine Sediment Reference Material”, National Research Council (NRC) of Canada, Ottawa, Canada), NIST SRM 2704 (“Buffalo River Sediment”, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA), NIST SRM 2710 (“Montana Soil”; “Highly Elevated Trace Element Concentrations”, NIST, Gaithersburg, MD, USA), and NIST SRM 2710a (“Montana I Soil”; “Highly Elevated Trace Element Concentrations”, NIST, Gaithersburg, MD, USA).

3.1.2. Sieved Soil

Bulk soil specimens to be sieved were placed in a modified sieving apparatus and shaken and tapped to allow the passage of the finer particles into the collection tubes. The apparatus (see Figure 3) consisted of 150 μm pore size disposable nylon screen (Miami Aqua-culture, Inc., Boynton Beach, FL, USA) in

plastic filter funnels (Thermo Scientific Nalgene, Pittsburg, PA, USA). Both the coarse ($> 150\ \mu\text{m}$) and fine ($< 150\ \mu\text{m}$) fractions were kept, but only the fine fractions were used for further analysis and will be referred to as “sieved soil”. Note that not all specimens were sieved (sieved soil specimens are noted in their respective Sections).

Figure 3. Sieving apparatus using disposable nylon mesh in a plastic filter funnel.

3.1.3. Color Determination

Munsell color designations were determined for sieved soils by two independent observers under identical conditions (fluorescent lighting, grey paper background). Colorblindness and color vision tests were successfully completed before making determinations. The pages of color chips were placed over the soil specimen and a Munsell designation assigned to each soil. Designations were given as described in Section 2.2.5.

3.1.4. Spiking of Specimens, Reference Materials, and Calibration Standards

A representative sample of approximately 0.45 g of each calibration standard, RM, and specimen (either sieved or bulk, depending on the study, as noted in their respective Sections) was accurately weighed and scandium single element ICP-MS standard solution was added (“spiked”) as an internal

standard to a final concentration of 350 ppm. Additional internal standards (lutetium single element ICP standard solution at a final concentration of 1000 ppm and indium single element ICP-MS standard solution at a final concentration of 300 ppm) were used in some of the studies, as noted in their respective Sections. Samples were mixed thoroughly with a vortex touch mixer (Fisher Scientific, Pittsburg, PA, USA) and dried in air at 80 °C in an aluminum block dry bath incubator (Fisher Scientific, Pittsburg, PA, USA) for 16 hours (for smaller samples, less time was required). Dry samples resembled a hardened ball and were broken up using the vortex touch mixer to ensure dryness throughout. These will be referred to as “spiked soil”. “Spiked sediment filters” were prepared by spiking internal standard onto the sediment portion of sediment filters to a final concentration of 300 ppm In and 350 ppm Sc on the basis of the weight of the sediment. Sediment filters were dried for one hour at 50 °C.

3.1.5. Tape-Mounting of Specimens, Reference Materials, and Calibration Standards

Spiked RM, calibration standard, and sieved soil specimens were carefully re-homogenized using the vortex touch mixer. For each one, a 19 x 22 mm piece of Scotch removable poster tape #109 (3M, St. Paul, MN, USA) was affixed to a labeled 30 x 22 mm glass coverslip (Fisher Scientific, Pittsburg, PA, USA), leaving the tape liner on. The liner was folded back halfway to expose an area of adhesive approximately 19 x 10 mm (see Figure 4a). A small amount of each sample (approximately 10 mg) was deposited onto the exposed tape and shaken gently to distribute it evenly (see Figure 4b). The deposited sample was smeared and pressed gently to improve adhesion, and then tapped lightly to remove any excess that was not adhered. The liner was folded back down to act as a cover to prevent contamination or loss and was taped in place (see Figure 4c). The tape-mounted specimen was packaged carefully in weighing paper, and labeled (see Figure 4d). Note that not all specimens were tape-mounted.

Figure 4. Tape-mounting of soil specimens.

3.1.6. Milling and Pelletizing of Specimens, Reference Materials, and Calibration Standards

Spiked soils were homogenized and pulverized to a fine powder (approximately 1 μm particle size) in a high speed ball mixer mill with tungsten carbide jars and balls (Retsch, Newtown, PA, USA) for 30 minutes at 25 Hz. “Bulk pellets”, “RM pellets” and “calibrator pellets” were prepared from spiked bulk soil, spiked RM and spiked calibration standards, respectively. “Sieved pellets” were prepared from sieved, spiked soil, but it was only milled for 20 minutes. Milled powder was pressed into pellets 13 mm in diameter and approximately 2 mm thick using high grade stainless steel dies in a vacuum-assisted benchtop pellet press (Carver, Wabash, IN, USA) for five minutes at four metric tonnes. “Sediment filter pellets” were prepared by milling crumpled, spiked sediment filters for ten minutes and pelletizing in 6 mm dies for five minutes at 1.2 metric tonnes. The “RM filter pellets” were prepared by milling together a blank filter and an equal mass of spiked RM for ten minutes and pelletizing in 6 mm dies for five minutes at 1.2 metric

tonnes. All pellets were mounted onto an adhesive backing, packaged in weighing paper (Fisher Scientific, Pittsburg, PA, USA), and labeled.

3.2. Elemental Analysis

Reference materials were measured at the beginning and end of each analysis day, and periodically throughout the day. Calibration standards were measured at the beginning of each day. One or more specimen sample(s) from each region was selected to be measured again at the end of the day (“duplicate run”). Element menus for each study are described in their respective Sections.

3.2.1. LA-ICP-MS

3.2.1.1. Instrumental Parameters

Elemental analysis by LA-ICP-MS was performed using a quadrupole ELAN DRC II 6100 (Perkin Elmer LAS, Shelton, CT, USA) in standard mode. Cross-calibration of the dual detector was performed using a multi-element solution containing all elements in the pre-selected element menu. Argon at 16 L/min was used as the plasma gas with an argon auxiliary gas at 1 L/min and an RF power of 1500W. Data were acquired in peak-hopping mode. Solid sample introduction was provided by a LSX500 laser ablation system (Cetac Technologies, Omaha, NE, USA) with a 15 mJ maximum pulse energy, 5 ns pulse width, Q-switched 266 nm Nd:YAG laser, focused on the sample surface. Helium at 0.9 mL/min was used as the ablation and carrier gas to bring ablated particles from the ablation cell (approximately 17 mL), through Tygon tubing (approximately 1.4 m, Fisher Scientific, Pittsburg, PA, USA), to a “Y” connector to merge with the argon nebulizer gas flow of 0.9 L/min, and finally to the ICP torch. Parameters were optimized to maximize signal intensity of ^7Li , ^{139}La and ^{140}Ce and minimize oxides (ThO) and doubly-charged species (Ca^{2+}). Daily performance was tested using NIST SRM 612 (“Trace Elements in a Glass Matrix (3mm Wafer)”; “Nominal Trace Element Concentrations 50 mg/kg (ppm)”, NIST, Gaithersburg, MD, USA).

Four replicate measurements were performed at different locations on each sample. The ablation cell was purged and flushed with helium between samples. Data for each replicate measurement were acquired as a transient signal (counts per second versus time) for 150 seconds, in peak-hopping mode for each isotope in the element menu. For each measurement, the laser was started 20 seconds after the ICP-MS

acquisition began (“gas blank”). The laser ablation continued for 50 seconds, and then the ablation cell was flushed for 80 seconds to eliminate carry-over between measurements.

For pellets, the laser was operated in depth profile mode, in which the stage moved up at a rate of 1 $\mu\text{m/s}$. During each ablation, ten pulses per second (repetition rate, Hz) were delivered at 100% energy, with a spot size of 200 μm , providing approximately 3 mJ to the sample (9.5 J/cm^2) for each of the 500 laser pulses. For tape-mounted specimens, the laser was operated in line mode, in which the stage moved horizontally at a rate of 150 $\mu\text{m/s}$. During each ablation, five pulses per second were delivered at 30% energy, with a spot size of 200 μm , providing approximately 0.5 mJ to the sample (1.6 J/cm^2) for each of the 250 laser pulses.

3.2.1.2. Signal Processing and Quality Control/Figures of Merit

The data acquired with the ICP-MS instrument were processed with GLITTER 4.4 (GEMOC, Macquarie University, Sydney, Australia). The software integrated the counts per second signal (“cps”) over 30 seconds after the laser-to-sample coupling peak, subtracted the gas blank signal, and normalized the data using ^{45}Sc as the internal standard (“normalized, background-subtracted cps”) (see Figure 5). The GLITTER software also corrected for drift and calculated the concentration of each isotope in each of the replicate measurements using concentration values supplied on the NIST SRM 2704 certificate using a linear yield interpolation ratio single-point calibration scheme. In order to manually verify the linearity of the single-point calibration, calibration curves were generated from the normalized, background-subtracted cps of the calibration standards for a sub-set of the isotopes in the element menu.

Figure 5. Evolution of the laser ablation transient cps signal measured by the ICP-MS for a particular isotope, showing the gas blank and sample integration regions.

Minimum detection limits (MDL) for each element were calculated by GLITTER at the 99 % confidence level for each replicate measurement. The data were exported as a text file where MDL-filtered concentrations were reported in $\mu\text{g/g}$ (ppm). Text files were imported into Excel (Microsoft Corporation, Redmond, WA, USA), and any values below the MDL were assigned the MDL for that replicate (“MDL-corrected concentrations”). Mean values, standard deviations, and relative standard deviations (RSDs) for each isotope in each sample were estimated for the four replicate measurements. Bias for each standard was reported as a percent deviation of the measured concentration of each element from the certified concentration values. Reproducibility for each duplicate run was reported as a percent difference in the measured concentration of each set of four replicates, for each isotope. Linearity of detector response was confirmed for a sub-set of the element menu using the calibration standards to prepare a series of calibration curves. The overall MDL for each isotope was calculated by averaging the MDL over all measurements.

3.2.2. LIBS

3.2.2.1. Instrumental Parameters

LIBS experiments were conducted on a home-built system using a Tempest laser (New Wave Research, Fremont, CA, USA) with 29 mJ pulse energy, 4 ns pulse width, Q-switched 266 nm Nd:YAG. The un-attenuated laser beam was expanded from 4 mm to 12 mm, and collimated, via a Galilean telescope configuration, and then focused to 1.4 mm into the sample using a plano-convex lens of focal length (f) 150 mm, resulting in a laser spot size of 137.5 μm (and therefore a laser fluence of approximately 148 J/cm^2) at the sample surface. All focusing optics were UV-coated fused silica (Thorlabs Inc, Newton, NJ, USA). Timing of the laser flashlamp and Q-switches was controlled externally at a repetition rate of 0.667 pulses per second (Hz) using a 565 delay generator (Berkeley Nucleonics, San Rafael, CA, USA).

Each laser pulse produced a LIBS microplasma at the sample surface. The light emitted from the plasma was collected, at 90 ° to the incident laser irradiation, through two plano-convex lenses ($f = 75$ mm, Thorlabs Inc, Newton, NJ, USA), which collimated and focused the light onto a 50 μm diameter fiber optic cable connected to a Mechelle 5000 spectrometer with 1024 x 1024 time-gated iStar iCCD camera (Andor Technologies, South Windsor, CT, USA). The spectrometer and detector provided broadband (200 – 936 nm) emission spectra (intensity versus wavelength) with a resolving power of 5000. Optical alignment was adjusted to maximize signal intensity. Daily performance was tested using NIST SRMs 1831 (“Soda Lime Sheet Glass (1.2 % Al_2O_3)”) and 610 (“Trace Elements in a Glass Matrix (3mm Wafer)”; “Nominal Trace Element Concentrations 500 mg/kg (ppm)”, NIST, Gaithersburg, MD, USA) in air and in argon.

Sample surfaces were bathed in argon at a flow rate of 0.9 L/min. For each laser pulse, a gate delay time of 2.0 μs was used before opening the gate to begin signal acquisition, and the gate remained open for signal acquisition for 150 μs (integration time, or gate width). To enhance SNR, the spectra from 75 laser pulses were added together to obtain one accumulated spectrum per replicate measurement. Five replicate measurements were performed at different locations on each sample. For pellets, spot mode was used, in which the 75 pulses were fired at the same location. In order to remove any surface contamination and to initiate laser-to-sample coupling, three cleaning pulses were applied to the each location before beginning

each replicate measurement. For tape, line mode was used, in which the sample stage was moved at a speed of 250 $\mu\text{m/s}$.

3.2.2.2. Signal Processing and Quality Control/Figures of Merit

The spectra were saved as text files and imported into Mathematica 5.2 (Wolfram Research, Champaign, IL, USA). An algorithm written by Dr. Cleon Barnett was used to calculate maximum peak height (“intensity”) for each emission line (using user-defined peak windows), calculate the noise, subtract the background, normalize the peak intensity to that of the internal standard emission line (Sc II 361.4 nm), and plot each peak and background region for visual inspection (see Figure 6). These “normalized background-subtracted intensities” for each emission line were output in a .csv file.

Figure 6. A section of the LIBS spectrum from NIST SRM 2704 showing the background-subtracted intensities of the internal standard peak (Sc II 361.4 nm) and a nearby Cr I 360.5 nm peak.

Each file was imported into Excel (Microsoft Corporation, Redmond, WA, USA). Calibration curves were generated for each emission line from the normalized background-subtracted intensities of the calibration standards, and linear regression was used to calculate slopes and y-intercepts in order to calculate the concentrations for all samples. Limits of detection were calculated as three times the standard deviation of the noise in the neighboring blank (background) regions of the spectrum (see Figure 6). Any

values below the LOD were assigned the LOD before further processing (“LOD-corrected normalized background-subtracted intensities”). Mean values, and standard deviations for each emission line in each sample were estimated for the five replicate measurements. Precision was reported as relative standard deviation (RSD), which is the standard deviation divided by the average value, converted to percent. Bias for each standard was reported as a percent deviation of the measured concentration of each element from the certified concentration values. Reproducibility for each duplicate run was reported as a percent difference in the measured concentration of each set of five replicates, for each emission line.

For each replicate measurement, “emission line ratios” were generated using an algorithm written in Mathematica (Wolfram Research, Champaign, IL, USA) by Dr. Barnett, in which the LOD-corrected normalized background-subtracted intensity of one emission line was divided by that of another (for example, Ba II 493.4 / Mg I 517.3). The best emission line ratios were selected on the basis of high discrimination power and low error rates using an algorithm written in Mathematica (Wolfram Research, Champaign, IL, USA). These emission line ratios were used in most statistical treatments for discrimination and provenancing purposes.

3.2.3. μ XRF

Parameters for μ XRF, such as live seconds acquisition time and beam current, were adjusted for adequate counting statistics for trace elements and maximum X-ray detector dead time, respectively. Four replicate measurements were performed at different locations on each sample. Specific instrument configurations and parameters are listed in Section 5.2.

Each file was imported into Excel (Microsoft Corporation, Redmond, WA, USA). Calibration curves were generated for each X-ray line from the calibration standards, and linear regression was used to calculate slopes and y-intercepts in order to calculate the concentrations for all samples. Limits of detection were calculated as outlined in Gilfrich & Birks [69], Ernst [70] and detailed in a proposed ASTM Standard Method [48], and converted to concentration using the slopes and y-intercepts as above. Mean values and standard deviations for each X-ray line in each sample were estimated for the five replicate measurements. Bias for each standard was reported as a percent deviation of the measured concentration of each element

from the certified concentration values. Reproducibility for each duplicate run was reported as a percent difference in the measured concentration of each set of five replicates, for each X-ray line.

3.3. Statistical Analysis

Processed data were imported into statistical software to perform various statistical tests, depending on the study, as specified in their respective chapters. Student's *t*-test, Q-test, Z-score, and Horowitz Trumpet calculations were conducted using Excel. ANOVA with Tukey's Post-Hoc test and LDA using jackknife (leave-one-out) cross-validation were conducted using Systat 11 (Systat Software, Inc., Chicago, IL, USA). Normality assessment, PCA, and LDA using jackknife cross-validation were conducted using JMP (SAS Institute, Inc., Cary, NC, USA). Reference materials were not included in the training sets for model building, but were included in the testing analysis as negative controls since they were known to be different. Confidence levels of 95 % were used for all statistical analyses unless otherwise stated.

3.4. Imaging & Dimensional Measurements

Low magnification imaging and measurement of ablation lines was performed using an EZ4D optical stereomicroscope (Leica Microsystems, Bannockburn, IL, USA). High magnification 3D imaging and measurement of depth and volume of ablation craters was performed using a VHX-1000 digital microscope (Keyence Corporation, Itasca, IL, USA). Specific measurement parameters used for the studies are detailed in their respective Sections.

Laser spot size was measured by irradiating laser alignment paper (Zap-It Corporation, Salisbury, NH, USA) with a beam attenuated below the breakdown threshold, and measuring the spot diameter with the optical stereomicroscope. This process was repeated nine times, and the mean of the spot diameters was determined. The fluence at the sample surface was estimated after correcting for a 4 % loss of laser energy for each lens surface through which it passed.

4. Development of Quantitative LIBS and LA-ICP-MS Methods

Soil and sediment standard reference materials were used to develop and optimize sample preparation protocols and instrumental parameters for both LIBS and LA-ICP-MS to obtain good performance. Parallel studies for the quantitative analysis of the elemental composition of soil specimens using LA-ICP-MS and LIBS on the same standards and soil samples, would permit the direct comparison of the analytical methods to determine the utility of each of the methods and the relative discrimination power of each of the methods. Statistical tests were employed and a preliminary element menu for discrimination was determined. Two soil sample sets were used to test the methods. This section consists of the development and re-optimization of quantitative methods for generation of elemental profiles of soil specimens by LIBS and LA-ICP-MS. These methods will be validated in Section 5 and used to generate elemental profiles for use in the discrimination studies in Section 6.

4.1. Development of a Preliminary LIBS Method: USEPA NEIC Specimens

4.1.1. Materials and Methods

The sample set included ten contaminated soil samples, taken at various locations around an automotive battery manufacturing facility by the USEPA NEIC [71]. They were initially analyzed for environmental contaminants (Pb, Sn, Sb) using KOH fusion followed by ICP-OES and HCl digestion followed by ICP-MS [71], and more recently LA-ICP-MS [72].

Emission lines from Harvard [73], Wiley [74], and NIST [75] databases, as well as the literature, were compiled into a searchable database using Access (Microsoft Corporation, Redmond, WA, USA). Since there are many emission lines of various elements in close proximity, and since soil is such a complex matrix, confirmation of the identity of each line was important. This was done by spiking soil with each element of interest to see which lines increased in intensity. The PACS-2 RM was used since it has certified concentrations for a large number of elements in an intermediate concentration range (contrasted with NIST SRM 2710, which contains highly elevated concentrations of many elements). Once emission lines were confirmed, Ni, U, Se, Ca, Ba, Sr, Mg, V, Pb, Sn, Sb, and Cr were spiked into multi-element calibration standards from 10 – 3000 ppm (Pb, Sn and Sb up to 10 000 ppm). Previous work by Arroyo et

al [24] used yttrium and germanium as the internal standard for LA-ICP-MS pellets; however these produced a large number of interfering emission lines in LIBS spectra. Scandium was used instead, at a final concentration of 1000 ppm in the pellets. Bulk soil, RM and calibration standard pellets were prepared as described in Sections 3.1.1, 3.1.4, and 3.1.6, with the following exceptions: scandium was the only internal standard, spiked at 1000 ppm, RMs included only PACS-2 and NIST SRM 2710, and milling time was only 20 min.

Elemental analysis of the pellets was conducted by LIBS as described in Section 3.2.2, with the following exceptions: delay time 1.25 μ s, gate width 15 μ s, focal depth 1.5 mm, three cleaning pulses followed by accumulation of 100 pulses per location, in an ambient air atmosphere. Emission lines monitored included Sc I 390.8 (internal standard), Ca I 634.9, Fe I 495.8, Li I 812.6, Mg I 516.7, Na I 819.5, Pb I 405.8, and Sr II 407.8 nm. Sufficiently sensitive and reproducible emission lines could not be found for Sn or Sb at such low concentrations. An example of the LIBS spectrum of specimen 103 is shown in Figure 7.

Figure 7. Accumulated LIBS spectrum for 100 laser pulses on NEIC specimen 103.

4.1.2. Results & Discussion

Calibration curves were linear for most emission lines, including Pb I 405.8nm (see Figure 8a). Figures of merit for lead are summarized in Table 1. Good accuracy and precision were obtained for Pb,

though the LOD was high at 235 ppm, which was above the certificate concentration of Pb in PACS-2. Results for Pb by LIBS correlated well with the other three methods (LA-ICP-MS, KOH fusion followed by ICP-OES, HCl digestion followed by ICP-MS, see Figure 8b-d, respectively).

Figure 8. Plots for analysis of Pb by LIBS.

a. calibration curve for Pb I 405.8 nm, b-d. correlation plots comparing LIBS results with those obtained from LA-ICP-MS, KOH fusion ICP-OES, and HCl digestion ICP-MS, respectively.

It should be noted that Sc has an emission line at 406.1 nm, which is near the most important emission line for lead (405.8 nm). Most of the Pb concentrations in these specimens were above those normally seen in uncontaminated soils, and were unaffected by the neighboring Sc peak, but the detection of Pb in PACS-2 was affected. The small cluster of points near the origin in Figure 8 was below the LOD. A concentration of 1000 ppm of Sc was determined to be the maximum for this study, and in the future, lower concentrations should be used. This should help to improve the LOD for Pb by reducing the tail of the neighboring Sc peak.

Table 1. Figures of merit for analysis of Pb by LIBS and LA-ICP-MS.

Reference Material (RM)	PACS-2	NIST 2710
Certificate Concentration $\pm$ 95% CI (ppm) ^a	183 $\pm$ 8	5532 $\pm$ 80
LIBS	Pb I 405.8 nm	
LOD (ppm)	235	
Experimental Mean $\pm$ SD (ppm) (n=15)	< LOD	5480 $\pm$ 145
% Error (Bias)	n/a	1
LA-ICP-MS [72]	208Pb	
LOD (ppm)	0.01	
Experimental Mean $\pm$ SD (ppm) (n=8)	178 $\pm$ 5	5705 $\pm$ 141
% Error (Bias)	3	3

a. Certified concentration, significant figures and 95% confidence interval (95% CI) as reported on RM certificate

4.1.3. Conclusions

A preliminary quantitative analytical method was successfully developed for elemental analysis of soil by LIBS. Unlike the soil analysis reported in the literature, this preliminary method used a 266 nm Nd:YAG laser. This provides a more direct comparison to LA-ICP-MS, which often uses 266 nm ablation, and offers an additional matrix to be analyzed using LIBS systems designed for forensic glass examination. More method optimization work is necessary to lower the limits of detection for key elements such as Pb and to further improve SNR and precision.

4.2. Optimization of Sample Preparation Methods: Miami-Dade Specimens

4.2.1. Miami-Dade Specimens

Specimens were collected at 14 locations throughout Miami-Dade County in undisturbed areas at least 100 m from any roads. Further information on these sites can be found in Table 2. The sampling scheme is summarized in Figure 9. Each site was defined as a transect 100 m long. Six sub-plots (1 m x 1 m) were defined within each site (see Figure 9b). Six specimens were taken within each sub-plot, and the GPS coordinates for the center of the sub-plot were recorded. Each specimen was taken at a depth of 5 cm using an auger (corer) (see Figure 9c for an example of the collection). Six agricultural soil types were defined in this region by the United States Department of Agriculture (USDA). At least two sites were sampled per agricultural soil type (see map, Figure 9a). Note: specimens were taken in conjunction with a microbial project led by Dr. Mills, so in some cases, there was not enough soil left over for elemental analyses. Back

in the laboratory, each sample was dried overnight at 50 °C. One specimen from each site was split into three sub-samples for assessment of within-sample variation.

Table 2. Miami-Dade County specimen sampling locations and descriptions (see map in Figure 9).

	Site Code	Approximate Location	Soil Type (USDA)	General Description	Legend
Miami-Dade County Specimens	CS1	Card Sound Rd, Florida City	4	Gray, fine, wetland
	FC1	Fairchild TBG, Coral Gables	4	Gray, fine, sandy
	FIU1	FIU Preserve, Sweetwater	1	Black, sandy, forest
	KK	Krome Ave, North of Kendall Dr	3	Brown, sandy,
	KS8	Krome Ave, South of 8th St	2	Gray, fine, shells
	NW137	NW137 Ave, North of Hwy 27	2	Black, sandy
	OSP1	Oleta River State Park, North Miami	1	Brown, beige, fine, sandy
	OSP2	Oleta River State Park, North Miami	1	Black, white, sandy
	TREC	UF TREC, Florida City	5	Red-brown, fine mulch
	USDA2	USDA AARS SHRS, Coral Gables	5	Gold, gray, sandy
	USDA3	USDA AARS SHRS, Coral Gables	6	Gray, fine
	CC6	Canal 6, South of Krome Ave	2	Gray, fine
RMs	2704	NIST		Buffalo River Sediment
	2710	NIST		Montana Soil
	PACS-2	NRC Canada		Marine Sediment Standard

Figure 9. Miami-Dade County soil sampling strategy.

a. Map of Miami-Dade County, divided into six USDA-defined soil types, with locations of sampled sites (see Table 2 for descriptions). b. the FIU1 site showing six sub-plots. c. one sub-plot showing collection of six specimens collected using the auger.

4.2.2. Internal Standard

As discussed in Section 4.1.2, the 1000 ppm of Sc had to be reduced because of the close proximity of the LIBS Sc 406.1 nm peak to the Pb I 405.8 nm peak. In addition, LA-ICP-MS cps for Sc at 1000 ppm were much higher than most trace elements. Concentrations from 350 to 1000 ppm were tested. Addition of a second internal standard is recommended in case of unexpected interferences (see Section 2.3.4). Lutetium was suggested as a potential candidate, given that in LIBS it emits reasonably strongly and does not have many interferences [76]. Concentrations from 350 to 10 000 ppm were investigated. The 10 000 ppm spike proved to be too high in that Lu interfered with some emission lines in LIBS and the resulting

intensity was well above the trace and minor elements. Concentrations under 1000 did not result in high enough intensities in LA-ICP-MS (see Section 4.4). The optimal concentrations were determined to be 350 ppm for Sc and 1000 ppm for Lu, providing good accuracy and precision for both LA-ICP-MS and LIBS. Both were added to the Miami-Dade specimens, RMs and calibration standards.

4.2.3. Sieving Studies

In Section 6.3, sieved soil will be used in order to mimic the type of soil evidence encountered in cases where transfer occurs. As discussed in Section 2.3.2.1, particle size may affect the elemental composition, whether because of increased surface area, or the type minerals that are commonly found in smaller grain sizes. A study was conducted in order to determine the effects of sieving on the elemental composition of Miami-Dade County soils. The elemental compositions of sieved pellets were compared to those of bulk pellets from FIU1, FBG, USDA1, CC6, CS1, KK, TREC, and KS8 in two phases.

Phase I involved analysis of triplicate samples from FIU1,1,2f (FIU1, sub-plot 2, specimen f). Sieved pellets and bulk pellets were prepared from each of the three samples as described in Section 3.1 using only 350 ppm Sc as the internal standard. Analysis by LA-ICP-MS was performed as described in Section 3.2.1. A two-tailed Student's t-test at the 95 % confidence level using unequal variances was used to perform pair-wise comparisons for multiple elements; each sieved pellet was compared to each bulk pellet. Results are shown in Table 3. Differences between sieved and bulk pellets were seen in most of the elements: Mg, Al, Si, P, Ca, Ti, V, Mn, Fe, Cu, Sr, Ba, and U in all cases; and Li, Cr, Ni, Zn, As, Zr, Sn, Sb, Hg, Tl, and Th in some cases. Only Sb and Pb showed no difference in any of the comparisons.

Phase II involved analysis and comparison of one sieved and one bulk pellet from the same specimen of each of the remaining sites (FBG, USDA1, CC6, CS1, KK, TREC, and KS8). The FIU1 data from all three sieved pellets were combined and compared with combined data from all three bulk pellets. Results for the eight sites are shown in Table 4. Results differed greatly from site to site. Only Ti showed a difference in all of the comparisons.

Table 3. Phase I comparison of bulk and sieved pellets from FIU1,1,2f.

Two-tailed Student's t-test with unequal variances was used to calculate p-values for each isotope (Iso). Differences (diff) occurred when $p > 0.05$ (95 % confidence level).

Iso	Bulk1 versus Sieved1		Bulk1 versus Sieved2		Bulk1 versus Sieved3		Bulk2 versus Sieved2		Bulk2 versus Sieved3		Bulk3 versus Sieved3	
	p	diff	p	diff	p	diff	p	diff	p	diff	p	diff
⁷ Li	0.001	diff	0.015	diff	0.002	diff	0.065	no	0.026	diff	0.000	diff
²⁵ Mg	0.000	diff	0.002	diff	0.000	diff	0.002	diff	0.000	diff	0.000	diff
²⁷ Al	0.000	diff	0.002	diff	0.000	diff	0.003	diff	0.000	diff	0.000	diff
²⁹ Si	0.000	diff	0.000	diff	0.000	diff	0.017	diff	0.029	diff	0.000	diff
³¹ P	0.000	diff	0.000	diff	0.001	diff	0.013	diff	0.005	diff	0.007	diff
⁴² Ca	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
⁴⁷ Ti	0.000	diff	0.001	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
⁴⁹ Ti	0.000	diff	0.001	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
⁵¹ V	0.000	diff	0.000	diff	0.000	diff	0.001	diff	0.001	diff	0.000	diff
⁵² Cr	0.577	no	0.483	no	0.798	no	0.690	no	0.883	no	0.040	diff
⁵³ Cr	0.549	no	0.425	no	0.722	no	0.610	no	0.980	no	0.033	diff
⁵⁵ Mn	0.001	diff	0.026	diff	0.008	diff	0.013	diff	0.012	diff	0.019	diff
⁵⁷ Fe	0.000	diff	0.001	diff	0.000	diff	0.015	diff	0.004	diff	0.000	diff
⁶⁰ Ni	0.193	no	0.145	no	0.234	no	0.201	no	0.335	no	0.013	diff
⁶³ Cu	0.010	diff	0.007	diff	0.008	diff	0.016	diff	0.016	diff	0.006	diff
⁶⁶ Zn	0.003	diff	0.000	diff	0.000	diff	0.011	diff	0.010	diff	0.070	no
⁷⁵ As	0.053	no	0.778	no	0.175	no	0.074	no	0.011	diff	0.026	diff
⁸⁸ Sr	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
⁹¹ Zr	0.106	no	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
¹²⁰ Sn	0.150	no	0.043	diff	0.368	no	0.069	no	0.352	no	0.080	no
¹²³ Sb	0.153	no	0.809	no	0.643	no	0.388	no	0.334	no	0.249	no
¹³⁷ Ba	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff	0.000	diff
¹⁷⁶ Lu	0.019	diff	0.335	no	0.743	no	0.008	diff	0.027	diff	0.069	no
²⁰² Hg	0.136	no	0.003	diff	0.129	no	0.005	diff	0.500	no	0.344	no
²⁰⁵ Tl	0.036	diff	0.048	diff	0.014	diff	0.416	no	0.669	no	0.073	no
²⁰⁶ Pb	0.680	no	0.103	no	0.174	no	0.821	no	0.541	no	0.214	no
²⁰⁷ Pb	0.701	no	0.182	no	0.493	no	0.565	no	0.186	no	0.107	no
²⁰⁸ Pb	0.196	no	0.097	no	0.127	no	0.783	no	0.590	no	0.213	no
²³² Th	0.010	diff	0.000	diff	0.636	no	0.026	diff	0.047	diff	0.057	no
²³⁸ U	0.000	diff	0.003	diff	0.001	diff	0.004	diff	0.008	diff	0.005	diff

Table 4. Phase II comparison of bulk and sieved pellets from Miami-Dade County. Two-tailed Student's t-test with unequal variances was used to calculate p-values for each isotope. Differences (diff) occurred when $p > 0.05$ (95 % confidence level).

	FBG 1,1,2f	USDA 1,1,6d	CC6 1,1,1b	CS1 1,1,1c	KK 1,1,5a	TREC 1,1,6c	KS8 1,1,6a	FIU1 (Phase I)
Isotope	diff?	diff?	diff?	diff?	diff?	diff?	diff?	diff?
⁷ Li	no	diff	no	no	no	diff	diff	diff
²⁵ Mg	diff	diff	no	no	no	diff	no	diff
²⁷ Al	diff	diff	no	no	no	diff	diff	diff
²⁹ Si	diff	diff	no	no	no	diff	diff	diff
³¹ P	diff	diff	no	no	no	diff	diff	diff
⁴² Ca	diff	diff	no	no	no	diff	no	diff
⁴⁷ Ti	diff	diff	diff	diff	diff	diff	diff	diff
⁴⁹ Ti	diff	diff	diff	diff	diff	diff	diff	diff
⁵¹ V	diff	diff	no	diff	diff	diff	diff	diff
⁵² Cr	diff	no	no	no	no	diff	diff	no
⁵⁵ Mn	diff	diff	no	no	no	diff	diff	diff
⁵⁷ Fe	diff	diff	no	no	no	diff	diff	diff
⁶⁰ Ni	diff	no	diff	diff	no	diff	diff	diff
⁶³ Cu	no	no	no	diff	diff	diff	no	diff
⁶⁶ Zn	no	diff	no	diff	no	diff	diff	diff
⁷⁵ As	diff	diff	no	no	no	diff	diff	diff
⁸⁸ Sr	diff	diff	diff	no	no	no	diff	diff
⁹¹ Zr	diff	diff	diff	no	diff	diff	diff	diff
¹²⁰ Sn	no	diff	diff	no	diff	diff	diff	no
¹²³ Sb	no	diff	diff	no	no	diff	no	diff
¹³⁷ Ba	diff	diff	diff	no	diff	diff	diff	diff
¹⁷⁶ Lu	diff	diff	diff	diff	diff	diff	diff	diff
²⁰² Hg	diff	diff	diff	diff	diff	no	diff	diff
²⁰⁵ Tl	diff	diff	no	diff	no	diff	diff	no
²⁰⁶ Pb	diff	diff	no	diff	diff	diff	diff	no
²⁰⁷ Pb	diff	diff	no	diff	diff	diff	diff	no
²⁰⁸ Pb	diff	diff	no	diff	diff	diff	diff	no
²³² Th	diff	diff	no	diff	no	diff	diff	no
²³⁸ U	diff	diff	diff	diff	no	diff	no	diff

Sample preparation was nearly identical for both sieved and bulk pellets, with only two exceptions. The first was the milling time. Sieved pellets were milled for 20 minutes, while bulk pellets were milled for 30 minutes. This was done to reduce particle size in bulk samples to be comparable to those in sieved

samples milled for a shorter time (see Section 4.2.4). It is unlikely that this affected the elemental composition, but to be safe, elements found in the milling jars that could be possible contaminants (W, C and Co) were not used in this study. Elements near the MDL were also not used. The other difference was the sieving itself. All materials used were plastic, including the mesh screen, sieving cup, and receiving tubes (see Figure 3). A fresh piece of mesh and new tubes were used for each specimen. Funnels and mesh-holders were washed thoroughly between uses; therefore no contribution to trace elements was expected. All pellets were analyzed on the same day.

During this investigation, it was observed that pellets prepared from sieved soil were stronger, more uniform, and produced more defined craters with less cracking. Data obtained from the sieved pellets were also of better quality, with better precision and SNR.

In conclusion, the particle size of the specimens to be compared does have an effect on the elemental composition, even from the same location. It is therefore very important to compare specimens of the same particle size fraction when performing forensic soil examinations using elemental analysis. Bulk evidence specimens should be compared to bulk known specimens, and transfer evidence specimens should be compared to sieved known specimens. The improved performance of the sieved pellets indicated that the particle size of the milled powder needed to be further reduced in the milling of bulk samples, especially for LIBS.

4.2.4. Milling Studies

The initial milling protocol of 20 minutes at 25 Hz was taken from Arroyo et al. [24], which was optimized for LA-ICP-MS analysis. Because of the characteristics of the laser and focusing optics, the beam quality in the LA system was much better than in the home-built LIBS system. In addition, the laser energy in the LIBS system was approximately ten times higher (29 mJ per pulse, compared to 3 mJ with LA). Even though the same fundamental principles apply, the LIBS plasma conditions and resulting shockwave are more intense, and require a more robust pellet to withstand the ablation without cracking or spallation.

The goal was to obtain optimal pellet quality in the shortest amount of time. Pellet quality was determined by visual examination before and after ablation, and by analytical precision of the LIBS and

LA-ICP-MS measurements (RSDs). A milling study were undertaken, in which Miami-Dade samples were milled at 20, 30, and 45 min, and 25 and 30 Hz and pressed into pellets. No difference in SNR was observed for any of the milling methods. Results for precision are shown in Figure 10. No difference in pellet quality or LIBS precision was observed between 25 Hz and 30 Hz (green versus purple in the Figure). 30 minutes (green) provided a significant improvement in precision over 20 minutes (red), but 45 minutes (orange) did not provide an additional improvement over all (precision for some emission lines improved, while others suffered). Precision of the 30 minute milling time for the bulk pellets (green) was very similar to that of the sieved pellets (blue). Optimal pellet quality was achieved for milling times of 30 minutes or more, which was similar to that of the sieved pellets. Therefore, 30 minutes at 25 Hz (green) was deemed optimal and used for all subsequent bulk specimens, calibration standards and RMs.

Figure 10. Evaluation of the LIBS precision (RSD) obtained on pellets prepared using five different milling methods for various emission lines.

“Current method” refers to the original method by Arroyo et al [24] before it was re-optimized.

4.2.5. Miami-Dade County Bulk Pellets, Sieved Pellets, and Sieved Soil for Use in Further Studies

On the basis of the above studies, a final sample preparation protocol was determined and used on the Miami-Dade County specimens to prepare bulk pellets, sieved pellets, and sieved soil for use in further studies. Calibration standards were spiked with internal standard and then with Ba, Ca, Cr, Cu, Fe, Li, Mg,

Mn, Pb, Sr, Ti, and V at concentrations ranging from 10 - 1200 ppm, and up to 7000 ppm for matrix elements (Ca, Fe, Mg, Ti) and those present at high concentrations in some RMs (Cu, Pb). Representative 0.45 g samples of bulk soil from all Miami-Dade specimens, as well as the calibration standards and RMs, were spiked, milled, and pelletized as described in Sections 3.1.4 and 3.1.6, using Sc (350 ppm) and Lu (1000 ppm) as the internal standards. These will be referred to as “bulk pellets”. Note: samples from CS1 and FIU1, as well as the RMs and calibrators used for these studies, were prepared prior to the milling study (Section 4.2.4); therefore were only milled for 20 minutes. All other bulk specimens were milled for 30 minutes.

The remaining soil, from each Miami-Dade specimen was sieved to less than 150 μm as described in Section 3.1.2. The < 150 μm fraction will be referred to as “sieved soil”. The number of sieved samples was reduced because some specimens did not have enough soil left over after other analyses, or the specimens were too coarse and did not yield enough of the < 150 μm fraction.

Of the sieved soil specimens, 0.45 g samples from CC6, HA, KNT, FIU1, FBG, USDA1, KK, TREC and KS8 were spiked, milled, and pelletized as described in Sections 3.1.4 and 3.1.6, using Sc (350 ppm) and Lu (1000 ppm) as the internal standards, but were milled for only 20 minutes. These will be referred to as “sieved pellets”.

4.3. Re-Optimization of the LIBS Method

Calibration pellets and RM pellets described in Section 4.2.5 were used to determine figures of merit for optimization of the quantitative LIBS method.

4.3.1. LIBS Parameters

Various emission lines were monitored in order to optimize the LIBS method by changing the following parameters while analyzing soil RMs:

- Gate width (from 3.5 to 500 μs)
- Gate delay (from 1 to 50 μs)
- Focal depth (from 0 mm at the surface to 1.7 mm into the sample)
- Number of pulses to accumulate per replicate (from 25 to 100)

- Ablation atmosphere (air or argon from 265 to 1000 mL/min)
- Ablation mode (spot mode with pre-ablation pulses or line scan at step distances of 0.01 to 0.05 mm per pulse)

The criteria used in optimization of experimental parameters were: high signal to noise ratio (SNR), high precision (measured as low RSD), high accuracy (measured as low bias), as well as linearity of calibration plots (measured as R^2) for all emission lines in the element menu. Below is a discussion of the choice of parameters.

One advantage of LIBS is that it can be done in an ambient atmosphere. However oxygen radicals from air can quench the plasma and shorten the overall lifetime. An argon atmosphere promotes ionization and prolongs the life of the plasma, which should enhance the integrated signal. This was observed by a marked increase in SNR for most emission lines when argon was used. The optimal flow rate was determined to be 900 - 1000 mL/min.

The number of laser pulses accumulated per replicate should be sufficient to improve SNR while minimizing analysis time and damage to the sample. For spot mode, the more pulses, the deeper the crater, which can reduce in the amount of light reaching the detector, and may drill right through the bottom of the pellet to the sample stage below. In addition, as the crater deepens, the “surface” retreats from the optimal focal depth, resulting in a reduction in the laser coupling efficiency. For hard, smooth, transparent matrices, such as glass, a number of laser pulses required to obtain an optimal coupling is much larger, and often the first 25 or 50 pulses are discarded. This is less of an issue with soils, but performance for the second 25 or 50 pulses was still observed to be better than the first. In line mode, the more pulses, the longer the line, which reduces the surface area available for subsequent analyses. The optimal number of pulses, by far, was 75 pulses, followed by 50, 25 and then 100 pulses (see Figure 11a). Because the repetition rate of the laser was limited to 0.667 Hz because of the duty cycle of the ICCD detector (the time required to collect the data for a full spectrum, process it into a digital file, and reset the detector for the next pulse), 75 pulses resulted in an analysis time of just under two minutes per replicate measurement.

Longer integration times result in increased signal intensity, but may also result in increased noise since the plasma and emissions decay over time. Since different species emit optimally in different temporal regions of the plasma, two distributions were observed in that some emission lines seemed to have optimal SNRs and RSDs in the early part of the plasma (5 μ s) while other emission lines favored the longer integration times of 150 μ s (see Figure 11b). The performance at intermediate integration times was poor. Since most of the emission lines were neutral and performed optimally at 150 μ s, including some of the more difficult lines, such as Pb I 405.8, an integration time of 150 μ s was chosen.

Long delay times would remove the continuum and even longer delays would favor neutral over ionic emission. However, the longer the delay, the lower the overall emission intensity. Optimization is a delicate balance between increased signal and reduced background. The optimal delay times were 1.5, followed by 1.25, 2.0 and 15 μ s, which were all approximately even (see Figure 11c). Interestingly some trace emission lines, for example Cu 324.7, Cr 360.5, Pb 368.3, and Na 819.5 nm, began to appear only after 2.0 μ s, when interferences peaks (presumably ionic species or continuum emission) disappeared. The performance of the other peaks was not significantly diminished by using 2.0 rather than 1.5, so 2.0 μ s was chosen as the delay time.

Focal depth in the sample is important in terms of the quality of the laser-sample interaction and spot size. When the laser is focused at the surface (a depth of 0 mm), the spot size is at its minimum and the energy density at its maximum, but in general the laser-sample coupling is poor because of a reduction in the area of the sample that is irradiated. The deeper the focal depth, the larger the spot size and the better the coupling, but there is a limit where the spot becomes too large and the resulting energy density becomes too low. Depths of 1.3 mm and 1.5 mm into the sample surface were nearly equally optimal (see Figure 11d), therefore 1.4 mm was chosen.

Figure 11. Histograms for optimization of LIBS parameters. a. number of pulses to accumulate per replicate spectrum, b. integration time, c. delay time, d. focal depth. Frequency of optimal occurrence calculated by counting the number of emission lines experiencing maximal SNR or minimal RSD at that particular parameter value.

Spot mode was initially chosen for its simplicity of implementation, and ability for more analyses per pellet. Surface contamination from pellet preparation and handling was removed by three pre-analysis cleaning pulses to remove the surface layer of the pellet and improve laser-to-sample coupling. Different ablation modes were considered to address possible heterogeneity within a pellet. In theory, if the particle size after milling is sufficiently small with respect to the laser spot size and if the powder pressed into a pellet is sufficiently well-mixed, this should not be an issue. Line mode using 0.010 and 0.025 mm steps (distance between each pulse) showed no improvement in SNR, but gave consistently worse RSDs than the 0.050 mm step (see Figure 12). Line lengths of 0.75 mm, 1.88 mm, and 3.75 mm were obtained for 75 shots with the 0.010 mm, 0.025 mm, and 0.050 mm steps, respectively. Larger steps were not evaluated since the resulting line lengths would severely limit the number of replicate analyses that could be done on

a single pellet. No appreciable difference in SNRs or RSDs was seen between line mode using 0.05 mm step (orange) and spot mode with three pre-analysis cleaning shots (blue), suggesting that the horizontal variation was no greater than the vertical variation in a pellet pressed with milled powder, and that spot mode was sufficient to account for within-pellet heterogeneity.

Figure 12. Evaluation of the precision (RSD) obtained for spot mode and line scan mode (raster) at various speeds for analysis of pellets by LIBS.

4.3.2. LIBS Data Analysis

As discussed in Section 2.5, background-subtraction and normalization is necessary in order to be able to compare spectra qualitatively or achieve quantitative results. The background signal was determined as the average intensity of a background region adjacent to the peak of interest (see Figure 6). It was important to verify that the background region of each emission line was indeed blank for each specimen, as one never knows what elements will be present in a soil specimen that could cause peaks to appear in this region. The background signal was subtracted from the intensity of the peak to obtain “background-subtracted intensity”.

Two normalization methods were investigated, and compared to non-normalized data to determine the optimal method. For background-subtracted intensities with no normalization (see Figure 13a), although R^2 was 0.95, linearity was quite poor. Bias for NIST SRM 2710 (green points, circled) was also poor. The first

normalization method was to divide the signal intensity by the mean intensity of the entire spectrum (total plasma light). Figure 13b shows a slight improvement in linearity and a large improvement in bias. The second method involved calculating the ratio of the background-subtracted intensity of each peak of interest over the background-subtracted intensity of an internal standard (scandium) added to the specimen (see Figure 13c). Linearity and bias were excellent. Although only data for Pb are shown, these normalization methods were tested for all elements. Non-normalized background-subtracted intensities were satisfactory for some emission lines (Li I 610.4 and Li I 670.7 nm), but gave poor results for all other emission lines. Total plasma light normalization provided some improvement over non-normalized data but still was not sufficient over all. Normalization with the internal standard (Sc) provided the best results in terms of linearity and bias for most emission lines. Scandium was ultimately chosen as the primary internal standard because of its higher sensitivity and slightly improved analytical performance over lutetium. For LIBS, four Sc and two Lu emission lines were monitored: Sc I/II 327.0, Sc II 361.4, Sc I 390.7, Sc I 402.0, Lu 339.7, and Lu 547.7 nm. The Sc I 390.8 and Sc II 361.4 nm lines provided the best bias, LODs and RSDs, but Sc II 361.4 nm was ultimately chosen because of improved sensitivity and linearity of normalized calibration curves.

Figure 13. Normalization strategies applied to background-subtracted LIBS emission line intensities. a. no normalization, b. normalization to the mean intensity of the entire spectrum, c. normalization to the internal standard (scandium).

Calibration curves were prepared as described in 3.2.2.2. Some examples of calibration curves are shown in Figure 14 (Ba II 493.4, Cr I 425.4, Pb I 405.8, Sr II 421.6 nm). The RMs were plotted to determine the accuracy of the calibration curves. Correlation coefficients (R^2) were greater than 0.90 for all monitored emission lines, with most near 0.99. The upper calibration limits (see Table 5, column 2) represent the concentration of the highest calibration standard that provided a linear curve containing at least three points. For some of the major elements (Ca, Fe, Mg, Ti), linearity continued beyond this range, even as far as the concentrations present in NIST SRM 2704, with good bias (see Table 5, column 4). Some emission lines are very strong emitters and are therefore very sensitive at trace concentrations, but prone to self-absorption. For example, Li I 670.7 and Ba II 493.4 nm upper calibration limits were quite low at 77

ppm and 307 ppm, respectively, so Li I 610.4 and Ba II 614.2 nm were also monitored for concentrations up to approximately 1200 ppm.

Figure 14. Examples of LIBS calibration curves for some emission lines of Ba, Cr, Pb, and Sr. Scandium-normalized background-subtracted intensity data were used. RMs were plotted to show the bias. Vertical error bars represent one standard deviation of the measured values over five replicates. Horizontal error bars on the SMs represent the uncertainty of the certified values listed on the certificate. In many cases, error bars cannot be seen over the data points because of the high precision.

The choice of emission lines to monitor was on the basis of the following criteria: high precision and SNR, linear response over the concentration range of interest (no self-absorption), lack of interfering peaks, and existence of a reliable nearby background region. The identity of the emission lines were confirmed as described in Section 4.1.1. The following emission lines were monitored: Ba II 493.4, Ba II 614.2, Ca I 643.9, Cr I 360.5, Cr I 425.4, Cu I 324.8, Fe I 360.9, Fe I 495.8, Li I 610.4, Li I 670.7, Mg I 517.3, Mg I 518.4, Mn I 404.1, Pb I 405.8, Sr II 407.8, Sr II 421.6, Ti I 336.1, and V I 437.9 nm. A summary of the figures of merit for these emission lines is given in Table 5.

Table 5. LIBS figures of merit for emission lines monitored.
Data were background-subtracted and normalized to Sc II 361.4 nm before conversion to concentrations using linear regression.

Emission Line (nm)	Upper Cal. Limit (ppm)	Precision (RSD %)	NIST SRM 2704 Certified Conc. (ppm)	Measured Conc. (ppm) ^b	Bias (%) ^c	LOD (ppm)	Literature LOD (ppm) ^f
Ba II 493.4	307	5	414 ± 12	487 ± 31	18	6.7	3 [77] - 296 [78]
Ba II 614.2	1185	6	414 ± 12	429 ± 27	4	5.7	3 [77] - 296 [78]
Ca I 643.9	7287	8	26000 ± 300	28960 ± 2423	11 ^d	166	12 [79] - 14 [77]
Cr I 360.5	1183	6	135 ± 5	439 ± 31	227	11	2 [77] - 54 [80]
Cr I 425.4	1183	7	135 ± 5	266 ± 26	99	33	2 [77] - 54 [80]
Cu I 324.8	1177	7	98.6 ± 5.0	84 ± 20	-15	14	3.3 [81] - 80 [82]
Fe I 360.9	7512	5	41100 ± 1000	39732 ± 2513	-4 ^d	475	7 [79] - 500 [83]
Fe I 495.8	7512	6	41100 ± 1000	71857 ± 3225	74 ^d	1377	7 [79] - 500 [83]
Li I 610.4	1178	8	~50 ^a	464 ± 36	n/a ^e	0.7	n/a
Li I 670.7	77	10	~50 ^a	116 ± 15	n/a ^e	2.7	n/a
Mg I 517.3	7069	5	12000 ± 200	10784 ± 537	-10 ^d	30	9 [77-79]
Mg I 518.4	7069	5	12000 ± 200	9877 ± 611	-18 ^d	9.9	9 [77-79]
Mn I 404.1	1185	7	555 ± 19	771 ± 53	38	11	55 [84] - 100 [83]
Pb I 405.8	1178	15	161 ± 17	216 ± 31	34	19	10 [85] - 298 [32]
Sr II 407.8	1181	6	~130 ^a	260 ± 19	n/a ^e	8.3	7 [77] - 52 [78]
Sr II 421.6	1181	4	~130 ^a	220 ± 11	n/a ^e	1.8	7 [77] - 52 [78]
Ti I 336.1	1242	4	4570 ± 180	3601 ± 210	-21 ^d	146	6 [77]
V I 437.9	1191	7	95 ± 4	237 ± 18	157	28	2 [77]

^a Informational (approximate) values only (not certified by NIST)

^b One standard deviation is reported

^c Bias was calculated as the percent error between measured and certified concentrations of NIST SRM 2704

^d Extrapolated (outside calibration range)

^e Not calculated due to lack of certified values

^f Numbers in parentheses indicate the literature source

Limits of detection were consistent with the lower values reported in the literature (see Table 5, column 7). Most were below 33 ppm, which is two to three orders of magnitude above the MDLs achieved with LA-ICP-MS (see Table 6 in Section 4.4), but all were well below the NIST SRM 2704 certified concentrations so LIBS is sufficiently sensitive enough. The higher LODs for major elements Ca, Fe and Ti

are the result of less sensitive emission lines being used to avoid self-absorption, and do not represent the lowest possible LODs achievable for those elements under these experimental conditions. Lower LODs could be obtained if necessary by using more sensitive emission lines.

Very good precision was achieved; all elements except for Pb had RSDs of 10 % or less. Lead is a poor emitter, even using its strongest emission line (Pb I 405.8). Bias was good overall; with most emission lines exhibiting a bias of less than 38 % (see Table 5, column 6). Chromium exhibited a poor bias for NIST SRM 2704, but as can be seen from the calibration curve (see Figure 14b), the bias was quite good for the other two RMs. Vanadium was not included in the discrimination studies (see Section 6). Concentrations of Li and Sr were not certified, but an informational value was provided. Another possible source of the high bias for some elements may be that sand is not a perfect matrix match for soil, especially soils that are not silica-based. For discrimination, precision is the more important the bias, and since the precision of LIBS is similar to that of LA-ICP-MS (see Table 6 in Section 4.4), LIBS has the potential to be very useful in discrimination studies.

4.4. Re-Optimization of the LA-ICP-MS Method

Calibration pellets and RM pellets described in Section 4.2.5 were used to determine figures of merit for re-optimization of the quantitative LA-ICP-MS method.

A LA-ICP-MS method was developed by Arroyo et al. [24]. Only a few modifications were required. The calibrator used by Arroyo et al was NIST SRM 2710, which has levels of contaminants, including lead, arsenic, copper, manganese, and zinc, that are highly elevated over baseline soil concentrations. Since NIST SRM 2704 was deemed to be more representative of normal soils, it was used instead. As discussed in Section 4.1.1, Ge and Y were not useful internal standards for LIBS, so Sc and Lu were investigated for use as alternatives. The only isotope of scandium is ^{45}Sc . Possible interferences may include $^{28}\text{Si}^{16}\text{O}^1\text{H}$, $^{29}\text{Si}^{16}\text{O}$, and $^{13}\text{C}^{16}\text{O}_2$, but as mentioned in Section 2.4.2.3, oxides are predominantly seen in solution ICP-MS, and are far less prevalent with LA. For lutetium, ^{176}Lu was used, even though it was not the most abundant isotope (only 2.6 %), since ^{175}Lu has possible interferences from Yb, Hf, and TbO. Scandium was chosen for normalizing the data since it provided slightly better bias over data normalized with Lu. Lutetium was retained as a backup internal standard. Isotopes to monitor were chosen for high relative

abundance (except for the matrix elements) and minimal interferences. The following isotopes were monitored: ^{27}Al , ^{75}As , ^{137}Ba , ^{42}Ca , ^{111}Cd , ^{59}Co , ^{63}Cu , ^{57}Fe , ^{202}Hg , ^7Li , ^{25}Mg , ^{55}Mn , ^{60}Ni , ^{31}P , $^{206,207,208}\text{Pb}$, ^{123}Sb , ^{45}Sc , ^{82}Se , ^{29}Si , ^{120}Sn , ^{88}Sr , ^{232}Th , $^{47,49}\text{Ti}$, ^{205}Tl , ^{238}U , ^{51}V , ^{89}Y , ^{66}Zn , and ^{91}Zr , as these elements were listed in the NIST SRM 2704 certificate. A summary of the figures of merit for these isotopes is given in Table 6.

Table 6. LA-ICP-MS figures of merit for isotopes monitored, using MDL-corrected concentrations obtained using ^{45}Sc as internal standard and NIST SRM 2704 as calibrator.

Isotope	Precision (RSD %)	NIST SRM 2710 Certified Conc. (ppm)	Measured Conc. (ppm) ^b	Bias (%) ^c	MDL (ppm)
^{27}Al	3	64400 ± 800	63426 ± 3217	2	0.04
^{137}Ba	2	707 ± 51	666 ± 64	6	0.01
^{42}Ca	2	12500 ± 300	11170 ± 688	11	4
^{63}Cu	9	2950 ± 130	1540 ± 216	48	0.01
^{57}Fe	6	33800 ± 1000	33383 ± 2212	1	0.3
^7Li	7	n/a ^d	47.8	n/a ^d	0.02
^{25}Mg	3	8530 ± 420	8788 ± 455	3	0.06
^{55}Mn	6	10100 ± 400	9740 ± 636	4	0.02
$^{206,207,208}\text{Pb}$	8	5532 ± 80	13263	140	0.004
^{88}Sr	3	330 ^a	311 ± 24	6	0.003
$^{47,49}\text{Ti}$	4	2830 ± 100	2809 ± 74	1	0.3
^{91}Zr	14	n/a ^d	173 ± 60	n/a ^d	0.03
^{51}V	5	76.6 ± 2.3	72.4 ± 7.0	6	0.01
^{238}U	6	25 ^a	24.3 ± 2.2	3	0.001

a. Informational (approximate) values only (not certified by NIST)

b. One standard deviation is reported

c. Bias was calculated as the percent error between measured and certified concentrations of NIST SRM 2710

d. Not calculated due to lack of certified values

Bias was 11 % or better for most elements. Note that Cu and Pb were highly elevated in NIST SRM 2710 compared to normal soil levels, and the levels in the calibrator (NIST SRM 2704), which may explain the high bias for these elements. Detection limits and precision were excellent; more than sufficient for use in discrimination. High RSDs were seen with Zr, which is susceptible to the nugget effect since it is often found in very hard minerals that may not be fully milled.

As described in Section 3.2.1.2, the single-point calibration obtained with the GLITTER software was verified by generating a series of calibration curves for a sub-set of the element menu. Linearity was very

good for all calibrated elements (see Figure 15 for examples of Sr, Ba, Cu, and Pb). Using linear regression to calculate the concentrations on the basis of the normalized background-subtracted cps, bias and precision compared well to the bias and precision obtained using the single-point calibration.

Figure 15. Examples of LA-ICP-MS calibration curves using Sc-normalized background-subtracted cps data for a. Ba, b. Cr, c. Pb and d. Sr.

4.5. Conclusions

Sample preparation methods for preparation of homogeneous sieved and bulk pellets have been optimized for use in LIBS and LA-ICP-MS analysis. Quantitative multi-element methods of analysis have been developed for both LIBS and LA-ICP-MS. Detection limits on the order of 10 ppm were obtained for LIBS and 10 ppb for LA-ICP-MS. Precisions between 4 and 15 % RSD were obtained for LIBS and between 2 and 9 % for most elements in LA-ICP-MS. While LA-ICP-MS provided higher precision and lower detection limits, the figures of merit for LIBS were still quite good. Therefore LIBS should be capable of generating elemental profiles of sufficient quality for use in forensic discriminations (see Section 6) at much lower purchasing and operating costs compared to LA-ICP-MS. As many elements and

emission lines should be measured as possible. Methods should be validated for use in forensic laboratories (see Section 5).

5. Method Validation: Inter-Laboratory Comparison

Once the methods were developed and optimized (see Section 4), the next step was to test and validate their robustness and utility in the forensic community. An inter-laboratory comparison exercise was designed and carried out for LA-ICP-MS, LIBS, and μ XRF [86]. Micro XRF was added because it is currently available in more forensic laboratories than the other two techniques, and is currently being used for elemental analysis of other matrices, such as glass.

A test kit was submitted to each of nine participating laboratories at local, state, and federal crime labs in the USA, Canada, and Germany. Participants were members of the Elemental Analysis Working Group (EAWG), a National Institute of Justice-funded effort consisting of crime laboratories at the USA local, state, and federal levels, as well as international laboratories. Participants were asked to analyze the specimens according to the methods provided, using the equipment available to them in their laboratories, and to report their results in order to compare the analytical performance of μ XRF, LIBS and LA-ICP-MS methods between laboratories. Data were compiled, possible issues with the methods were addressed, and the applicability of LIBS as a low-cost alternative is discussed [86].

5.1. Test Kits

Each test kit contained the following:

- Analysis and reporting instructions.
- Pressed pellets, individually packaged and labeled, of the following specimens: PACS-2, NIST SRM 2710, NIST SRM 2710a, and NIST SRM 2704 (see Section 3.1.1 for descriptions). For LIBS and μ XRF, four additional in-house calibrator pellets (Cal-1 through Cal-4) were included.
- A list of concentrations of scandium internal standard, as well as each element in the calibrator pellets (see Table 7).

Pellets were prepared as described in Sections 3.1.4 and 3.1.6, with the following exceptions: to ensure homogeneity between pellets, each specimen and calibrator was spiked and homogenized in a large batch containing enough material for all laboratories, and then split into 0.5 – 0.6 g sub-samples, each of which was pressed into an individual pellet. Larger masses (compared with 0.45 g) were used to create

thicker pellets that would withstand shipping and ablation with different laser systems without fear of boring right through the pellets.

Table 7. Concentrations provided in each test kit, reported in ppm ($\mu\text{g/g}$).

Element	PACS-2	NIST2704	NIST2710	NIST2710a
Sc ^a	349.5	373.5	358.2	369.3
Element	Cal-1	Cal-2	Cal-3	Cal-4
Ba	84.7	986	10.0	511
Cr	30.3	84.8	159	407
Cu	51.2	994	497	3472
Fe	866	1559	7306	368
Li	12.1	26.9	101	2.1
Mg	562	1255	63.3	7005
Mn	306	1000	2981	7.0
Pb	103	599	2977	3.2
Sr	79.5	981	509	4.7
Ti	560	1054	61.0	3018
Zr	126	1003	531	26.5
Sc ^a	373.1	371.5	370.9	371.1

a. Internal standard

5.2. Elemental Analysis by Participating Laboratories

Of the nine laboratories, six used LA-ICP-MS, five used μXRF and two used LIBS, with some laboratories using multiple techniques. Participants were asked to use the equipment available in their laboratories to report concentrations, in each specimen, for the quantitative elements (concentrations of calibrators provided), as well as the raw data for the qualitative elements, listed in Table 8. Many participants measured additional elements or isotopes. Table 9 and Table 10 contain summaries of the instrumental configurations, parameters, and any additional elements used by each participant.

Table 8. Required elements for analysis by participants.

Technique	Quantitative Elements	Qualitative Elements
LA-ICP-MS	²⁷ Al, ¹³⁷ Ba, ⁴² Ca, ⁷ Li, ²⁵ Mg, ⁵⁵ Mn, ^{206,207,208} Pb, ⁴⁵ Sc, ⁸⁸ Sr, ^{47,49} Ti, ⁵¹ V	
LIBS	Ba, Cr, Cu, Fe, Li, Mg, Mn, Pb, Sr, Ti, Zr	Al, Ca, Co, Si, V
μXRF	Cr, Cu, Fe, Mn, Pb, Sr, Ti, Zr	Al, Ca, Si, Zn

The LA-ICP-MS participants were asked to optimize their operating parameters to minimize oxides and doubly-charged species and to measure signals in transient mode with a 30 second gas blank, 60 second specimen ablation, and 30 second flush. Laser ablation using depth profile or spot mode was

recommended, with a spot size of 200 μm and a repetition rate of 10 Hz. The NIST SRM 2704 pellet was to be analyzed with four replicate measurements at the beginning and end of the sample set for use as the calibrator for calculation of specimen concentrations using GLITTER (see Section 3.2.1.2). All other specimens were to be measured five times.

Since LIBS configurations differ significantly between systems, the instrumental parameters listed in Section 3.2.2.1 and the emission lines listed in Section 4.3.2 were provided as a reference only. Optimization of the instrumental parameters (laser wavelength, energy, focal depth, gate delay, and gate width) and choice of emission lines were left up to the user to achieve the best possible linearity of response, LODs, and precision for their system. Any difficulties in obtaining reliable data for a particular emission line were to be reported. Five replicate measurements were to be made on each pellet. Participants were asked to background-subtract and normalize the peak intensities to the Sc internal standard, and then construct calibration curves using the calibrator pellets in order to determine the specimen concentrations.

Participants using μXRF optimized live seconds acquisition time for adequate counting statistics for trace elements, and beam current for maximum X-ray detector dead time. Five replicate measurements were to be made on each pellet. Participants were asked to construct calibration curves using the calibrator pellets in order to determine the specimen concentrations.

Table 9. LA-ICP-MS instrumentation and parameters used by each laboratory. Required isotopes were specified in the instructions (see Table 8). Additional isotopes monitored by participants are listed here.

Lab ID	Instrumentation & Parameters	Additional Isotopes Monitored
A-ICP	ICP-MS: Perkin-Elmer Elan DRC II, 1500W Laser ablation: New Wave UP213, 213nm, He carrier gas 1L/min, spot size 60µm, energy 17.5J/cm ² , tubing length 0.9m, ablation cell ~80mL	
B-ICP	ICP-MS: Thermo Element 2, 1250W Laser ablation: New Wave UP193SS, 193nm, He 0.6L/min, spot size 120µm, energy 9.5J/cm ² , tubing length 1m, ablation cell ~40mL	⁶ Li, ⁵⁷ Fe, ²⁹ Si, ²³⁸ U
D-ICP	ICP-MS: Thermo Element 2 XR, 1190W Laser ablation: New Wave UP193FX, 193nm, Ar 0.785L/min, He 0.415L/min, spot size 150µm, energy 2.69J/cm ² , tubing length 0.3m, ablation cell 33mL	¹⁰⁷ Ag, ⁷⁵ As, ²⁰⁹ Bi, ⁴⁴ Ca, ⁵⁹ Co, ⁶³ Cu, ⁵⁷ Fe, ⁶⁹ Ga, ³⁹ K, ²⁴ Mg, ⁶⁰ Ni, ³¹ P, ⁸⁵ Rb, ¹²¹ Sb, ²⁹ Si, ¹¹⁸ Sn, ⁶⁶ Zn
E-ICP (includes E.3 & E.5)	ICP-MS: Perkin-Elmer Elan DRC II 6100, 1500W Laser ablation: CETAC LSX500, 266nm, He carrier gas 0.9L/min, spot size 200µm, energy 9.2 J/cm ² , tubing length 1.4m, ablation cell ~17mL	⁷⁵ As, ¹¹¹ Cd, ⁵⁹ Co, ⁶³ Cu, ⁵⁷ Fe, ²⁰² Hg, ⁶⁰ Ni, ³¹ P, ¹²³ Sb, ⁸² Se, ²⁹ Si, ¹²⁰ Sn, ²³² Th, ²⁰⁵ Tl, ²³⁸ U, ⁶⁶ Zn, ⁹¹ Zr
F-ICP	ICP-MS: Agilent 7700x, 1540W Laser ablation: New Wave UP193FX, 193nm, He carrier gas 0.9L/min, spot size 150µm, energy 10J/cm ² , tubing length 4m, ablation cell ~25mL	

Table 10. μ XRF and LIBS instrumentation and parameters used by each laboratory. Required elements were specified in the instructions (see Table 8). Specific LIBS emission lines for the required elements and any additional elements monitored by LIBS and μ XRF participants are listed here. TC: Time constant.

Lab ID	Instrumentation & Parameters	Emission Lines (LIBS) & Additional Elements Monitored
A-XRF	μXRF: EDAX Eagle III, poly-capillary, Rh tube, 37% dead time, TC 17 μ s, 500 live seconds acquisition time, beam: 45 kV, 210 μ m.	Al, Ca, Mg, Si, Zn
B-XRF	μXRF: Orbis PC, 45% dead time, TC 12.6 μ s, 1200 live seconds acquisition time, beam: 50 kV, 2mm.	Al, Ba, Br, Ca, Si, W, Zn
D-XRF	μXRF: Phillips XL30 + IXRF, poly-capillary, Rh tube, 12-20% dead time, TC 16, 500 live seconds acquisition time, beam: 45 kV, 150 μ m.	Al, Ba, Ca, Si, Zn
A-LIBS	LIBS: Nd:YAG laser, 266nm, 29mJ (148J/cm ²), focused 1.4mm into the specimen, 75 shots per replicate after 3 cleaning shots, gate delay 2.0 μ s, integration time 150 μ s, Andor Mechelle 5000 broadband spectrometer with iStar ICCD camera (resolution 5000).	Lines Used: Ba II 493.4, Ba II 614.2, Ca I 643.9, Cr I 425.4 ^c , Cu I 324.8 ^b , Cu I 521.8, Fe I 360.9 ^b , Li I 670.8, Mg I 516.7 ^c , Mg I 518.4, Mn I 404.1, Pb I 405.8, Sc II 361 ^a , Sr II 407.8, Sr II 421.6, Ti I 336.1, V I 437.9, Zr I 468.8, Additional Lines: Cr I 360.5, Cu I 324.8, Fe I 495.8, Fe I 532.2, Li I 610.4, Mg I 517.3, Pb I 368.3
B-LIBS	LIBS: Applied Spectra RT100BB, Nd:YAG laser, 266nm, 7mJ, focused at specimen surface, 100 shots per replicate, gate delay 0.2 μ s, integration time 5 μ s, Catalina broadband spectrometer	Lines Used: Ba II 493.4, Ba II 614.2, Cu I 324.8, Fe I 360.9, Mg I 516.7, Mg I 518.4, Pb I 405.8, Sr II 407.8, Sr II 421.6, Ti I 336.1 Additional Lines: Li I 670.8, Mn I 404.1

a. Internal standard

b. Linear calibration curves obtained only at low concentrations

c. Linear calibration curves obtained only at high concentrations

5.3. Data Compilation & Statistics

The accuracy and precision reported by each laboratory were compared to the inter-laboratory results as well as to the certified values for PACS-2, NIST SRM 2704 (μ XRF and LIBS only), NIST SRM 2710, and NIST SRM 2710a. Since PACS-2 and the NIST SRMs are RMs, there are certified concentrations available for many elements. Data reported for each element in each RM included the study mean (inter-laboratory), study standard deviation (inter-laboratory), certified concentration, acceptance range, and intra-laboratory standard deviations of the measurements. The study mean and study standard deviation were calculated as the mean and standard deviation of the reported concentrations of each isotope or emission line in the study from each of the participant laboratories. Precision was expressed as a relative standard

deviation (RSD, %). Accuracy was represented by bias (percent error). The acceptance range was defined by the high and low acceptance values (high limit and low limit shown in Figure 16 and Figure 18). These limits were calculated as the certified concentration $\pm 3 \sigma$ for a 99.7 % confidence level, where σ represents a target standard deviation on the basis of the Horwitz Trumpet (which is determined using the certified concentration) [61]. Note that for elements with no certified concentration available, acceptance ranges were not calculated because of the limited number of participants. In addition, Z-scores were also calculated, but the numerical values were not shown since the acceptance range data shown in Figure 16 and Figure 18 provided the same information.

For μ XRF, LODs were calculated on data from all specimens from laboratory A-XRF as outlined in Gilfrich and Birks [69], Ernst [70] and detailed in a proposed ASTM Standard Method [48]. Limits of detection for LIBS and LA-ICP-MS were calculated as described in Sections 3.2.2.2 and 3.2.1.2, but for LIBS, only data from laboratory A-LIBS were used. For elements that were monitored using more than one emission line, average values were used: Ba (II 493.4, II 614.2), Cu (I 324.8, I 521.8), Mg (I 516.7, I 518.4), and Sr (II 407.8, II 421.6) (see Section 5.4). Laboratory B-LIBS experienced problems with emission line selection, resolution, and detection as a result of insufficient method optimization (see Section 5.4), therefore laboratory B-LIBS results were not included in any of the calculations or in the summary tables (Table 11 and Table 13), but are shown in Figure 18 for illustrative purposes.

5.4. Results & Discussion

The results for the elemental analysis of soil were separated into two main groups on the basis of the technique used. The first group was composed of six laboratories that performed elemental analysis by LA-ICP-MS. The second group was composed of laboratories that conducted elemental analysis by μ XRF (three laboratories) or LIBS (two laboratories). The data in Figure 16, Figure 18, and Table 11 through Table 13 summarize the individual and inter-laboratory results for the elements of interest and provide a method for evaluation of the performance across the laboratories and for the different techniques.

Table 11. Summary of the inter- and intra- laboratory precision, expressed as relative standard deviation (RSD), and limits of detection (LODs) for the three techniques across all laboratories.

nc: not quantified in the calibrator, nd: not detected, nr: not analyzed by any laboratory (not a required or additional element), n/a: not enough participants reported data for calculation of standard deviation.

Element	RSD (%)					LOD (ppm)		
	Inter-Laboratory		Intra-Laboratory					
	LA-ICP-MS	μXRF	LA-ICP-MS	μXRF	LIBS ^a	LA-ICP-MS	μXRF	LIBS ^a
Al	9	nc	3	nc	nr, nc	12	740	nr, nc
Ba	9	nc	4	nd	6	0.061	nd	24
Ca	12	nc	4	nc	nc	23	56	nc
Cr	17	14	11	4	33	0.15	23	31
Cu ^b	nr	24	nr	3	76	nr	32	237
Fe ^b	7	3	4	2	6	4.3	16	574
Li ^c	7	nr	4	nr	8	0.33	nr	0.25
Mg ^c	7	n/a	3	7	5	2.9	1526	253
Mn	9	9	4	5	8	0.095	41	157
Pb	19	18	5	4	17	0.033	64	63
Si ^b	26	nr	6	nc	nc	21	nr, nc	nc
Sr	19	25	6	5	6	0.023	30	14
Ti	9	11	6	5	7	0.90	43	82
V ^c	5	nr	3	nc	nc	0.055	nr	nc
Zr ^b	nr	27	nr	8	30	nr	42	95

a. Does not include laboratory B-LIBS (see Section 5.4)

b. Not a required LA-ICP-MS element (not measured by some or all laboratories, see Table 9)

c. Not a required μXRF element (not measured by some or all laboratories, see Table 10)

Table 11 provides a comparison of inter-laboratory and intra-laboratory precision, as well as LODs, for each of the three techniques. As expected, inter-laboratory precision was greater than intra-laboratory (within-run) precision (see Table 11). Intra-laboratory precision for μXRF and LIBS were greater than for LA-ICP-MS, but the observed level of precision may still be sufficient for forensic comparisons (see Section 6). The LODs for μXRF and LIBS were higher than those for LA-ICP-MS, but were sufficiently low for the analysis of most elements of interest in soil. It is worth pointing out that the LIBS LODs reported here do not reflect the lowest achievable LODs, as discussed in Section 4.3.2.

Table 12. LA-ICP-MS inter-laboratory summary for three RMs.
Results reported as concentrations in ppm ($\mu\text{g/g}$) unless otherwise specified. n/a: no certified or informational value reported on RM certificate.

Isotope	NIST SRM 2710			NIST SRM 2710a			PACS-2		
	Cert. Conc. $\pm 95\% \text{ CI}^{\text{a}}$	Inter-Lab Mean $\pm \text{SD}^{\text{c}}$	Bias (%)	Cert. Conc. $\pm 95\% \text{ CI}^{\text{a}}$	Inter-Lab Mean $\pm \text{SD}^{\text{c}}$	Bias (%)	Cert. Conc. $\pm 95\% \text{ CI}^{\text{a}}$	Inter-Lab Mean $\pm \text{SD}^{\text{c}}$	Bias (%)
^{27}Al	64400 $\pm$ 800	61189 $\pm$ 1663	-5	59500 $\pm$ 500	62671 $\pm$ 6808	5	66200 $\pm$ 3200	53552 $\pm$ 7271	-19
^{137}Ba	707 $\pm$ 51	670 $\pm$ 33	-5	792 $\pm$ 36	847 $\pm$ 130	7	n/a	848 $\pm$ 59	n/a
^{42}Ca	12500 $\pm$ 300	11384 $\pm$ 877	-9	9640 $\pm$ 450	9379 $\pm$ 1012	-3	19600 $\pm$ 1800	16290 $\pm$ 2704	-17
^{52}Cr	39	35.0 $\pm$ 2.2	-10	23 $\pm$ 6	36 $\pm$ 12	56	90.7 $\pm$ 4.6	81.4 $\pm$ 8.5	-10
$^{57}\text{Fe}^{\text{b}}$	33800 $\pm$ 1000	36051 $\pm$ 1855	7	43200 $\pm$ 800	51750 $\pm$ 6360	20	40900 $\pm$ 600	37094 $\pm$ 1657	-9
^7Li	n/a	44.1 $\pm$ 3.0	n/a	n/a	30.1 $\pm$ 1.8	n/a	32.2 $\pm$ 2.0	31.3 $\pm$ 2.3	-3
^{25}Mg	8530 $\pm$ 420	8776 $\pm$ 653	3	7340 $\pm$ 380	7333 $\pm$ 720	-0.1	14700 $\pm$ 1300	13414 $\pm$ 413	-9
^{55}Mn	10100 $\pm$ 400	10401 $\pm$ 569	3	2140 $\pm$ 60	2252 $\pm$ 310	5	440 $\pm$ 19	366 $\pm$ 28	-17
$^{206,207}\text{Pb}$	5532 $\pm$ 80	5719 $\pm$ 965	3	5520 $\pm$ 30	6235 $\pm$ 1604	13	183 $\pm$ 8	171 $\pm$ 27	-7
$^{29}\text{Si}^{\text{b}}$	289700 $\pm$ 1800	356492 $\pm$ 74816	23	311000 $\pm$ 4000	399167 $\pm$ 149870	28	280000	268244 $\pm$ 56069	-4
^{88}Sr	330	335 $\pm$ 78	2	255 $\pm$ 7	267 $\pm$ 56	5	276 $\pm$ 30	225 $\pm$ 31	-18
$^{47,49}\text{Ti}$	2830 $\pm$ 100	2845 $\pm$ 181	0.5	3110 $\pm$ 70	3326 $\pm$ 430	7	4430 $\pm$ 320	3858 $\pm$ 294	-13
^{51}V	76.6 $\pm$ 2.3	73.8 $\pm$ 1.8	-4	82 $\pm$ 9	87.6 $\pm$ 8.6	7	133 $\pm$ 5	115 $\pm$ 5	-13

a. Certified concentration, significant figures and 95% confidence interval (95% CI) as reported on RM certificate

b. Not a required LA-ICP-MS element (not measured by some or all laboratories, see Table 9)

c. One standard deviation (SD)

Table 13. μ XRF and LIBS inter-laboratory summary for four RMs.

Results reported as concentrations in ppm ($\mu\text{g/g}$) for each element (Ele) unless otherwise specified. nd: not detected, nr: not analyzed by any laboratory (not a required or additional element), n/a: no certified or informational value reported on RM certificate.

Ele	NIST 2710				NIST 2710a				PACS-2				NIST 2704			
	Cert. Conc. $\pm$ 95% CI ^b	μ XRF Inter-Lab Mean $\pm$ SD ^c	μ XRF Bias (%)	LIBS Bias (%) ^a	Cert. Conc. $\pm$ 95% CI ^b	μ XRF Inter-Lab Mean $\pm$ SD ^c	μ XRF Bias (%)	LIBS Bias (%) ^a	Cert. Conc. $\pm$ 95% CI ^b	μ XRF Inter-Lab Mean $\pm$ SD ^c	μ XRF Bias (%)	LIBS Bias (%) ^a	Cert. Conc. $\pm$ 95% CI ^b	μ XRF Inter-Lab Mean $\pm$ SD ^c	μ XRF Bias (%)	LIBS Bias (%) ^a
Ba ^d	707 $\pm$ 51	nd	nd	-5	792 $\pm$ 36	nd	nd	-3	n/a	nd	n/a	n/a	414 $\pm$ 12	nd	nd	-7
Cr	39	nd	nd	-91	23 $\pm$ 6	nd	nd	nd	90.7 $\pm$ 4.6	51 $\pm$ 9	-43	-10	135 $\pm$ 5	nd	-11	31
Cu	2950 $\pm$ 130	2071 $\pm$ 148	-30	-43	3420 $\pm$ 50	2303 $\pm$ 186	-33	-55	310 $\pm$ 12	216 $\pm$ 33	-30	11	98.6 $\pm$ 5.0	83 $\pm$ 54	-16	-45
Fe	33800 $\pm$ 1000	31699 $\pm$ 985	-6	-34	43200 $\pm$ 800	37883 $\pm$ 1065	-12	-49	40900 $\pm$ 600	36417 $\pm$ 432	-11	-52	41100 $\pm$ 1000	35829 $\pm$ 1327	-13	-52
Li ^d	n/a	nr	nr, n/a	n/a	n/a	nr	nr, n/a	n/a	32.2 $\pm$ 2.0	nr	nr	248	50	nr	nr	196
Mg ^d	8530 $\pm$ 420	6268	-27	-15	7340 $\pm$ 380	5617	-23	-10	14700 $\pm$ 1300	9990	-32	-43	12000 $\pm$ 200	7126	-41	-28
Mn	10100 $\pm$ 400	10202 $\pm$ 304	1.0	16	2140 $\pm$ 60	2052 $\pm$ 48	-4	63	440 $\pm$ 19	528 $\pm$ 69	20	123	555 $\pm$ 19	550 $\pm$ 98	-0.9	146
Pb	5532 $\pm$ 80	7073 $\pm$ 233	28	-7	5520 $\pm$ 30	9366 $\pm$ 762	70	-6	183 $\pm$ 8	403 $\pm$ 88	120	-60	161 $\pm$ 17	259 $\pm$ 102	61	-30
Sr	330	352 $\pm$ 29	7	74	255 $\pm$ 7	267 $\pm$ 17	5	85	276 $\pm$ 30	292 $\pm$ 119	6	14	130	210 $\pm$ 91	61	63
Ti	2830 $\pm$ 100	3232 $\pm$ 324	14	16	3110 $\pm$ 70	3392 $\pm$ 431	9	5	4430 $\pm$ 320	4852 $\pm$ 317	10	-5	4570 $\pm$ 180	4796 $\pm$ 800	5	-8
Zr	n/a	137 $\pm$ 30	n/a	n/a	200	252 $\pm$ 34	26	85	n/a	213 $\pm$ 59	n/a	n/a	300	290 $\pm$ 128	-3	9

a. Does not include laboratory B-LIBS (see Section 5.4)

b. Certified concentration, significant figures and 95% confidence interval (95% CI) as reported on RM certificate

c. One standard deviation (SD)

d. Not a required μ XRF element (not measured by some or all laboratories, see Table 10)

Figure 16. Summary of LA-ICP-MS performance on various elements for all laboratories. Blue: PACS-2; red: NIST SRM 2710; green: NIST SRM 2710a; diamond: measured value (with arrow: value above chart range); solid line: certified or informational value (see Table 12); dotted line: high and low limits. Error bars represent one standard deviation (n=4). Missing data points indicate non-detects, except Fe (not a required element; not measured by laboratories A-ICP or F-ICP). Missing limits indicate no certificate values available.

In general, all LA-ICP-MS laboratories had good performance in terms of accuracy and precision (see Figure 16 and Table 12). With the exception of a few elements, all laboratories were within the control criteria for the inter-laboratory comparison. Many of the unacceptable results were not far out of the acceptable range (see Figure 16). An overall negative bias was observed for all elements in PACS-2 (see Table 12). This may have been a result of under-estimation of internal standard concentration (if Sc was present at non-zero levels in the specimen before addition of the internal standard), unequal ablation efficiencies, and/or suppression of ionization from marine salts.

When using single point calibration for an ICP-MS without automatic cross-calibration of the dual detector, manual calibration of detector response was crucial when element concentrations varied widely, as they do in soils. For example laboratory E-ICP, which did not cross-calibrate for all elements in the menu, analyzed the specimens twice (only one analysis is included in calculations and summary tables, as laboratory E.5-ICP) and observed reproducible results (data not shown), but accuracy was poor for some elements (Al, Ba, Pb, and Sr). Upon examination of the transient signals, a problem with the dual detector cross-calibration was identified. Figure 17 shows the transient signals for three isotopes of Pb. A sudden change in counts per second (cps) can be seen for ^{206}Pb and ^{207}Pb at the threshold where the detector switches from analog to digital mode (2 million cps), while ^{208}Pb shows a normal transient signal in analog mode. Clearly ^{206}Pb and ^{207}Pb concentrations will be inaccurate, but even ^{208}Pb concentrations would be inaccurate if the calibrator were measured in digital mode. A dual detector cross-calibration was performed before a third analysis of the specimens, which resulted in improved accuracy (post-cross-calibration results reported as laboratory E.3-ICP). The most significant improvements were seen with Pb and Sr (see Figure 16 and Table 12). Improvements were seen for the 11 required elements in 18 of 30 reported concentrations.

Figure 17. LA-ICP-MS transient signals for the three Pb isotopes. Digital to analog crossover occurs at 2×10^6 cps. a. before cross-calibrating the dual detector, b. after cross-calibration.

Figure 18. Summary of μ XRF and LIBS performance on various elements for all laboratories. Blue: PACS-2; red: NIST SRM 2710; green: NIST SRM 2710a; purple: NIST SRM 2704; diamond: measured value (with arrow: value above chart range); solid line: certified or informational value (see Table 13); dotted lines: high and low limits. Error bars represent one standard deviation (n=5). Missing data points indicate non-detects except Li, Ba and Mg (not required elements; not measured by some or all laboratories, see Table 10). Missing ranges indicate no certificate values available. †: Laboratory B-LIBS is shown here for illustrative purposes, but was not included in any of the summary tables.

Selection of suitable emission lines and optimization of instrumental parameters were crucial for LIBS, and indeed were the main issues for laboratory B-LIBS. Parameters and emission lines were carefully optimized by laboratory A-LIBS as described in Section 4.3. However, since laboratory B-LIBS used a completely different instrumental configuration (see Table 10), their use of the same parameters and emission lines as A-LIBS, without further optimization, produced less than favorable results. This was manifested as non-detects for some elements (Co, Cr, Li, and Zr) and spectral interference for other elements (Pb, and the internal standard Sc), resulting in poor agreement with the certified values and with the other laboratories (see Figure 18 and Table 13). Given more time to optimize, good results could have been achieved by laboratory B-LIBS.

Laboratory A-LIBS used average values when two lines were measured for a particular element. However it is common for some lines to perform better at low concentrations and others to perform better at high concentrations (see footnotes to Table 10) [22]. For example, Mg I 518.4 was a relatively strong-emitting line and exhibited some self-absorption (non-linearity) at higher concentrations and would therefore be suitable only for analysis at lower concentrations. Mg I 516.7, on the other hand, is a more weak-emitting line and was not sensitive in the low concentration ranges, but exhibited no self-absorption at higher concentrations. Since the Mg concentration ranges in this study were relatively high, the use of Mg I 516.7 alone, rather than the average of the two lines, would have improved the accuracy of the measurements. Therefore it is recommended that multiple emission lines be monitored, if possible, for each element.

Calibration curves generated by laboratories A-XRF (see Figure 19 for examples for four X-ray lines) and A-LIBS using the calibrators were very linear. Bias was high for Li by LIBS (see Figure 18 and Table 13), which may be because of a matrix effect. While calibration curves generated using the calibrators (made of silica sand) were very linear, the RMs (made of soil with other major elements present) did not plot along the fit line. For some specimens, Ba, Cr, Li, and Mg were not reliably detected by some laboratories at low concentrations, particularly near the LOD (see Figure 18). Li is typically not detected by μ XRF because of its low energy line ($K\alpha$, 54.3 eV) [47,87]. Both μ XRF and LIBS exhibited some problems detecting Cr since some of the specimens had Cr concentrations near the LOD. For LA-ICP-MS,

it should be noted that ^{52}Cr is subject to a polyatomic interference generated by $^{40}\text{Ar}^{12}\text{C}$. This may have contributed to the poorer precision, higher bias, and outliers observed in the raw data (two outliers were removed after Q-testing [61]). Future studies should monitor both ^{52}Cr and ^{53}Cr to evaluate this interference. One possible contribution to poor Cr performance for all three techniques may be the “nugget effect”, resulting in large and unevenly distributed particles. Attempts were made to minimize this through homogenization, however harder minerals that are more difficult to mill may still pose problems (e.g., chromite and many minerals containing silicon or zirconium) [47,88].

Figure 19. Examples of μXRF calibration curves using background-subtracted peak area (counts) data for Fe, Mn, Sr, and Ti.

5.5. Recommendations for Forensic Laboratories

The present study was limited to sufficiently sensitive elemental analysis techniques already available in some forensic laboratories (μXRF and, to a lesser extent, LA-ICP-MS), and to LIBS which was evaluated here for its potential applicability as a cost-effective alternative. Each of the three was capable of generating elemental data of sufficient quality for use in forensic soil examinations, as well as other

forensic examinations requiring elemental analysis. With each of the techniques there were a number of configuration options available that affect the quality of the results.

LA-ICP-MS provided the lowest detection limits, but is also the most expensive and requires the most maintenance and training. High resolution instruments are available, but these are even more expensive, require even more maintenance and expertise, and were not necessary to achieve good results for soils (see results from laboratories A-ICP, E-ICP, and F-ICP). The choice of laser in the ablation unit is the primary consideration. Forensic laboratories may choose lasers in the UV wavelength range because of improved laser-to-sample coupling and reduced specimen damage (cracking and spallation) with transparent matrices, such as glass [42,43] and reduced fractionation for complex and mineral matrices such as soil and rock [24]. The range of available laser spot sizes is also important, with smaller spots causing less damage and providing higher spatial resolution, and larger spots providing a more representative measurement of non-homogeneous specimens and higher intensity signals because of the increased mass ablated. If there is a dual detector and it is not automatically cross-calibrated, a manual cross-calibration must be performed for all elements in the soil element menu prior to measurement by LA-ICP-MS.

Micro XRF is currently the most commonly available of the three in forensic laboratories, though it is not commonly used for soil analysis. In this study, μ XRF provided good detection limits for major and minor elements in soils, but was not able to detect many trace elements, nor those in the low mass range. Training requirements are less intense than with LA-ICP-MS. Most laboratory instruments with capillary optics and energy-dispersive X-ray detectors (EDX, or EDS) will provide adequate performance if they are capable of at least 35 kV excitation energy [48]. Further considerations for μ XRF configurations, such as X-ray tube and focusing optics, are discussed in a proposed ASTM method for forensic glass analysis by μ XRF [48].

LIBS provided detection limits in the ppm range, which was sufficient for many trace elements in soils. It arguably requires the least maintenance and operator training of the three. It should be noted that successful method development does require some additional expertise: one must carefully optimize acquisition parameters such as integration time, gate delay, laser focal depth, and number of laser shots to accumulate per analysis for elements of interest in the each matrix (in this case, soil). Furthermore, the

identity of potential emission lines should be confirmed, spectral interferences identified, and linear dynamic ranges determined. Since the concentrations are not known in advance, multiple emission lines (strongly and weakly emitting) should be monitored for each element. The configuration options available in a LIBS instrument must be carefully considered, before purchasing, with the applications or matrices in mind. UV lasers may be chosen for the same reasons as stated above, but IR lasers will also work for some applications. Limits of detection (LODs) for trace elements can also be enhanced with the use of a more sensitive and reliable detector. Spectral resolution is perhaps the most important variable for complex matrices such as soil and steel, while less important for matrices with simpler spectra such as glass and plant material. For this reason, the choice of spectrometer (spectrograph) is important.

Portable XRF and LIBS instruments can be less sensitive and reliable than their laboratory counterparts, but may still provide adequate results for some applications, such as field screening [32,84].

5.6. Conclusions

This inter-laboratory study permitted a direct comparison of the analytical performance between different elemental analysis techniques and between participant laboratories using the same technique. Direct analysis of soil produced limits of detection of 0.01-23 ppm for LA-ICP-MS, 0.25-574 ppm for LIBS, and 16-4400 ppm for μ XRF for elements useful in forensic discrimination. Inter-laboratory precision for most elements was 19 % or less for LA-ICP-MS and 25 % or less for μ XRF, which is encouraging for a first inter-laboratory exercise where a fully standardized method and analytical protocol was not used by the participants. The statistics, particularly for μ XRF and LIBS, would have benefited from more participants. Still, the results obtained in this study allowed each participant to identify potential sources of uncertainty and fine-tune their methods to work towards the optimization and development of standardized methods of analysis. Quantitative analysis was possible with all three techniques through external calibration.

For forensic analysis, the precision, cost, and specimen consumption of an analysis are important. Of the three methods evaluated here, LA-ICP-MS provided the most precise and accurate data but is the most expensive. Intra-laboratory precision for most elements was 6 % RSD or less for LA-ICP-MS, 8 % or less for μ XRF, and 17 % or less for LIBS. While μ XRF is non-destructive, LA-ICP-MS and LIBS remove only

microgram amounts of mass (quasi-non-destructive) still allowing for future reanalysis of the pellets. LIBS & μ XRF are less expensive and easier to operate and maintain than LA-ICP-MS, and field-portable units are now commercially available for both. All three techniques can be used for analysis of additional matrices (glass, paint, metals, and inks).

The elemental analysis methods presented here are an additional tool for the forensic examination of soil. Forensic (or other) scientists may find the information useful in choosing the elemental analysis technique most suited to their needs, and to identify important considerations for method development and validation. The next step would be an inter-laboratory study using these methods for forensic comparisons, which has already been done with glass analysis by the members of the EAWG [89].

6. Development of Methods for Discrimination Based on Elemental Profiles

In this Section, methods for discriminating soils specimens using elemental profiles generated from LIBS and LA-ICP-MS were developed with various statistical analysis techniques. For the preliminary development of discrimination using LIBS data, environmental samples, previously analyzed by LA-ICP-MS, were used to compare the performance of the two techniques. Miami-Dade County specimens were used to refine and test the methods in three subsequent studies. Discriminations made using elemental data were compared to discriminations made using color and agricultural soil type. For all studies, triplicate specimens and duplicate analyses were used as positive controls that should be easily associated. The RMs were used as negative controls since they were known to originate from different sources and should be easily distinguished from the specimens and from one another. For LA-ICP-MS, elemental profiles consisted of MDL-corrected concentrations (see Section 3.2.1.2) for all monitored isotopes. For LIBS, elemental profiles consisted of emission line ratios (see Section 3.2.2.2) for all monitored emission lines, except where noted. The effectiveness of LIBS as a lower-cost alternative to LA-ICP-MS for discrimination was evaluated.

6.1. USEPA NEIC Specimens: Development of a Preliminary LIBS Discrimination Method

The sample set from NEIC was described in Section 4.1.1. Although the samples were collected as part of an environmental study, they were used here in a forensic context to develop a LIBS discrimination method. The elemental profile consisted of normalized background-subtracted intensities generated for the specimens and controls as described in Section 4.1.1.

Since the samples originated from the same region, but differed in their distance from the contamination source, it was hypothesized that the three contaminants (Pb, Sn, and Sb) would be good discriminators. Since only one of the three, Pb, could be reliably detected by LIBS, additional elements were investigated for the purposes of discrimination as described in Section 4.1.1. Pairwise comparison was performed using ANOVA with Tukey's HSD, followed by Student's t-test, as described in Section 3.3. All ten specimens were successfully discriminated on the basis of their normalized elemental concentrations. A positive control, consisting of a duplicate sample of specimen number 110, was correctly associated. The PACS-2 pellet was used as a negative control, and was successfully discriminated from all specimens. Twelve

pellets resulted in 66 possible comparison pairs. Lead I 405.8 was not the most discriminating emission line, but still provided 55 % discrimination, meaning that of all possible pairs that should have been discriminated, 55 % were discriminated by Pb. A summary of the discrimination power of the emission lines investigated is shown in Table 14.

Table 14. Discrimination of NEIC specimens using LIBS and L-ICP-MS.

LIBS		
Emission Line	Indistinguishable Pairs	% Discrimination
All Elements	0	100
Ca I 643.9nm	17	74
Sr II 407.8nm	23	65
Fe I 495.8nm	26	61
Pb I 405.8nm	30	55
Mg I 516.7nm	37	44
Na I 819.5nm	42	36
Li I 812.6nm	61	8
Total Possible Pairs	66	
LA-ICP-MS		
Isotope	Indistinguishable Pairs	% Discrimination
All Isotopes	0	100
¹²⁰ Sn	11	83
²⁰⁸ Pb	14	79
¹²¹ Sb	23	65
Total Possible Pairs	66	

In conclusion, 100% of the contaminated soil samples taken from different locations were distinguished from each other by both LIBS and LA-ICP-MS. No type I or type II errors were detected. This was more of an environmental forensics case, so further work should be done using more forensically relevant sample set and additional statistical tools should be evaluated.

6.2. Miami-Dade County Specimens: Proof of Principle

This proof of principle study was performed in order to show that the laser-based elemental analysis methods could indeed be used to discriminate between soil samples known to be very different. The samples were taken from two sites at opposite ends of Miami-Dade County, having different colors and textures, and belonging to two different agricultural soil types. Various statistical techniques were explored in order to discriminate between the two sites.

6.2.1. Materials & Methods

The Miami-Dade County sample set was described in Section 4.2.1. The first two sites to be sampled were CS1 and FIU1 (see map, Figure 9a). They were separated by approximately 45 km (28 miles), easily discriminated on the basis of texture and color (see Appendix 1), and belonged to different agricultural soil types. Bulk pellets from these sites, as well as calibrator and RM pellets, were prepared as described in Section 4.2.5. Elemental profiles were generated using LIBS and LA-ICP-MS methods as described in Section 4.3 and 4.4, respectively.

Emission line ratios (see Sections 2.5 and 3.2.2.2) were used for LIBS, resulting in $n(n-1)/2$ possible ratios, where n is the number of emission lines (e.g., for 20 emission lines this would mean 190 ratios). This is far too many to put into any model. Even for LA-ICP-MS, where isotope concentrations are used without ratios, if all elements monitored were used in the element menu for discrimination, this would take a long time; for univariate pair-wise comparisons, the process must be repeated for each element and then the results for all elements must be tabulated, and for multivariate analyses, the software would take a long time to process such a large amount of data. In addition, for many statistical tests, the number of observations (the number of replicate analyses in this case) must be greater than the number of variables (the number of isotopes or emission line ratios in this case). In addition, the most discriminating elements will provide real differences and result in a more robust discrimination, while elements that are not truly discriminating may contribute noise and artifacts, and increase the likelihood of model over-fitting and Type I and Type II errors. Therefore only the most discriminating elements should be used in the discrimination element menu.

The most discriminating emission line ratios were initially identified by an algorithm (see Section 3.2.2.2); however, the most discriminating emission line ratios were found to be too discriminating; positive controls were incorrectly discriminated. Emission line ratios further down the list that correctly associated positive controls and correctly distinguished negative controls were chosen. The isotopes and emission line ratios chosen for the element menus were good discriminators while contributing few or no Type I or II errors. Pair-wise comparisons (ANOVA with Tukey's HSD, followed by Student's t-test) were

used for the initial discriminations. Multivariate methods were then developed using PCA for visualization and LDA for classification.

6.2.2. Results & Discussion

For LIBS, the following emission line ratios were chosen for the discrimination element menu: Sr II 421.6/Ti I 336.1, Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Fe I 495.8/Mg I 518.4, and Li I 610.4/Mg I 517.3. For LA-ICP-MS, the following isotopes were chosen for the discrimination element menu: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{88}Sr , $^{47,49}\text{Ti}$, ^{238}U , and ^{51}V . The concentrations of each emission line and isotope observed in the specimens were well above the LODs, (see Table 5 & Table 6).

Normalized LIBS spectra from CS1 (green) and FIU1 (blue) specimens were shown in Figure 2. Differences were seen in Ba II 493.4, Fe I 495.8, Mg I 517.3 and Mg I 518.4 nm, and a group of titanium (I) emission lines. Pair-wise comparison was used to correctly associate all specimens from the same site and discriminate all specimens from different sites. Positive controls were correctly associated and negative controls were correctly distinguished, resulting in no Type I or Type II errors. Similar results were obtained for LA-ICP-MS

For Both PCA and LDA, each replicate measurement is considered as a separate observation. The LIBS dataset included 340 LIBS measurements and the LA-ICP-MS dataset included 188 measurements. Looking at the PCA scores plot (see Figure 20), a clear separation can be seen between the CS1 (green) and the FIU1 (blue) specimens, as well as each of the negative controls. Three PCs, representing over 97 % of the variation in the data, were required in order to visualize these differences.

Figure 20. PCA score plots for CS1 (green) and FIU1 (blue) generated from LIBS data. The following discrimination element menu was used: Sr II 421.6/Ti I 336.1, Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Fe I 495.8/Mg I 518.4, and Li I 610.4/Mg I 517.3. Numbers and different shades denote the different sub-plots. Ellipses were added to show the groupings but do not carry any statistical weight.

For LA-ICP-MS, clear separation was also seen using PCA (see Figure 21). The increased precision of the technique resulted in tighter groupings, and only two PCs, representing 93 % of the total variation, were required. Note that the numbers in both figures represent the sub-plot number.

Figure 21. PCA score plots for CS1 (green) and FIU1 (blue) generated from LA-ICP-MS data. The following discrimination element menu was used: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{88}Sr , $^{47,49}\text{Ti}$, ^{238}U , ^{51}V , ^{91}Zr . Numbers and different shades denote the different sub-plots. Ellipses were added to show the groupings but do not carry any statistical weight. The inset is a zoomed-in view of the CS1 and FIU1 clusters to show detail.

In CS1, the specimens from the six sub-plots seemed to be evenly distributed in the LIBS PCA, while only slightly separated in the LA-ICP-MS. This was likely because of the difference in precision of the two methods, and may indicate that there is not much variation in the site overall. It is interesting to note that CS1 was a wetland site near the Everglades. Even in the dry season the soil was often inundated, and the water may have provided a means of redistributing minerals and trace elements over the entire region. In FIU1, within-site variation was seen with both LIBS and LA-ICP-MS data. This variation was larger than the within-method variation, even for LIBS, and was attributed to the overall heterogeneity of the site. In Figure 20, from the LIBS data, if the points were not colored blue and the identities were unknown, the FIU specimens could be split into two groups, with the upper group containing sub-plots 2 (specimens C through F) and 4, and the lower group containing the rest. Perhaps even a third group could be imagined in the middle, containing the rest of sub-plot 2 and specimen B from sub-plot 3. In Figure 21, from the LA-

ICP-MS data, the result of this heterogeneity is more of a continuum, with sub-plots 2 and 4 at one end, 5 and 6 at the other, and 1 and 3 smeared throughout. Interestingly, specimens from sub-plots 2 and 4 were paler in color than the rest of FIU (see Appendix 1), which may imply difference in mineral composition that was detected in the elemental profiles.

Recall that PCA is an unsupervised technique, so no prior knowledge of specimen identity was used in generating the PCs. In addition, no error rates were generated. In contrast, LDA is a supervised classification technique, and the identity of each replicate measurement on each specimen in the training set was specified in order to generate the linear discriminant function. A leave-one-out (jackknife) cross-validation was used to test the prediction accuracy of the function, which provided a correct classification rate. For LIBS, a correct classification rate of 99.4 % was obtained for 340 replicates. The confusion matrix is shown in Table 15, with the numbers along the diagonal representing correct classifications and the off-diagonal numbers representing misclassifications. The two misclassifications belonged to FIU1 but were incorrectly classified as belonging to NIST SRM 2704; one replicate measurement of a specimen from sub-plot 1 and one from sub-plot 2. This was observed as a slight overlap in the LDA canonical plot (see Figure 22). A correct classification rate of 100 % was obtained for LA-ICP-MS, and tight, clearly distinct groupings were observed in the LDA canonical plot (see Figure 23).

Table 15. Confusion matrix for LDA with leave-one-out cross-validation on 340 LIBS replicates from CS1 and FIU1 pellets, as well as RM pellets.

		Predicted Origin					Total
		CS1	FIU1	2704	2710	PACS-2	
True Origin	CS1	135	0	0	0	0	135
	FIU1	0	133	2	0	0	135
	2704	0	0	25	0	0	25
	2710	0	0	0	20	0	20
	PACS-2	0	0	0	0	25	25

Figure 22. LDA canonical plots for CS1 (green) and FIU1 (blue) generated from LIBS data. The following discrimination element menu was used: Sr II 421.6/Ti I 336.1, Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Fe I 495.8/Mg I 518.4, and Li I 610.4/Mg I 517.3. The different shades denote the different sub-plots.

Figure 23. LDA canonical plots for CS1 (green) and FIU1 (blue) generated from LA-ICP-MS data. The following discrimination element menu was used: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{88}Sr , $^{47,49}\text{Ti}$, ^{238}U , ^{51}V , and ^{91}Zr . The different shades denote the different sub-plots.

6.2.3. Conclusions

Using both univariate and multivariate statistical techniques, specimens from CS1 were discriminated from FIU1 specimens. A correct classification rate over 99 % was obtained for LIBS, and 100% for LA-ICP-MS. Although LA-ICP-MS resulted in better precision, LIBS provided the same information, making it fit for purpose. The inherent heterogeneity of a site may be an important factor in the success of a forensic examination of soil by any method, and should be investigated further (see Section 6.3).

6.3. Miami-Dade County Specimens: A More Challenging Test & Sieving to Mimic Transfer Evidence

As discovered in Section 6.2, some sites can be quite heterogeneous, and differences in color may indicate differences in composition that may be observed in the analytical profiles. This study was performed in order to examine the effects of soil color, texture, and distance on discrimination. Variation between sites is what the forensic examiner hopes for, but variation within a site, within a sub-plot, and

within a specimen must all be examined in order to determine how variable the soil is in a given location and how it differs from other locations. For example, if a questioned specimen originated from sub-plot 2 of FIU1, but known specimens were taken only from sub-plots 5 and 6, and no other knowledge existed about the FIU1 site, the conclusion might be that the questioned specimen did not originate from FIU1. But what does this really mean? On the basis of the analysis, all one could really say is that the questioned specimen did not originate from sub-plots 5 and 6 of FIU1. If only a few known specimens were also taken from CS1, one could say that the elemental profile of the questioned specimen was more similar to that of FIU1 than CS1, but results would be inconclusive. It was only by well-characterizing the sites that a more complete picture of their inherent variation was obtained.

The previous study (see Section 6.2) was performed to prove that the methods worked for specimens from obviously different sources. A more challenging test was required in order to determine how well the laser-based elemental analysis methods would perform under more realistic scenarios. Normally examination by color is performed first, and only if no differences are found do the examiners continue on to other techniques. Specimens were taken from three sites having similar color and texture, two of which were close together and belonged to the same agricultural soil type, and the third at the other end of Miami-Dade County and belonging to a different agricultural soil type. Fewer specimens were used per site, and were sieved to mimic transfer evidence. Discrimination methods were fine-tuned for this more challenging sample set.

6.3.1. Materials & Methods

The Miami-Dade County sample set was described in Section 4.2.1. Three sites were selected containing fine grey soils: KNT and CC6 belonged to the same agricultural soil type and were separated by less than 8 km (5 miles), and HA, which belonged to a different agricultural soil type and was separated by 43-50 km (27-31 miles) from the other two (see Figure 9a). Sieved pellets from these sites were prepared as described in Section 4.2.5. Elemental profiles were generated using LIBS and LA-ICP-MS methods as described in Section 4.3 and 4.4, respectively. Principal components analysis was used for visualization and LDA was used for classification. Strategies for choice of discrimination element menu were improved.

6.3.2. Results & Discussion: LIBS

Poor choice of element menu can affect the discrimination results. Using the algorithm and choosing the most discriminating emission line ratios that had no Type I or type II errors in the controls, as described in Section 6.2, resulted in the following discrimination element menu: Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Ti I 336.1, Sr II 421.6/Ti I 336.1, and Sr II 421.6/V I 437.9. As shown in the PCA scores plot (Figure 24a), separation between HA (red) and the other two sites could be seen, but there was significant overlap between KNT (green) and CC6 (blue), even with the use of three PCs. The discrimination element menu for CS1 and FIU1 from the Proof of Principle study (Sr II 421.6/Ti I 336.1, Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Fe I 495.8/Mg I 518.4, and Li I 610.4/Mg I 517.3, Section 6.2) was applied, to see if the separations could be improved (see Figure 24b). Comparing the two Figures, it was obvious that the Proof of Principle discrimination element menu provided much better separation. At this point the choice of discrimination element menu seemed somewhat arbitrary, and was relied on a lot of trial and error. A more systematic and intuitive ratio selection tool was required.

Figure 24. PCA score plots for HA (red), KNT (green), and CC6 (blue) generated from LIBS data on sieved pellets. The following discrimination element menus were used: a. Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Ti I 336.1, Sr II 421.6/Ti I 336.1, and Sr II 421.6/V I 437.9; b. Sr II 421.6/Ti I 336.1, Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Fe I 495.8/Mg I 518.4, and Li I 610.4/Mg I 517.3. Numbers and different shades denote the different sub-plots.

First, histograms were generated for each emission line by binning the background-subtracted normalized intensities of all specimens from the three sites. Emission lines showing multimodal distributions were considered as potentially good discriminators. Figure 25 shows an example of Cr I 360.5 and Fe I 360.9 with bimodal distributions. Then using the emission line ratios, the average levels for each site were calculated. It was observed that most barium and magnesium-containing ratios varied little between the three sites; therefore they were considered poor discriminators. The most discriminating ratios appeared to be those containing calcium, chromium, iron, and to a lesser extent lithium, strontium and possibly manganese, which agreed with what was observed with the histograms.

Figure 25. Histograms showing the frequency of the normalized LIBS intensities for all specimens. Bimodal distributions were seen for Cr and Fe emission lines.

A bivariate plot of Ca I 643.9/ Fe I 360.9 versus Cr I 360.5/ Sr II 421.6 showed good separation of the RMs used as controls and of the three sites, though some overlap was seen with KNT (green) and CC6 (blue, see Figure 26a). In addition, HA sub-plot 6 has gotten closer to the rest of HA. Looking again at the average levels of each site, Fe/Li ratios seemed to differ between the two sites, as did Ba/Fe. Three-dimensional plots of Ca I 643.9/ Fe I 360.9 and Cr I 360.5/ Sr II 421.6 versus Fe I 495.8/ Li I 670.8 (see Figure 26b) and versus Ba II 493.4/ Fe I 360.9 (see Figure 26c) showed KNT (green) separating more from CC6 (blue) and HA (red) grouping more tightly together and further from KNT.

Figure 26. Bivariate and trivariate plots used to for element menu selection for HA (red), KNT (green), and CC6 (blue) generated from LIBS data on sieved pellets.

a. Ca I 643.9/ Fe I 360.9 versus Cr I 360.5/ Sr II 421.6, b. Ca I 643.9/ Fe I 360.9 and Cr I 360.5/ Sr II 421.6 versus Fe I 495.8/ Li I 670.8, c. Ca I 643.9/ Fe I 360.9 and Cr I 360.5/ Sr II 421.6 versus Ba II 493.4/ Fe I 360.9. Numbers and different shades denote the different sub-plots.

PCA was performed with all four ratios (Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Li I 670.8, and Ba II 493.4/ Fe I 360.9), for an even better separation (see Figure 27). The first two PCs explained 93.2 % of the variation (see Figure 27b), while the third PC added only 4.3 % and was only necessary to separate the RMs (see Figure 27a). Analysis by LDA was performed using the same four ratios, and resulted in 11 misclassifications out of 200 replicates, for a 94.5 % correct classification rate.

For both PCA and LDA, negative controls were easily separated, and the positive controls clustered together with no misclassifications. The confusion matrix for the three Miami-Dade County sites is shown in Table 16, with the numbers along the diagonal representing correct classifications and the off-diagonal numbers representing misclassifications. Five CC6 replicates from sub-plot 6 were misclassified as originating from KNT, and six KNT replicates (five from sub-plot 1 and one from sub-plot 6) were misclassified as originating from CC6. These were observed as a slight overlap of the two sites in the canonical plot (Figure 28). This was not surprising since they were of the same color, soil type, and texture, and located less than 8 km apart. In addition, HA specimens seemed to cluster more tightly than the other two sites, underlining the previous observation that within-site heterogeneity is site-specific. The numbers in the Figures represent the sub-plot number (and are also colored with different shades), and it was interesting to note that specimens from the same sub-plot tended to cluster together somewhat, indicating that differences may be detectable even within a site.

Figure 27. PCA score plots for HA (red), KNT (green), and CC6 (blue) generated from LIBS data on sieved pellets.

The following discrimination element menu was used: Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Li I 670.8, and Ba II 493.4/ Fe I 360.9. Numbers and different shades denote the different sub-plots. RMs were also plotted as negative controls: NIST SRM 2710 (peach), NIST SRM 2704 (orange), and PACS-2 (brown). a is the full plot and b is zoomed in showing only HA, KNT, and CC6.

Figure 28. LDA canonical plot for HA (red), KNT (green) and CC6 (blue) generated from LIBS data on sieved pellets.

The following discrimination element menu was used: Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Li I 670.8, and Ba II 493.4/ Fe I 360.9. Numbers and different shades denote the different sub-plots. Misclassified specimens are circled.

Table 16. Confusion matrix for LDA with leave-one-out cross-validation on 200 LIBS replicates from HA, CC6 and KNT sieved pellets.

		Predicted Origin			Total
		CC6	HA	KNT	
True Origin	CC6	65	0	5	70
	HA	0	55	0	55
	KNT	6	0	69	75

6.3.3. Results & Discussion: LA-ICP-MS

Discrimination by LDA was performed with the LA-ICP-MS data using the same ratios used for the previous study (see Section 6.2), and resulted in five misclassifications out of 172 replicates, for a 97.1 % correct classification rate. Other element menus were investigated (e.g., adding Mn, removing Ti, adding

Pb), but the original element menu was the best in terms of visual separation and correct classification rate. Negative controls were easily separated, and the positive controls clustered together with no misclassifications. The confusion matrix for the three Miami-Dade County sites is shown in Table 17, with the numbers along the diagonal representing correct classifications and the off-diagonal numbers representing misclassifications. Five CC6 replicates (three from sub-plot 6 and two from sub-plot 5) were misclassified as originating from KNT. These are circled in the canonical plot (Figure 29), but do not touch any KNT points. Although it would appear that these replicates group with CC6, their distance from the centroid of CC6 was actually further than the distance to the centroid of KNT, which is why they were assigned to KNT. The slight improvement over LIBS in correct classification rate and separation in the plots was likely because of the increased precision of LA-ICP-MS. This high precision also resulted in more pronounced clustering of individual sub-plots (denoted by the different numbers and shades of a particular color) of CC6, and to a lesser extent KNT, as compared to LIBS. This highlights the importance of proper characterization of a site of interest by taking known specimens throughout the region where the transfer is thought to have occurred. For example, if known specimens were only taken from KNT sub-plots 1, 2 and 5, then a questioned sample from sub-plot 3 might be excluded.

Figure 29. LDA canonical plots for HA (red), KNT (green) and CC6 (blue) generated from LA-ICP-MS data on sieved pellets.

The following discrimination element menu was used: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{88}Sr , $^{47,49}\text{Ti}$, ^{238}U , ^{51}V , and ^{91}Zr . Numbers and different shades denote the different sub-plots. Misclassified specimens are circled.

Table 17. Confusion matrix for LDA with leave-one-out cross-validation on 172 LA-ICP-MS replicates from HA, CC6 and KNT sieved pellets.

		Predicted Origin			Total
		CC6	HA	KNT	
True Origin	CC6	59	0	5	64
	HA	0	44	0	44
	KNT	0	0	64	64

6.3.4. Conclusions

Heterogeneity was important, and the degree of heterogeneity appeared to be site-specific. Forensic examiners and collectors of evidence need to consider this when collecting known specimens in order to

properly characterize a site. Choice of discrimination element menu was also important. The most discriminating emission lines for one region may not necessarily be discriminating for another region because of differences in the underlying mineralogy, sediment deposits or human activity. Strategies were developed for the selection of discrimination element menus for LIBS and LA-ICP-MS data. High correct classification rates (94.5 % for LIBS and 97.1 % for LA-ICP-MS) were obtained using LDA on this challenging sample set with similar soils from nearby locations. As many additional elements as possible should be monitored to avoid missing elements that may provide the key to discrimination. This way if an interesting element is later discovered, the statistical analysis can be re-processed to use it without running the samples again.

6.4. Miami-Dade County Specimens: Characterizing the Selected Areas Throughout the County

In order to have a more complete picture of the entire Miami-Dade County, the remaining specimens were analyzed and elemental profiles generated. Discrimination studies were performed using Munsell color scale and elemental analysis. As mentioned previously, color is usually the first analysis performed by a forensic examiner. Since specimens of similar color may not have the same mineralogy or human activity, elemental analysis may provide additional or complimentary information. Specimens from different locations that could not be distinguished by color were analyzed by elemental analysis to see if they could be discriminated. Specimen descriptions can be found in Table 2.

6.4.1. Color Analysis: Munsell Color Scale Measurements

Munsell color designations were determined as described in Section 3.1.3 for sieved soils (see Section 4.2.5). Rather than providing a table of Munsell designations for each soil, the figures in Appendix 1 were generated to better illustrate the results of the color analysis. Each figure represents pages in the Munsell Soil Color Book. The orange numbers represent the sub-plot the specimens were taken from. A number on a colored square (chip) indicates that the soil specimen was assigned that color, while a number between two chips indicating that the specimen was assigned an intermediate color.

The first thing noted was the variation in color within each site, with some sites being quite homogeneous (e.g., HA), and others very heterogeneous (e.g., CC6, KNT). False exclusions may arise if

the known specimens are taken too far from the transfer location. For example, without proper characterization of the site, specimens from FIU1 sub-plots 2 or 4 would likely be discriminated from FIU1 sub-plots 3 and 6 (see Appendix 1). This is especially important in single transfer events from a specific location (for example, stepping in a muddy puddle in an otherwise dry area), and may be less important in cases where the transfer occurs continuously throughout an area (for example, walking through a backyard), though in all cases better characterization of the site will yield greater confidence in the results. For example, after characterizing FIU1 and CS1 by taking numerous specimens, one can be more confident in saying that a specimen originating from FIU1 would not be incorrectly associated with CS1 since there is no overlap in the color of any of the known specimens. In cases where there is significant overlap between the color designations of one site and those of another site, this would indicate that they are similar and cannot be discriminated by color alone. For example, USDA2 and USDA3 display a near total overlap and would be considered indistinguishable. This is not surprising, given that they are only one kilometer apart on the same estate. It will be interesting to see whether they can be discriminated by elemental analysis.

6.4.2. Elemental Analysis

Bulk pellets were prepared from all sites, as described in Section 4.2.5, except for HA and KNT, which did not have enough specimens with bulk soil left over. Composite pellets were prepared for some sites, containing equal amounts of soil from each of the sub-plots. Calibrator and RM pellets were also prepared as above. Elemental profiles were generated using LIBS and LA-ICP-MS methods as described in Section 4.3 and 4.4, respectively. Averages for all replicates of all pellets for each site are provided in Table 18. Interestingly, FIU1 was high in Si and Al, but low in Ca, which suggests that it may not be completely limestone, which may help to explain the clear discrimination seen in Section 6.2.

Table 18. Summary of the elemental profiles of each Miami-Dade County site. Average (Avg) concentrations and standard deviations (SD) for each isotope by LA-ICP-MS on bulk pellets, reported in ppm (µg/g). Values preceded by “<” indicate less than MDL.

Isotope	CC6		CS1		FC		FIU1	
	Avg	±SD	Avg	±SD	Avg	±SD	Avg	±SD
²⁷ Al	7290	2186	1109	839	2297	182	12739	26801
⁷⁵ As	3.9	0.4	1.1	0.4	2.2	0.3	2.4	1.5
¹³⁷ Ba	80	12	22	2	12	1	31	66
⁴² Ca	151760	17797	415352	51389	95618	23649	8917	5809
¹¹¹ Cd	0.16	0.04	<0.03	n/a	0.13	0.03	0.07	0.06
⁵⁹ Co	2	1	4	5	11	3	39	12
⁵² Cr	19	4	10	2	15	2	55	23
⁶³ Cu	8	3	4	6	10	3	3	2
⁵⁷ Fe	6393	578	1439	220	1485	147	4243	1696
²⁰² Hg	0.14	0.03	0.3	0.5	0.8	0.2	2.9	0.6
⁷ Li	21	9	1.1	1.0	6	0	27	12
²⁵ Mg	4476	800	3165	248	1340	709	676	558
⁵⁵ Mn	138	50	90	13	31	5	111	409
⁶⁰ Ni	6	1	3	1	4	1	21	10
³¹ P	487	75	149	62	295	33	180	400
²⁰⁶ Pb	27	8	10	13	17	3	14	6
²⁰⁷ Pb	27	8	9	12	17	3	13	6
²⁰⁸ Pb	28	8	9	12	18	3	12	6
¹²³ Sb	2.5	0.8	0.04	0.02	0.14	0.05	0.4	1.3
²⁹ Si	27925	11582	2501	1595	102434	25038	501303	77759
¹²⁰ Sn	2.0	1.8	0.2	0.1	1.3	0.4	0.7	0.2
⁸⁸ Sr	820	108	1556	189	1012	130	56	32
²³² Th	1.1	0.3	1.3	0.2	0.6	0.1	2.5	0.4
⁴⁷ Ti	301	88	63	40	167	15	942	219
⁴⁹ Ti	303	90	65	42	166	15	921	218
²⁰⁵ Tl	0.12	0.02	0.02	0.01	0.04	0.00	0.3	1.4
²³⁸ U	0.6	0.2	0.5	0.3	1.3	0.1	2.2	1.7
⁵¹ V	12	1	4	2	7	1	16	6
⁶⁶ Zn	35	13	3	1	12	5	15	19
⁹¹ Zr	10	3	3	2	12	2	85	28

Table 18, continued.

Isotope	KK		KS8		NW137		OSP1	
	Avg	±SD	Avg	±SD	Avg	±SD	Avg	±SD
²⁷ Al	12379	2122	3004	502	12497	6333	1484	521
⁷⁵ As	2.6	2.3	4.7	0.7	5.5	2.2	4	4
¹³⁷ Ba	27	2	59	6	40	16	14	5
⁴² Ca	106107	8840	181779	8037	47384	7266	82796	58047
¹¹¹ Cd	0.27	0.08	0.09	0.03	0.9	1.2	0.11	0.03
⁵⁹ Co	4	1	0.6	0.4	5	3	8	5
⁵² Cr	49	11	11	1	31	9	13	4
⁶³ Cu	8	2	4	1	34	26	7	5
⁵⁷ Fe	5430	858	9397	1518	7787	2849	3852	3890
²⁰² Hg	0.16	0.03	0.08	0.01	1.0	1.2	0.3	0.2
⁷ Li	36	9	4	1	17	10	3	1
²⁵ Mg	1286	141	3260	465	1832	735	948	471
⁵⁵ Mn	68	14	130	27	66	37	32	12
⁶⁰ Ni	10	2	3	1	9	3	3	1
³¹ P	576	91	206	48	718	271	327	177
²⁰⁶ Pb	32	6	26	8	73	60	18	13
²⁰⁷ Pb	32	6	26	8	73	62	18	13
²⁰⁸ Pb	33	6	26	8	73	60	18	13
¹²³ Sb	0.3	0.0	0.15	0.04	1.9	1.6	0.3	0.1
²⁹ Si	98147	11980	8733	1964	53492	38914	130060	104197
¹²⁰ Sn	1.0	0.2	0.4	0.1	1.3	0.7	0.5	0.3
⁸⁸ Sr	621	56	576	79	289	60	813	674
²³² Th	2.5	0.3	0.5	0.1	1.8	0.9	0.4	0.1
⁴⁷ Ti	762	91	138	28	618	287	187	62
⁴⁹ Ti	772	93	139	28	619	289	187	63
²⁰⁵ Tl	0.09	0.01	0.10	0.01	0.16	0.07	0.08	0.06
²³⁸ U	1.9	0.2	0.4	0.1	1.3	0.3	2.5	1.8
⁵¹ V	12	1	7	2	21	7	6	2
⁶⁶ Zn	44	18	9	2	910	979	22	9
⁹¹ Zr	28	5	5	1	19	6	28	33

Table 18, continued.

Isotope	OSP2		TREC		USDA2		USDA3	
	Avg	±SD	Avg	±SD	Avg	±SD	Avg	±SD
²⁷ Al	1098	235	66293	9816	2230	255	2200	677
⁷⁵ As	3.7	0.9	16	4	0.8	0.1	6.0	2.4
¹³⁷ Ba	14	6	41	10	4	1	23	9
⁴² Ca	101069	37879	17297	19280	3346	1937	136075	45102
¹¹¹ Cd	0.11	0.05	0.09	0.04	0.07	0.04	0.23	0.05
⁵⁹ Co	3	1	8	2	56	22	6	3
⁵² Cr	13	3	145	25	8	2	26	8
⁶³ Cu	8	1	25	10	3	4	28	7
⁵⁷ Fe	2252	424	34173	6197	1657	192	2928	455
²⁰² Hg	0.18	0.08	0.2	0.1	1.8	0.6	0.17	0.04
⁷ Li	2	1	64	10	3	1	3	1
²⁵ Mg	2368	915	3384	535	147	55	2577	1131
⁵⁵ Mn	36	9	652	117	36	11	63	11
⁶⁰ Ni	2	1	46	8	4	2	6	3
³¹ P	262	24	306	118	48	14	1498	248
²⁰⁶ Pb	27	20	43	9	6	2	27	8
²⁰⁷ Pb	28	21	43	9	7	2	27	8
²⁰⁸ Pb	28	21	44	9	7	2	27	8
¹²³ Sb	0.8	0.4	0.5	0.1	0.06	0.03	1.0	0.6
²⁹ Si	55470	23638	112943	27394	436327	57973	55148	36797
¹²⁰ Sn	0.5	0.2	3.0	0.7	0.4	0.1	0.9	0.1
⁸⁸ Sr	796	294	103	100	24	14	718	167
²³² Th	0.3	0.1	12	3	0.5	0.1	0.6	0.2
⁴⁷ Ti	130	77	3940	915	278	36	178	66
⁴⁹ Ti	131	79	3976	943	280	37	180	67
²⁰⁵ Tl	0.02	0.01	0.47	0.10	0.01	0.00	0.05	0.01
²³⁸ U	1.0	0.3	2.4	0.6	0.2	0.0	1.6	0.7
⁵¹ V	5	1	76	17	9	1	7	1
⁶⁶ Zn	52	27	24	5	7	2	52	20
⁹¹ Zr	7	4	124	35	42	20	9	4

6.4.2.1. Discrimination using LA-ICP-MS

The optimized discrimination element menu for LA-ICP-MS was determined to be ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{55}Mn , ^{88}Sr , ^{49}Ti , ^{238}U , and ^{51}V . Principal components analysis was used for visualization and LDA for classification.

The PCA score plots are shown in Figure 30. Duplicate pellets, represented by open circles, associated well with their sister pellets. Composite samples for FC1, KK, FIU1 represented by open diamonds, plotted near the middle of their respective clusters. Three PCs, expressing 87.7 % of the variation, were required to provide reasonable separation. Because there were so many sites, it was difficult to find an angle in a 3D plot in which all discriminated sites could be clearly seen. In Figure 30a, showing PC1 versus PC2, the three RMs (brown, black, and gray), as well as TREC (pink), were well separated. It should be noted that TREC was also the most different in color (the only reddish soil, see Appendix 1). Zooming in on the lower left corner, Figure 30b is obtained. Here it is clear that further separation was possible for FIU1 (indigo), CC6 (peach), USDA2 (light green), and CS1 (orange). Figure 30c is a plot showing PC2 versus PC3 (if one imagines the x and y-axes to be PC1 and PC2, respectively, then PC3 represents the z-axis). Now KS8 (yellow) was also separated. In addition, the overlap between KK (teal) and NW137 (light blue) in Figure 32b was resolved in Figure 32c using PC3, though there was a small cluster of NW137 quite far away from the rest. OSP1 (red) separated somewhat, but there was clearly a large degree of within-site heterogeneity, as each small cluster of points represented one sub-plot. FC1 (dark green), OSP2 (magenta), and USDA3 (purple) could not be separated from one another in any view. FC1 was located only a few km from USDA3. Although USDA3 (purple) overlapped with some sites, it was clearly separated from USDA2 (light green), showing that elemental analysis can be used to discriminate sites that were indistinguishable by color alone. No groupings were seen within the various agricultural soil types. For example, CS1 (orange) and FC1 (dark green) from USDA soil type 4 did not group together, nor did KS8 (yellow), NW137 (light blue), or CC6 (peach) from soil type 2. Soil types for each site are shown in Table 2 and Figure 9.

Figure 30. PCA score plots generated from LA-ICP-MS data of Miami-Dade County bulk pellets. The following discrimination element menu was used: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{55}Mn , ^{88}Sr , ^{49}Ti , ^{238}U , and ^{51}V . a. the full plot of PC1 versus PC2, b. PC1 versus PC2 zoomed in without TREC or the RMs, c. PC2 versus PC3 without TREC or the RMs.

The LDA canonical plots showed very similar trends and are therefore not shown. The confusion matrix is shown in Table 19, with 28 misclassifications out of 384 replicates, for a 92.7 % correct classification rate. As with the PCA results, the misclassifications were between FC1, OSP1, OSP2, USDA3, and NW137. Hierarchical clustering was also attempted, with similar results, but is not shown

because of space limitations. In all cases USDA2 and USDA3 were clearly distinguishable from one another by LA-ICP-MS.

Table 19. Confusion matrix for LDA with leave-one-out cross-validation on 384 LA-ICP-MS replicates from Miami-Dade County bulk pellets. Zeroes were left out to make it easier to read.

		Predicted Origin												
		CC6	CS1	FC1	FIU1	KK	KS8	NW137	OSP1	OSP2	TREC	USDA2	USDA3	Total
True Origin	CC6	20												20
	CS1		24											24
	FC1			20					8	3			1	32
	FIU1				116									116
	KK					32								32
	KS8						28							28
	NW137							20				4		24
	OSP1								12			8		20
	OSP2									20				20
	TREC										28			28
	USDA2											20		20
	USDA3			4									16	20

6.4.2.2. Discrimination using LIBS

The optimized discrimination element menu for LIBS was determined to be Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Li I 610.3/Mg I 517.3, Sr II 421.6/Ti I 336.1, Fe I 495.8/Mg I 518.4. Spectra from LIBS analysis of USDA2 (red, dotted) and USDA3 (black, solid) specimens are shown in Figure 31. Intensities were scaled to the height of the Sc II 360.5 nm peak. Five replicates of each were plotted to show that the variation within a pellet was much less than the variation between the two specimens. Differences were seen in Ba II 493.4, Fe I 495.8, Mg I 517.3 and Mg I 518.4 nm, and a group of titanium (I) emission lines. Principal components analysis was used for visualization and LDA for classification.

Figure 31. LIBS spectra of USDA2 (red, dotted line) and USDA3 (black, solid line) specimens. Five replicate measurements of each are shown.

The PCA score plots are shown in Figure 32. Three PCs, expressing 90 % of the variation, were required in order to provide reasonable separation. Because of the large number of sites and the reduced precision of LIBS compared to LA-ICP-MS, groupings were not detected as easily. As with LA-ICP-MS results, the three RMs (brown, black, and gray), as well as TREC (pink), were well separated (Figure 32a). Zooming in on the rest of the plot (see Figure 32b), some clustering was seen for KS8 (yellow), FIU1 (indigo), NW137 (light blue), and CC6 (orange). As with the LA-ICP-MS data, OSP1, OSP2, USDA3, and FC1 could not be separated. USDA3 and FC1 were located approximately 5 km apart, and OSP1 and OSP2 were less than 300 m apart.

Figure 32. PCA score plots generated from LIBS data of Miami-Dade County bulk pellets. The following discrimination element menu was used: Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Li I 610.3/Mg I 517.3, Sr II 421.6/Ti I 336.1, Fe I 495.8/Mg I 518.4. a. the full plot, b. zoomed in without TREC or the RMs. See Figure 30 for legend.

The confusion matrix from LDA of the LIBS data is shown in Table 20, with 128 misclassifications out of 565 replicates, for a 77.3 % correct classification rate. Most of the misclassifications were between FC1, OSP1, OSP2, and USDA3. Because of the reduced precision of LIBS data, some additional misclassifications were seen with all sites. Looking at the LDA canonical plot (see Figure 33), groupings were slightly better than with the PCA scores plot. No overlap was seen between USDA2 and USDA3, but because of the way the predictions are made (on the basis of the distances from the centroids of each group), the three circled replicates of USDA2 were misclassified with USDA3. These represented only three of the 50 USDA2 replicates, 40 of which were correctly classified.

Figure 33. LDA canonical plots generated from LIBS data of Miami-Dade County bulk pellets. The following discrimination element menu was used: Ba II 493.4/Mg I 517.3, Ca I 643.9/Mg I 518.4, Li I 610.3/Mg I 517.3, Sr II 421.6/Ti I 336.1, Fe I 495.8/Mg I 518.4. See Figure 30 for legend. USDA2 specimens misclassified as USDA3 are circled.

Table 20. Confusion matrix for LDA with leave-one-out cross-validation on 565 LIBS replicates from Miami-Dade County bulk pellets. Zeroes were left out to make it easier to read.

		Predicted Origin										Total
		CS1	FC1	FIU1	KS8	NW137	OSP1	OSP2	TREC	USDA2	USDA3	
True Origin	CS1	48	2									50
	FC1	6	31		1			5			17	60
	FIU1			58					1	1		60
	KS8				64	2	9					75
	NW137				3	48	4					55
	OSP1		10	4	1	5	12	13		1	4	50
	OSP2		3					44			8	55
	TREC								55			55
	USDA2			2				2	3	40	3	50
	USDA3		9		3			6			37	55

6.4.3. Conclusion

Separation of USDA2 and USDA3, which could not be discriminated by color alone, was possible using LIBS and LA-ICP-MS with PCA and LDA. Similar grouping trends were observed for both LIBS and LA-ICP-MS, with LA-ICP-MS providing better precision and higher correct classification rates (92.7%, compared to 77.3% for LIBS). Although perfect discrimination was not achieved, this approach could be used to narrow down a search region in a forensic investigation without a crime scene. No correlation was observed between USDA-defined agricultural soil type and elemental composition.

6.5. Conclusions

Discrimination methods were developed for elemental profiles generated by LIBS and LA-ICP-MS. In a contaminated area, specimens at varying distances from the contamination source were 100 % discriminated by ANOVA with Tukey's post-hoc testing. In a proof of principle study, specimens from two very different locations were discriminated using ANOVA with Tukey's post-hoc testing, PCA and LDA at 99 % or better. In a more challenging and realistic study, sieved soil specimens from three similar sites were discriminated using both PCA and LDA at 94.5 % or better. In a larger study, 14 sites throughout Miami-Dade County were discriminated using PCA and LDA at 77.3 % or better. Specimens from two nearby sites that could not be distinguished by color (USDA2 and USDA3) were distinguishable by elemental profile. Most of the indistinguishable specimens originated from sites located within a few kilometers of each other: OSP1 and OSP2; USDA3 and FC1; CC6 and KNT.

The results of these studies showed that discrimination of specimens from different locations was possible using either LA-ICP-MS or LIBS with univariate statistical tools, such as ANOVA, and with multivariate statistical tools, such as PCA and LDA. Although the precision of LIBS was not quite as good as that of LA-ICP-MS, and correct classification rates were lower, similar information was obtained with both techniques.

Spatial heterogeneity, in both elemental profile and color, was determined to be site-specific; some sites showed very tight groupings while others showed a larger spread, with tighter groupings at the within-sub-plot and within-sample level, highlighting the importance of proper characterization of each site to determine the within-site variation.

The discrimination element menu was important in achieving the best possible discrimination, and various approaches were investigated for determining the optimal combination of elements. In a real forensic case, optimization of the element menu should be performed on the known specimens only (i.e., generating the model using the training set of known specimens). Once a good separation of the known specimens is achieved, only then should the model be tested using the questioned specimen(s).

7. Expanding the Sample Set: Different Soil Types & Different Geographic Regions

From a legal standpoint, one could argue that if the main source of elemental variation in soils is its mineral content, which is largely derived from its underlying lithology, then the elemental profiles from calcareous lithologies in Miami-Dade County could be similar to those obtained in Washington. If elemental profiles were used to associate a suspect to a particular location, and the evidence were presented in court, this is surely something a defense attorney would point out, in order to weaken the confidence in that association. It is therefore necessary to explore the variations in a particular lithology over larger distances in order improve confidence in an association.

From a geological standpoint, calcareous lithologies account for much of the primary underlying bedrock in Florida, as well as the central states and southwest Washington (see Figure 34). However, much of the United States contains other primary lithologies, which contain different minerals, some of which are quite hard (for example, quartz, zircon, and granite). These may be more difficult to mill and/or couple to the laser irradiation, resulting in reduced pellet homogeneity and analytical performance. In addition, the element menu may need to be adjusted to reflect the differences in chemical composition. Basaltic lithologies account for regions in the mountainous regions of the western USA and to a smaller extent around Lake Superior. Granitic lithologies are more widespread, common along the west coast, in Idaho, east of the Appalachians, New England, and small pockets in Missouri and Tennessee. The primary lithologies of the remaining regions in the USA are argillaceous, which are mostly shales. In eastern Canada and neighboring regions in the northeastern USA, glacial till is the dominant lithology. Soil samples from regions with these additional primary lithological types were tested to determine whether they were compatible with the methods developed in Sections 4 and 6. Analytical methods were re-optimized to accommodate these different lithologies. Within particular lithologies, forensic discrimination was performed.

Figure 34. Map of the USA showing the primary lithologies for specimens available from the USGS archives for the North American Soil Geochemical Landscapes Project. Lithologies shown: granitic (yellow and pale yellow), basaltic (orange), argillaceous (blue and light blue), and calcareous (green). Specimens used in this chapter are circled.

7.1. USGS Specimens

The United States Geological Survey (USGS) has archives of soil and rock samples taken as part of various studies that are available for analysis upon request. The North American Soil Geochemical Landscapes Project took soil samples at various depths, including surface samples, throughout the conterminous USA in 2010 [4,90]. Sampling density for this study was the highest of any large-scale study to date, at one site per 1600 km² [21]. Sub-samples of surface soil specimens were obtained from the USGS archives from regions with three lithologies: granitic, basaltic, and argillaceous (see Figure 34). The granitic group included nine specimens from one region in South Carolina (SC) and two neighboring regions in Idaho: 15 from ID1 and 31 from ID3, which also encompassed some parts of WA and Oregon. The basaltic group included 15 specimens from one region in California (CA), and ten specimens from one region in Oregon (OR) next to ID3. The argillaceous group included 12 specimens from one region in Montana (MT2) and 19 specimens from one region in Ohio (OH), which encompassed some parts of West Virginia. Note that the ID3 region contained some samples with basaltic lithology, which will become important later.

Samples were spiked with scandium and indium, milled, and pelletized as described in Sections 3.1.4 and 3.1.6. Since the 30 minute milling time was optimized using Miami-Dade County specimens, which were calcareous and not particularly hard minerals, milling time was revisited when the USGS and RCMP specimens were received, since these contained much harder minerals (for example, quartz, zircon, and granite). Three specimens of each soil type (granitic, basaltic, and aluminosilicate) as well as two additional calcareous specimens, were prepared using 30 minutes and 45 minutes at 25 Hz. Analysis by LIBS and LA-ICP-MS was performed on each as described in Sections 3.2.1 and 3.2.2. No appreciable difference in precision was observed, therefore milling for 30 minutes was deemed sufficient.

7.2. RCMP Specimens

Specimens were taken throughout Canada as part of a study undertaken by the Royal Canadian Mounted police (RCMP). Specimens were prepared with indium as the only internal standard, using the pellet method. Pellets were obtained from a sub-set of the specimens from southeastern Canada, made up largely of glacial tills from granitic and argillaceous sources in Quebec (QC), New Brunswick (NB) and Nova Scotia (NS) (see Figure 35). Elemental profiles were measured by LA-ICP-MS and LIBS as described in Sections 3.2.1 and 3.2.2.

Figure 35. Map of southeastern Canada showing the locations of the specimens used in this study. Quebec (red), New Brunswick (yellow), and Nova Scotia (blue).

7.3. Results & Discussion

7.3.1. Discrimination of USGS Specimens

7.3.1.1. Granitic Lithologies

The USGS granites were analyzed first. Principal components analysis scores plots were generated for both LIBS (Ca, Cu, Li, Mg, Si, Sr, Ti, and V) and LA-ICP-MS (Ca, Cr, Li, Mg, Rb, Si, Sr, Ti, V, and U) data. There was a clear separation between the SC (blue) and the Idaho specimens, though ID1 (red) and ID3 (orange) specimens overlapped (see Figure Figure 36a). Upon further examination of the lithologies, the SC specimens were true granites, while the Idaho specimens were granodiorites. This slight difference in mineral composition, as well as the large distance that separates the two regions, and the differences in surrounding lithologies may have been factors.

Figure 36. PCA score plots of granitic, and some basaltic, specimens from SC (blue), ID1 (red) and ID3 (orange and yellow) regions.

a. LA-ICP-MS data, b. LA-ICP-MS data zoomed in and rotated, c. LIBS data zoomed in and rotated. Most specimens to the left of the green line were granitic (dots), while most specimens to the right were basaltic (triangles).

Zooming in on the Idaho specimens (see Figure 36b and c), there was still no clear differentiation between ID2 and ID3 regions with either LA-ICP-MS or LIBS data, most likely because they were located right next to one another. Another look at the lithologies revealed that there were some basaltic areas within ID3, especially along the border with OR and WA. There was a grouping of the basaltic samples (triangles) on the right of each PCA scores plot. Overall, differences were greatest for specimens separated by large

distances. On a more local scale, differences in lithology seemed to drive the differences in elemental profile, but other local effects also contributed.

7.3.1.2. Argillaceous and Basaltic Lithologies

Additional basaltic specimens from the OR region adjacent to ID3 were taken, as well as another set of specimens from CA, as shown on Figure 34. Argillaceous specimens were taken from the MT2 and OH regions, as well as a few specimens from other sampled regions (ID, WA, AZ, MT, and ND) that turned out to be argillaceous as well. Analysis by PCA was performed in order to visualize the data and try to distinguish between the two different lithologies (see Figure 37). The following discrimination element menu was used: Ca, Mn, Th, U, and V, with the first three PCs representing 91 % of the variation. Basaltic specimens were plotted as triangles and argillaceous as diamonds. Each region was represented by a different color: yellow for OR, orange for ID3, light green for CA, dark green for AZ, magenta for MT2, purple for OH, and black for the others.

Figure 37. PCA score plot from LA-ICP-MS data of argillaceous (diamond) and basaltic (triangle) specimens. Points were plotted as yellow for OR, orange for ID3, light green for CA, dark green for AZ, magenta for MT2, purple for OH, and black for the others.

As in the previous section, the two lithologies showed separate groupings. In Figure 37, the argillaceous specimens (diamonds) plotted on the left and the basaltic specimens (triangles) on the right with a few exceptions. Most of the argillaceous specimens were shales. The AZ and ID3 argillaceous specimens near the center of the plot (dark green diamonds and orange diamonds, respectively) were not actually shales, but siltstone and sandstone. In addition, these two argillaceous AZ and ID3 specimens both originated very close to basaltic regions, and therefore it would not be surprising to find some basaltic contributions in their elemental profiles.

Focusing on just the shales, the element menu was then adjusted to see if the OH shales could be distinguished from the MT2 shales. Analysis by PCA was then performed using the optimal element menu (P, Ti, Ba, and Pb, see Figure 38). Excellent separation between the two regions was observed with the exception of one specimen (four replicates of MT10942). Therefore it is not likely that shales from the two regions would be confused.

Figure 38. PCA score plot from LA-ICP-MS data of shales from MT2 (magenta) and OH (purple) regions.

7.3.1.3. Forensic Discrimination of Ohio Shales

A sub-set of five specimens (OH1788, OH4860, OH5884, OH8956, and OH9980) taken from a relatively densely-sampled region in OH (see Figure 39a) were used to determine the forensic applicability within a small region. The maximum distance between specimens was 42.2 km (26.2 miles) and the minimum distance was 4.1 km (2.6 miles) between OH4860 and OH8956. While the element menu used in the previous section was meant to unify the OH shale specimens in contrast with the MT2 shale specimens, a different element menu that highlighted the differences between these five OH shale specimens was required here. Looking at the data, differences were seen in a large number of elements, so the element menu was refined to achieve good separation between the specimens and tight clustering of the four

replicates of each specimen. The optimal discrimination element menu was determined to be Li, Mg, Al, P, Cu, and U. Analysis by LDA was performed on the five specimens using this discriminant element menu and the canonical plot is shown in Figure 39b. Each of the specimens was clearly separated from all others. The confusion matrix is shown in Table 21, with no misclassifications (a 100 % correct classification rate). Forensic discrimination was therefore possible for these specimens. If this had been a real case, more known specimens from each location would have been taken in order to get a better idea of the variation within a location and therefore better confidence in any resulting association or discrimination.

Figure 39. Forensic examination of five shale specimens from Ohio.

a. Map of southern Ohio showing the locations of the five specimens (circled). b. LDA canonical plot generated from LA-ICP-MS data of the five specimens. The following discrimination element menu was used: Li, Mg, Al, P, Cu, and U.

Table 21. Confusion matrix for LDA with leave-one-out cross-validation on 20 LA-ICP-MS replicates from the five OH shale specimens in Figure 39.

The following discrimination element menu was used: Li, Mg, Al, P, Cu, and U.

		Predicted Origin					Total
		OH1788	OH4860	OH5884	OH8956	OH9980	
True Origin	OH1788	4	0	0	0	0	4
	OH4860	0	4	0	0	0	4
	OH5884	0	0	4	0	0	4
	OH8956	0	0	0	4	0	4
	OH9980	0	0	0	0	4	4

7.3.2. Discrimination of RCMP Specimens

Many combinations of elements were tested for the discrimination element menu, and the most discriminating were chosen. Principal components analysis scores plots were generated for both LIBS (Al, Ba, Cr, Cu, Mg, Mn, and Sr, see Figure 40) and LA-ICP-MS (Al, Ba, Cr, Mn, Sb, Se, Sr, Ti, U, and V, see Figure 41) data. In both plots, some clustering was seen for the QC (red) and NS (blue) specimens, while NB (yellow) specimens overlapped both. Nova Scotia specimens were taken from two areas: the mainland (Truro, Halifax, Dartmouth, Liverpool, and Lunenburg) and Cape Breton Island (all other NS specimens). The mainland specimens were colored a lighter blue in the plots, and some slight clustering was observed. The primary lithologies were mainly glacial tills, but the secondary lithologies were granites (dots), granites mixed with argillaceous minerals (X), argillaceous minerals mixed with granites (+), and mixtures of argillaceous, granites and calcareous minerals (*). In the LA-ICP-MS PCA scores plot (Figure 41), granites mixed with argillaceous minerals (X) dominated the left side of the plot, while the mixtures containing calcareous minerals were on the right. Granites (dots) and argillaceous minerals mixed with granites (+) were in the center. Trends in the LIBS PCA scores plot (Figure 40) were not as clear as with LA-ICP-MS.

Figure 40. PCA scores plot of RCMP specimens using LIBS data.

Quebec (red), New Brunswick (yellow), and Nova Scotia (Mainland: blue, Cape Breton Island: light blue) using LIBS data. The following element menu was used: Al, Ba, Cr, Cu, Mg, Mn, Sr. Primary lithology was glacial till; secondary lithology was granites (dots), granites mixed with argillaceous minerals (X), argillaceous minerals mixed with granites (+), and mixtures of argillaceous minerals, granites and calcareous minerals (*).

Figure 41. PCA scores plot of RCMP specimens using LA-ICP-MS data.

Quebec (red), New Brunswick (yellow), and Nova Scotia (Mainland: blue, Cape Breton Island: light blue) using LA-ICP-MS data. The following element menu was used: Al, Ba, Cr, Cu, Mg, Mn, Sr. Primary lithology was glacial till; secondary lithology was granites (dots), granites mixed with argillaceous minerals (X), argillaceous minerals mixed with granites (+), and mixtures of argillaceous minerals, granites and calcareous minerals (*).

Given that these regions were glacial tills (see Section 2.2.2); it was not surprising that they were on the whole not well-separated, since the movement of the glaciers served to redistribute minerals over a broad area. Local variations from other sources seemed to exist, but were subtle. These findings may also apply to parts of the northeastern USA that were also subject to glaciations periods.

7.3.2.1. Forensic Discrimination of a Sub-Set of the RCMP Specimens

A forensic study was conducted on the region with the highest sampling density: the northern part of Cape Breton Island (known as the Industrial Cape Breton region), including the communities of Sydney (3 specimens), North Sydney (1 specimen), Sydney Mines (1 specimen), and Glace Bay (1 specimen) (see inset in Figure 35 for their locations). Analysis by LDA was performed on the LA-ICP-MS data.

The following element menu was used initially: As, Cd, Cr, Cu, Li, P, Pb, Rb, Sb, Sn, Ti, U, and Zn. No misclassifications were obtained, and the replicates of the four communities were clearly separated from one another on the canonical plot. Lead was the most discriminating element, providing a significant separation of Sydney mines from the rest of the communities. Other highly discriminating elements included Zn and Cu. The least discriminating elements were Sn, Ti, and U. These were removed and the resulting LDA re-analysis yielded very similar results; once again with clear separations and no misclassifications. The LDA was re-analyzed further with Pb also removed in order to see if the communities could still be distinguished. The separations were less obvious on the canonical plot, but all replicates were still correctly classified. Instead of removing Pb, other less discriminating elements (Cd, Cr, Li, and Rb) were removed. Obvious separations were observed, with no misclassifications. Using all element menus, a 100 % correct classification rate was obtained.

Figure 42. LDA canonical plots generated from LA-ICP-MS data of RCMP Industrial Cape Breton region. All communities had glacial till primary lithologies. North Sydney's secondary lithology consisted of argillaceous minerals mixed with granites (+), while the other communities consisted of mixtures of argillaceous minerals, granites and calcareous minerals (*). The following discrimination element menus were used: a. As, Cd, Cr, Cu, Li, P, Pb, Rb, Sb, Sn, Ti, U, and Zn; b. As, Cd, Cr, Cu, Li, P, Pb, Rb, Sb, and Zn; c. As, Cd, Cr, Cu, Li, P, Rb, Sb, and Zn; d. As, Cu, P, Pb, Sb, and Zn.

Table 22. Confusion matrix for LDA with leave-one-out cross-validation on 24 LA-ICP-MS replicates from RCMP Industrial Cape Breton region bulk pellets for all element menus listed in Figure 42.

		Predicted Origin				Total
		Glance Bay	North Sydney	Sydney	Sydney Mines	
True Origin	Glance Bay	4	0	0	0	4
	North Sydney	0	4	0	0	4
	Sydney	0	0	12	0	12
	Sydney Mines	0	0	0	4	4

The primary lithology of all four communities was glacial till. The secondary lithology of North Sydney consisted of argillaceous minerals mixed with granites (+), while the rest of the communities consisted of mixtures of argillaceous minerals, granites and calcareous minerals (*), with Sydney in particular possibly containing gypsum (calcium sulfate dihydrate). These lithological differences did not seem to play a significant role using this particular element menu, and calcium was not a discriminating element. Looking at the spatial distance between the communities, one could suppose that North Sydney and Sydney Mines would be the most similar, but this was not the case. Another factor to consider was industrial land use. Glance Bay was once a coal mining city. Sydney Mines was named for its coal mines, and was later home to large steel manufacturing plants. Sydney was also a steel city. North Sydney has always been primarily a port community. None of these factors seemed to fully explain the trends; therefore it was likely that the differences resulted from other local factors.

In conclusion, specimens from each of the four communities were discriminated at a 100 % correct classification rate. However, the number of samples was small: one specimen (measured in four replicates) per community for all but Sydney (3 specimens, each measured in four replicates) was not really enough to properly characterize these communities. It was possible to discriminate specimens from these communities, but the 100 % correct classification rate was considered a preliminary result, with more specimens needed from each community for a more accurate discrimination.

7.3.3. The Bigger Picture

Looking at specimens from multiple regions of each of the main lithologies, a snapshot can be obtained of the variation that can be expected throughout the USA and Eastern Canada. A summary of the concentrations of each lithological type, by region, is given in Table 23. All data were obtained by LA-ICP-MS analysis of bulk, milled, pelletized specimens from the Miami-Dade (see Section 6), USGS, and RCMP sample sets. Elements like Mg, P, Ca, Ti, and Zn showed large differences between regions, and it was not surprising that these were commonly selected for discrimination element menus. Other elements, such as Tl, and Hg, showed very little variation.

Miami-Dade County specimens were much lower in most elements than the other regions, with the exception of Ca and Sr (Lu was not reported since it was spiked as an internal standard for some specimens). The basaltic specimens from ID3 (OR/WA/ID) were much higher than all other specimens in Co, Ni, Cu, Zn, As, Se, Cd, Sb, and Lu, which helped to explain why it separated from the rest of the Idaho specimens in Section 7.3.1. Some of these elements showed very little variation in all other specimens, which further underscored the importance of monitoring as many elements as possible, even the ones that have not been particularly discriminating in the past. Within a particular lithology, significant differences were seen according to region. This indicated that local effects far outweighed the underlying lithology in their contributions to the elemental profile. Examples of such effects may include mixing with other nearby lithologies, human activity (e.g., industry, farming, mining, soil erosion, or urban development) and natural phenomena (e.g., dust storms, tsunamis, earthquakes, volcanic eruptions, or glaciation).

Table 23. Summary of elemental profiles for the major lithological types (Lith), by region (Reg).

Average (Avg) LA-ICP-MS concentrations and standard deviations (SD) reported for each isotope (Iso) in ppm ($\mu\text{g/g}$) for n pellets analyzed (4 replicate measurements/pellet). Two-letter abbreviations indicate states or provinces except for SE (Southeastern). The secondary lithology of glacial till was mixtures of argillaceous and granite.

Lith.	Calcareous		Argillaceous			Glacial Till	Granitic		Basaltic	
Reg.	FL (Miami) n=96	Other (MN, AZ, WA) n=6	MT n=14	OH n=19	Other (ID, WA, AZ, MT, ND) n=9	SE Canada (NS, NB, QC) n=41	ID/ WA n=28	SC n=8	OR/ WA/ ID n=12	CA n=16
Iso.	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD	Avg $\pm$ SD
⁷ Li	20 $\pm$ 19	30 $\pm$ 16	31 $\pm$ 6	51 $\pm$ 20	35 $\pm$ 14	36 $\pm$ 11	45 $\pm$ 20	18 $\pm$ 4	23 $\pm$ 7	30 $\pm$ 9
²⁵ Mg	1811 $\pm$ 1362	10879 $\pm$ 6097	10955 $\pm$ 6484	4828 $\pm$ 2189	14298 $\pm$ 12917	9055 $\pm$ 3834	12923 $\pm$ 10896	2231 $\pm$ 1285	9738 $\pm$ 5011	16575 $\pm$ 13480
²⁷ Al	10204 $\pm$ 16543	70624 $\pm$ 47944	72232 $\pm$ 18467	70338 $\pm$ 17650	75657 $\pm$ 31097	51779 $\pm$ 14684	101166 $\pm$ 40375	93985 $\pm$ 42770	67485 $\pm$ 27216	126482 $\pm$ 27985
²⁹ Si	214639 $\pm$ 217172	573673 $\pm$ 272284	738689 $\pm$ 159313	647611 $\pm$ 143884	565952 $\pm$ 218284	258260 $\pm$ 124773	611074 $\pm$ 213852	921488 $\pm$ 162698	433138 $\pm$ 160965	532208 $\pm$ 213716
³¹ P	333 $\pm$ 350	1170 $\pm$ 530	817 $\pm$ 222	789 $\pm$ 348	1360 $\pm$ 557	1045 $\pm$ 652	1134 $\pm$ 554	553 $\pm$ 228	1434 $\pm$ 529	1270 $\pm$ 480
⁴² Ca	92484 $\pm$ 106999	25526 $\pm$ 16688	16396 $\pm$ 18035	3878 $\pm$ 2945	28294 $\pm$ 27173	7768 $\pm$ 6530	20442 $\pm$ 11980	3014 $\pm$ 2889	26951 $\pm$ 15961	27902 $\pm$ 25665
⁴⁷ Ti	757 $\pm$ 1001	5093 $\pm$ 2204	4382 $\pm$ 479	7046 $\pm$ 1727	8242 $\pm$ 8790	4093 $\pm$ 2108	5732 $\pm$ 2693	11731 $\pm$ 6872	8433 $\pm$ 4730	9325 $\pm$ 4601
⁴⁹ Ti	759 $\pm$ 1011	5077 $\pm$ 2128	4300 $\pm$ 447	7194 $\pm$ 1821	8224 $\pm$ 8684	4148 $\pm$ 2154	5692 $\pm$ 2616	11857 $\pm$ 7057	8530 $\pm$ 4768	9316 $\pm$ 4644
⁵¹ V	16 $\pm$ 19	85 $\pm$ 28	95 $\pm$ 23	91 $\pm$ 21	144 $\pm$ 131	78 $\pm$ 23	82 $\pm$ 48	92 $\pm$ 47	185 $\pm$ 126	220 $\pm$ 105
⁵² Cr	40 $\pm$ 39	57 $\pm$ 14	104 $\pm$ 34	87 $\pm$ 22	140 $\pm$ 262	69 $\pm$ 31	82 $\pm$ 140	58 $\pm$ 25	35 $\pm$ 18	146 $\pm$ 85
⁵³ Cr	32 $\pm$ 44	56 $\pm$ 14	106 $\pm$ 36	87 $\pm$ 22	141 $\pm$ 267	67 $\pm$ 32	83 $\pm$ 149	58 $\pm$ 27	28 $\pm$ 24	152 $\pm$ 89
⁵⁵ Mn	101 $\pm$ 162	760 $\pm$ 290	762 $\pm$ 295	1248 $\pm$ 721	987 $\pm$ 507	977 $\pm$ 600	1138 $\pm$ 349	1122 $\pm$ 776	4538 $\pm$ 5417	1832 $\pm$ 550
⁵⁷ Fe	6595 $\pm$ 8339	33542 $\pm$ 13554	36618 $\pm$ 12175	35107 $\pm$ 9812	52508 $\pm$ 41755	33904 $\pm$ 8766	41046 $\pm$ 18279	43855 $\pm$ 19967	64573 $\pm$ 27344	76337 $\pm$ 28154
⁶⁰ Ni	13 $\pm$ 14	19 $\pm$ 4	30 $\pm$ 9	32 $\pm$ 10	55 $\pm$ 96	29 $\pm$ 16	26 $\pm$ 31	21 $\pm$ 13	299 $\pm$ 439	88 $\pm$ 49

Table 23, continued.

Lith.	Calcareous		Argillaceous			Glacial Till	Granitic		Basaltic	
Reg.	FL (Miami) n=96	Other (MN, AZ, WA) n=6	MT n=14	OH n=19	Other (ID, WA, AZ, MT, ND) n=9	SE Canada (NS, NB, QC) n=41	ID/ WA n=28	SC n=8	OR/ WA/ ID n=12	CA n=16
Iso.	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD	Avg ± SD
⁶³ Cu	9±12	30±12	23±9	21±8	34±31	37±21	20±11	27±14	9019 ±16034	65±23
⁶⁶ Zn	78±323	277±521	76±23	87±29	74±39	96±57	90±30	69±24	2168±3518	92±27
⁷⁵ As	4±4	6±2	9±3	13±4	9±9	13±10	6±3	4±1	2121±3582	4±1
⁸² Se	1.0±0.4	6±15	0.7±0.3	0.9±0.2	0.6±0.3	1±1	1.0±0.5	1.3±0.3	37±61	1±1
⁸⁵ Rb	5±6	71±12	90±16	89±19	67±17	75±20	114±37	121±87	307±389	42±16
⁸⁸ Sr	459±463	305±107	200±96	112±93	246±121	89±52	437±190	186±158	266±193	415±269
⁹¹ Zr	44±45	635±306	696±390	880±323	495±253	399±526	579±324	4352±4777	727±423	408±156
¹¹¹ Cd	0.2±0.3	0.6±0.8	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.0	15184 ±25093	0.2±0.1
¹¹⁸ Sn	1.1±1.1	2±0.5	2±0.3	3±1	2±1	13±17	2±1	5±6	2±1	2±0.5
¹²⁰ Sn	0.9±0.9	1.9±0.4	1.7±0.4	2.8±1.5	2.0±0.8	13±17	2±1	5±6	5±6	2±0.5
¹²³ Sb	0.5±0.8	0.9±0.4	0.9±0.3	0.9±0.3	0.6±0.2	0.6±0.4	0.6±0.4	0.6±0.2	8±12	0.4±0.2
¹³⁷ Ba	27±20	702±230	867±218	553±191	713±245	397±171	1088±347	1578±2215	607±466	768±306
¹⁷⁶ Lu	n/a	0.9±0.3	0.9±0.3	1.2±0.3	0.8±0.3	0.6±0.4	0.8±0.5	3±2	25±41	0.7±0.2
²⁰² Hg	1.1±1.3	2±2	1.1±0.4	0.9±0.4	0.9±0.4	0.1±0.1	1.2±0.6	3±1	2±2	0.7±0.6
²⁰⁵ Tl	0.1±0.1	0.4±0.1	0.6±0.1	0.7±0.3	0.4±0.1	0.5±0.1	0.5±0.1	0.8±0.4	0.5±0.3	0.3±0.1
²⁰⁸ Pb	24±23	110±200	18±3	29±12	20±5	69±122	27±10	39±12	19±20	14±5
²³² Th	2±3	9±3	11±2	13±3	9±3	8±5	16±12	16±6	6±4	6±2
²³⁸ U	2±1	3±1	4±1	5±1	3±1	2±1	4±3	6±2	2±1	2±1

7.4. Conclusions

In general, differences were greatest for specimens separated by large distances, even within the same lithological type. Local effects far outweighed the underlying lithology in their contributions to the elemental profile. In answer to the question posed at the beginning of this section, it is highly unlikely that a calcareous soil specimen from Miami would be mistaken for one from Washington. This work provides the beginnings of a database of elemental profiles that may be useful to forensic examiners to determine the source (provenance) of a questioned soil specimen.

In addition, bulk elemental analysis of soils will find utility as a tool to narrow down the origin of a soil sample. Since lithological and elemental maps, models, and databases are available for many regions, it will be possible to eliminate certain areas from consideration as a possible source of an unknown sample and therefore narrow down a search area, for example. In this regard, where the soil did NOT originate from is almost as important as where the soil could have originated from.

8. Small Specimens: Tape-Mounting for Forensic Transfer Evidence

The above pellet methods were developed for bulk analysis; in effect large specimens (a minimum of approximately 0.47 g of soil) are required. For most environmental, geological, agricultural, and archaeological applications, and a few forensic applications, these methods are ideal. Pellets are homogeneous, robust, and can easily incorporate an additional internal standard. In discussing the methods with forensic examiners, however, a number of concerns were raised. The most important was that in cases involving transfer evidence, the soil specimens are quite small (on the order of tens of milligrams). Another concern was the amount of time and number of steps required to get the soil into pellet form. The pellet method takes two to four days, depending on the number of specimens to be processed. And lastly, forensic examiners prefer methods that minimize the potential for loss or contamination, and that are non-destructive, allowing for additional analyses by other confirmatory or complementary techniques, re-analysis by counter-experts, and archival storage. While pellets are easily stored and re-analyzed by other elemental analysis techniques, the milling process is destructive to certain properties of the soil, such as color and texture, and has the potential for loss (some powder adheres to the milling surfaces) and contamination (hard minerals in the soil may abrade small amounts of the milling surfaces).

Analysis of loose soil, as is done by the ChemCam on the Mars Rover is only practical in large spaces with large amounts of soil. If this were attempted on forensic specimens of only a few milligrams, the first laser pulse would blow the whole sample away. The soil particles must be fixed in order for the specimen to withstand repeated laser pulses. Some researchers have mounted powders on tape or adhesive SEM stubs [91-95] for elemental analysis by SEM, SEM/EDX, LIBS, and LA-ICP-MS. Cengiz et al [91] compared stub-mounted soil with pellets by SEM/EDX. Particles were coarsely sieved (< 5 mm), resulting in heterogeneous pellets, which far out-performed the stub-mounted soil in terms of precision. Pye & Croft [95] tested raster mode on stub-mounted soils by SEM, which improved precision over spot mode because of the heterogeneity of the soil. They also compared sieved and bulk soils using raster mode and found no difference in precision. Sun et al [92] mounted milled and diluted iron ore onto double-sided tape and measured Si and Mn with LIBS. Results were comparable to pellets. Anzano et al [93,94] placed milled geological materials on tape in a widely-spaced fashion and performed single-particle analysis by LIBS and

LA-ICP-MS. Results were inconclusive. None of these studies took into account the background contribution from the tape.

If the soil is not milled or diluted before mounting on tape, other analyses may be performed on the mounted sample, such as color, microscopy, and mineralogy. If a non-permanent adhesive is used, individual particles may be removed for further mineralogical analysis, such as density, polarized light microscopy or XRD. If a transparent adhesive is used on a transparent surface (e.g., a glass slide), optical microscopy can be performed directly on the tape-mounted soil using transmitted light. Methods were developed for a simple sample preparation procedure involving deposition of a thin layer of soil on adhesive tape. The LIBS and LA-ICP-MS methods were re-optimized to accommodate these tape-mounted samples. Analytical performance and discrimination capabilities were compared with the pellet method [96].

8.1. Method Development

Before attempting to reduce the sample preparation steps (which will be discussed in Section 8.2), spiked, milled RMs and calibration standards were used to determine whether quantitative analysis of tape-mounted specimens by LIBS and LA-ICP-MS would be feasible and to optimize the analysis parameters. These were mounted on tape as described in Section 3.1.5, but were not sieved since the particle size was already small. The methods for analysis of pellets by both LA and LIBS developed in Section 4 involved the acquisition of multiple laser pulses in depth profile or spot mode. This would bore through the thin layer of soil to the tape underneath after only a few laser pulses, and eventually the glass slide. The resulting signal would contain only low levels from the soil and large contributions from the tape, and possibly the glass. Therefore a line scan (or raster) was required in order to ablate a fresh portion of soil for each laser pulse. Line or raster mode has the added advantage of accounting for the heterogeneity of the deposited soil by averaging the signals over a larger number of particles. This is an advantage if the milling step (which homogenizes the particles) is to be eliminated from the processing. It also accounts for any small inhomogeneities of the internal standard [96].

8.1.1. Characterization of the Tape

The tape used was 3M Scotch Removable Poster Tape #109, which is composed of a 3.0 mil polyethylene film backing with synthetic acrylic adhesive on both sides, for a total thickness of 4.0 mils. The tape is lined on one side with a white silicone paper liner before being rolled onto the dispenser. The adhesive is a proprietary formulation, and no further information could be obtained. Tape blanks were analyzed in order to determine what was present and where possible interferences may occur.

In LIBS, a number of peaks were identified that occurred in the tape but not in soils: 386.2, 387.1 (see Figure 43), 419.7, and 516.5 nm (a shoulder on the Mg I 516.7 nm). These peaks were broader than elemental emission lines, and most likely a result of molecular emissions from the polymers. They were observed in tape (red) with accumulation of only one laser pulse, while they were not observed in the soil pellet (brown), even after five pulses. For soil mounted on tape, they were not observed with the first laser pulse (cyan), which means that only the soil was ablated. This ablation left the tape exposed, so the second pulse (dark blue) in the same location ablated the tape, resulting in tape peaks being observed. These wavelengths were monitored while optimizing the LIBS parameters on tape-mounted soils.

Figure 43. LIBS spectra of tape.

One laser pulse on blank tape (red), one laser pulse on NIST SRM 2704 mounted on tape (cyan), two laser pulses on NIST SRM 2704 mounted on tape (dark blue), and five laser pulses on a NIST SRM 2704 pellet (brown). Peaks found in the tape but not the soil are circled.

For LA-ICP-MS analysis of the blank tape, since it did not contain a quantified internal standard, semi-quantitative analysis was performed in mass scan mode and mass spectra were normalized to ^{14}C . The normalized spectra from ablation of blank cellulose nitrate filters (see Section 9.2) that had previously been

analyzed by microwave digestion ICP-MS were overlaid with the spectra from the blank tape for comparison (see Figure 44). The peak heights of most elements in the blank tape were lower than those of the blank filters, which in turn were < MDL by microwave digestion ICP-MS. Cr and Fe levels were close to the MDL (1.5 and 24 ppm, respectively). Silicon levels were approximately half of the filter levels (90 ppm). These levels were below the concentrations anticipated in soils, so were deemed negligible. No scandium or indium was detected in the tape blanks. Scandium was chosen as the internal standard since it was used in the pellets to which the tape-method would eventually be compared (see Section 8.3).

Figure 44. Mass spectra of blank tape (orange). Spectra of quantified blank filters (blue, see Section 9) were added for comparison. Spectra were normalized to ^{13}C and re-scaled.

8.1.2. LIBS Parameters for Tape-Mounted Soils

The repetition rate (0.67 Hz) was already quite low (limited by the duty cycle of the spectrometer and detector) so was not changed. All other parameters were kept as listed in Section 3.2.2.1 while the various line scan speeds were tested. The shortest line with the minimum tape contributions was obtained at a speed of 175 $\mu\text{m/s}$. At 0.67 Hz, this meant the sample stage was moving 261 μm between each laser pulse, which was the approximate diameter of a LIBS crater, and therefore a fresh portion of soil was being ablated with each laser pulse. Precision, linearity of calibrations curves, LODs and bias were comparable to those obtained with pellets, therefore the other parameters were left unchanged (see the “Milled, normalized” column in Table 24).

Table 24. A summary of the linearity (expressed as R^2), LODs, and bias (expressed as percent difference) for the three RMs by LIBS on spiked milled and non-milled soil either tape-mounted or pelletized. n/a: no concentration listed on certificate; ok: fairly linear with possible self-absorption (limit of linearity listed); SA: significant self-absorption; int: possible spectral interference.

		Milled, normalized		Non-milled, normalized	
		tape	pellets	tape	pellets
Ba II 493.4	linearity	excellent	ok	ok	poor
	R^2	1.00	0.91	0.99	0.62
	LOD	6	6	10	13
	bias 2710	-8	19	4	17
	bias 2704	2	-1.0	10	-12
	bias pacs2	n/a	n/a	n/a	n/a
Ba II 614.2	linearity	good	ok	ok	poor
	R^2	1.00	0.96	0.99	0.66
	LOD	25	33	51	58
	bias 2710	-8	1.2	-3	-7
	bias 2704	13	-7	17	-23
	bias pacs2	n/a	n/a	n/a	n/a
Ca I 393.366	linearity	ok	ok	good	good
	R^2	0.95	0.95	0.99	1.00
	LOD	26	31	29	38
	bias 2710	-38	-7	-43	-24
	bias 2704	-53	-53	-70	-56
	bias pacs2	-45	-38	-65	-57
Ca I 396.85	linearity	ok	ok	good	excellent
	R^2	0.97	0.93	1.00	1.00
	LOD	49	35	40	34
	bias 2710	-39	-9	-44	-25
	bias 2704	-54	-54	-70	-56
	bias pacs2	-47	-40	-66	-56
Ca I 643.91	linearity	good	good	ok	ok
	R^2	1.00	1.00	0.99	0.99
	LOD	131	109	81	114
	bias 2710	-3	6	-41	-28
	bias 2704	19	-19	-47	-46
	bias pacs2	1	-10	-57	-53
Cr I 360.5	linearity	good	excellent	ok	excellent
	R^2	0.99	1.00	0.98	1.00
	LOD	15	18	18	22
	bias 2710	50	147	45	169
	bias 2704	17	45	16	58
	bias pacs2	59	78	12	43

Table 24, continued.

		Milled, normalized		Non-milled, normalized	
		tape	pellets	tape	pellets
Cr I 425.4	linearity	good	excellent	ok (excellent to 200)	good
	R ²	0.98	1.00	0.97	1.00
	LOD	8	6	8	7
	bias 2710	-73	15	-93	-23
	bias 2704	-19	14	-19	5
	bias pacs2	-15	25	-45	-12
Cr I 357.869	linearity	ok	good	ok (excellent to 200)	ok (excellent to 200)
	R ²	0.98	0.99	0.95	0.96
	LOD	0.00	0.00	0.00	0.00
	bias 2710	65	201	39	164
	bias 2704	14	32	3	28
	bias pacs2	26	50	-27	3
Cu I 324.8	linearity	ok (excellent to 500)	ok (excellent to 500)	ok (excellent to 500)	ok (excellent to 500)
	R ²	0.78	0.47	0.65	0.90
	LOD	33	74	40	74
	bias 2710	-30	84	9	-7
	bias 2704	-379	-847	-304	-452
	bias pacs2	195	113	147	74
Cu I 521.8	linearity	good	good	good	excellent
	R ²	0.99	1.00	1.00	1.00
	LOD	228	470	418	382
	bias 2710	-34	-23	7	-59
	bias 2704	167	258	47	96
	bias pacs2	40	87	29	-30
Fe I 360.9	linearity	good	good	ok	excellent
	R ²	0.99	1.00	0.98	1.00
	LOD	308	476	386	523
	bias 2710	-60	-40	-61	-49
	bias 2704	-68	-61	-75	-65
	bias pacs2	-59	-54	-71	-66
Fe I 532.8	linearity	excellent	good	excellent	int
	R ²	1.00	1.00	1.00	0.98
	LOD	250	559	260	679
	bias 2710	-54	-26	-55	-32
	bias 2704	-57	-35	-66	-45
	bias pacs2	-48	-37	-63	-50

Table 24, continued.

		Milled, normalized		Non-milled, normalized	
		tape	pellets	tape	pellets
Fe I 495.8	linearity	ok	ok above 700	int	int
	R ²	0.98	0.94	0.96	0.78
	LOD	350	708	378	1028
	bias 2710	-48	-18	-45	-15
	bias 2704	-50	-27	-59	-39
	bias pacs2	-38	-25	-57	-44
Li I 610.4	linearity	excellent	good	int	good
	R ²	0.70	0.49	0.36	0.54
	LOD	12	8	15	8
	bias 2710	n/a	n/a	n/a	n/a
	bias 2704	724	725	520	433
	bias pacs2	598	730	333	292
Li I 670.8	linearity	SA	ok	ok	good
	R ²	0.81	0.94	0.91	0.99
	LOD	0.13	0.13	0.13	0.14
	bias 2710	n/a	n/a	n/a	n/a
	bias 2704	128	59	15	0.36
	bias pacs2	316	128	132	26
Mg 517.3	linearity	excellent	good	excellent	good
	R ²	1.00	1.00	1.00	0.99
	LOD	44	59	53	59
	bias 2710	-16	-16	-30	-38
	bias 2704	-26	-34	-56	-58
	bias pacs2	-8	-28	-39	-57
Mg 518.4	linearity	excellent	good	excellent	good
	R ²	1.00	1.00	1.00	1.00
	LOD	25	33	32	38
	bias 2710	-9	-14	-26	-37
	bias 2704	-21	-37	-55	-58
	bias pacs2	-2	-33	-39	-57
Pb I 368.3	linearity	ok	ok	ok to 600	ok to 600
	R ²	0.99	0.99	0.97	0.99
	LOD	119	138	174	189
	bias 2710	17	6	56	5
	bias 2704	126	194	77	115
	bias pacs2	194	227	-41	74
Pb I 405.8	linearity	ok (excellent to 600)	ok (excellent to 600)	ok to 600	ok to 600
	R ²	0.99	0.99	0.98	1.00
	LOD	47	60	61	75
	bias 2710	31	2	58	-5
	bias 2704	-14	-15	6	-66
	bias pacs2	44	25	-101	-55

Table 24, continued.

		Milled, normalized tape pellets		Non-milled, normalized tape pellets	
Sr II 407.8	linearity	good	ok	ok	ok
	R ²	1.00	0.97	0.92	0.93
	LOD	15	11	17	19
	bias 2710	11	37	7	26
	bias 2704	37	5	22	7
	bias pacs2	9	2	-13	-30
Sr II 421.6	linearity	excellent	good	ok	ok
	R ²	1.00	0.98	0.90	0.95
	LOD	11	7	11	7
	bias 2710	-7	6	-13	-2
	bias 2704	15	-1.5	14	-1.1
	bias pacs2	-14	-20	-28	-46
Ti I 336.1	linearity	good	good	ok	good
	R ²	1.00	0.98	0.97	0.98
	LOD	37	136	44	166
	bias 2710	-58	3	-69	13
	bias 2704	-70	-22	-80	-28
	bias pacs2	-59	-15	-79	-39
V I 437.9	linearity	good	excellent	good	excellent
	R ²	0.98	1.00	0.99	1.00
	LOD	10	14	11	16
	bias 2710	-32	66	-51	28
	bias 2704	-22	39	-49	23
	bias pacs2	-2	31	-44	-2
Zr I 468.8	linearity	ok	ok	good	good
	R ²	0.96	0.97	0.98	0.97
	LOD	88	116	79	175
	bias 2710	n/a	n/a	n/a	n/a
	bias 2704	-47	112	-63	20
	bias pacs2	n/a	n/a	n/a	n/a

8.1.3. LA-ICP-MS Parameters for Tape-Mounted Soils

LA-ICP-MS laser energies of 25, 30, 40, 60, and 100 % were tested. Energies above 40 % resulted in visible ablation of the tape. Of the remaining energies, 30 % provided the lowest RSDs. Spot sizes of 100 μm and 200 μm were tested. No difference in precision was observed, but calibration curves generated using 200 μm were more linear. Repetition rates of 1, 5 and 10 Hz were tested. One Hz (1 pulse per second) was too infrequent to generate a steady stream of particles and produced a transient signal that was not

stable. No difference was seen between 5 and 10 Hz, but 10 Hz ablated a line twice as long, so 5 Hz was chosen.

8.1.4. Conclusion

In conclusion, laser energy, speed, and length of the line scan were optimized for LIBS and LA-ICP-MS to provide the best precision and lowest tape contributions with the shortest line. The optimized LIBS method was five replicates per specimen, 75 pulses accumulated per replicate, 900 mL/min Ar, 100 % energy, focused 1.4 mm behind the surface, 0.67 Hz, line mode at 250 $\mu\text{m/s}$. The optimized LA-ICP-MS method was four replicates per specimen, 500 pulses per replicate, 30 % energy, 200 μm spot size, 50 s ablation at 5 Hz, line mode at 150 $\mu\text{m/s}$. Signal intensities for tape-mounted soil were lower than those for pellets, but the figures of merit were comparable when using spiked, milled soil for both.

8.2. Method Development: Reduction of Sample Preparation

Using spiked, milled soil, and spreading it onto tape rather than pressing it into a pellet does not really reduce the destructiveness, number of sample preparation steps or the time required to process the samples. The most basic preparation would involve spreading unprocessed soil directly onto tape. This creates a number of analytical challenges. The first challenge was heterogeneity and particle size. As discussed previously, bulk soils contain the full range of particle sizes, which would also be mounted to the tape. If the soil is not milled, ablation of large particles may result in large chunks (resulting in spikes in the LIBS and LA-ICP-MS signals) that skew the overall elemental profile and reduce the precision of the analyses. Sieved soils also resulted in more homogenous coverage of the tape. As with any transfer, the larger particles are less likely to adhere, and would be more likely to be lost in a non-systematic fashion during handling. For these reasons, in addition to the reasons discussed in Sections 2.3.2.1 and 4.2.3, sieved soils were used. Sieving is common in forensic examinations, especially when mineralogical analysis is performed, so it may not add any extra time to the overall processing.

8.2.1. Homogenization

The second challenge was the internal standard. As discussed previously (see Section 4.3.2), an internal standard must be added in order to achieve good analytical results. The internal standard must be

evenly distributed throughout the sample, but this may be difficult without milling the spiked soils to homogenize the internal standard. Since the internal standards used so far were in solution form, the volume added must be sufficient to just cover the soil particles, without being so excessive that it takes a long time to dry or would coat the sides of the tube instead of the particles. The required volume was approximately double the volume of scandium required for a 350 ppm final concentration. Therefore either an equal amount of water or a second internal standard can be added. The spiked soils were thoroughly homogenized using the vortex mixer, and then allowed to dry at 80 °C. Periodically throughout the drying process, the samples were re-vortexed. The drying time, which was the longest step, was much shorter than for the pellet process because of the reduced sample size and spike volumes. One to two hours was sufficient for these samples, while 8 - 16 hours were normally required for pellet samples. Once the spiked soils were dry, many had formed a hard ball at the bottom of the tube. This was broken up by shaking and homogenizing using the vortex touch mixer until a fine, uniform powder was obtained. Unlike milling, which pulverized the mineral grains to obtain a fine powder, shaking and homogenizing with the vortex touch mixer simply broke up the aggregated soil that had caked together while drying; the texture and mineral structure were unaltered.

In order to evaluate this technique, a study was undertaken to compare spiked (using Sc and In), non-spiked, milled and non-milled soil on tape and in pellets by LIBS. RMs and calibrations standards were used to evaluate precision, bias, LODs, and linearity of the calibration curves. Spiked homogenized samples were split, with one half being milled and one half not. Of these, each was split again, with one half mounted onto the tape, and the other half pressed into pellets. All samples were analyzed by LIBS and LA-ICP-MS.

As expected, LIBS calibration curves for non-spiked soil were non-linear, while those obtained from soils spiked with internal standard and normalized were greatly improved. Therefore internal standard was necessary. The only exception was for lithium, which was more linear when not normalized. Looking at only the spiked soil on tape, precision for non-milled soil was less than 10 % RSD for most emission lines, and in many cases the RSDs were better than the milled soil (see Figure 45). Comparison of tape and pellets for spiked, non-milled soils showed precisions for tape were similar to or better than those of pellets

(see Figure 45). Comparing the new tape method (spiked, non-milled) with the current pellet method (spiked, milled), the precision of the tape method was comparable or better for most emission lines in the three RMs. This was surprising given the homogeneity and robustness of the pellets, however there are two possible explanations. Firstly, the pellets are ablated in spot mode, where the plasma may be confined by the crater that is formed, resulting in a reduction in the amount of light reaching the detector (LIBS) or particles transported to the ICP (LA-ICP-MS). Secondly, the mass ablated for the tape method was much higher than for the pellets (see Section 8.2.2), resulting in higher signal intensities and therefore better precision.

Figure 45. Comparison of the precision (RSD, %) achieved by LIBS analysis of tape-mounted and pelletized, milled and non-milled soil.

The other figures of merit were shown previously in Table 24. Overall results are very similar for all four cases (milled, non-milled, tape, and pellets). R^2 was reported in the Table rather than showing a huge number of calibration curves. Because R^2 is not a perfect indicator of linearity, the linearity was verified by eye, and any leveling off because of potential self-absorption or other issues were indicated in the footnotes to the Table. In most cases the quality of the tape calibration curves was equivalent to that of the pellet curves. The LODs were very similar in all four cases. Bias improved in some cases and worsened in an approximately equal number of cases.

As described in Section 3.2.1.2, the single-point calibration obtained for LA-ICP-MS data with the GLITTER software was verified by generating a series of calibration curves for a sub-set of the element menu. Linearity was excellent, with R^2 values above 0.98 for all isotopes. Figure 46 and Figure 47 show examples of calibration curves obtained with the final methods for LIBS and LA-ICP-MS, respectively.

Figure 46. Calibration curves obtained from LIBS of calibration standards mounted on tape.

Figure 47. Calibration curves obtained from LA-ICP-MS of calibration standards mounted on tape.

8.2.2. Mass Removal Studies

Mass removal studies were then undertaken in order to determine the amount of material ablated. Pellets and tape-mounted soils were weighed before and after LA-ICP-MS and LIBS analyses. Since the masses removed for both tape and pellets were quite small, a large number of replicates were used in order to be able to measure the difference.

For tape, nine specimens from three Miami-Dade County sites (HA, CC6, KNT) were sieved, spiked, and mounted onto pre-weighed tape affixed to glass coverslips, as described in Sections 3.1.2, 3.1.4, and 3.1.5, and weighed. For LIBS, ten replicates were performed on each specimen using the method described in Section 8.1.4 and then they were weighed again. For LA-ICP-MS, five to six replicates were performed on each specimen using the method described in Section 8.1.4 and then they were weighed again. The size

of the tracks left by the ablation in line mode were measured with the stereomicroscope as described in Section 3.4, and averaged over all nine specimens.

For pellets, nine specimens of different soil types were spiked, milled, and pelletized, as described in Sections 3.1.4 and 3.1.6, and weighed. For LIBS, 75 replicates were performed on each specimen using the method described in Section 3.2.2.1 and then they were weighed again. For LA-ICP-MS, 38 replicates were performed on each specimen using the method described in Section 3.2.1.1 and then they were weighed again. The size of the craters left by the ablation in spot mode were measured with the digital microscope as described in Section 3.4, and averaged over all nine specimens.

Mass removed by each technique on each sample type was calculated and averaged over the nine specimens, then divided by the number of replicates to obtain the mass removed per replicate. To find the mass removed per analysis, the LIBS masses per replicate were multiplied by five since five replicates are used in a LIBS analysis, and the LA-ICP-MS masses per replicate were multiplied by 4. Results are summarized in Table 25. The amount of specimen required to prepare the different sample types is also provided.

Table 25. Summary of the amount of specimen required to prepare each sample type and the amount of soil removed with each analysis method.

Sample Type		Amount Required to Prepare Sample	Amount Removed per Analysis	
		Average $\pm$ SD	LIBS (5 Reps) ^a	LA (4 Reps) ^b
Pellets ($\varnothing=13\text{mm}$)	Mass (mg):	470 $\pm$ 50	0.10 $\pm$ 0.03 (n = 75)	0.45 $\pm$ 0.16 (n = 38)
	Volume (mm ³):	265 ^c	0.023 $\pm$ 0.009 (n = 75)	0.023 $\pm$ 0.009 (n = 38)
Tape	Mass (mg):	10 $\pm$ 4	0.8 $\pm$ 0.2 (n = 90)	1.7 $\pm$ 0.4 (n = 50)
	Area (mm ²):	190 ^d	38.9 $\pm$ 7.1 (n = 90)	11.0 $\pm$ 3.3 (n = 50)

a. 266 nm, 100 % energy (29 mJ), line mode, 175 $\mu\text{m/s}$, 0.67 Hz, 75 shots/rep 266 nm, focused 1.4 mm behind surface, 137.5 μm spot

b. 266 nm, 30 % energy (0.49 mJ), line mode, 150 $\mu\text{m/s}$, 50 s ablation @ 5 Hz, 500 shots/rep, 200 μm spot

c. Approximate pellet volume, given a 2 mm height

d. Approximate tape-mounted area, given a 19 x 10 mm exposed surface

8.2.3. Conclusion

In conclusion, the challenges in creating a sample preparation method for small specimens were addressed and a method for mounting soils on tape and analysis by LIBS and LA-ICP-MS was developed. Non-milled, tape-mounted soils performed as well as milled, pelletized soils. Elemental profiles were generated by LIBS and LA-ICP-MS with good precision using only 10 ± 4 mg of soil.

8.3. Discrimination: Comparison to Miami-Dade Sieved Pellets

Since the above tape-mounting methods were designed at the request of forensic examiners, they needed to be tested for their discrimination capabilities and compared to the results of the established pellet method [96].

8.3.1. Materials & Methods

Sieved soil specimens from HA, CC6 and KNT sites in Miami-Dade County were used. Pellets from these specimens had been previously prepared, analyzed by LA-ICP-MS and LIBS, and discriminated by PCA and LDA (see Section 6.3). In this section, sieved, spiked, tape-mounted samples were prepared by the tape-mounting method as described in Sections 3.1.2, 3.1.4, and 3.1.5, with the following exceptions: Sc and Lu were used as the internal standards. Only 10 ± 4 mg of sieved soil was required. Analysis by LA-ICP-MS and LIBS was as described in Section 8.1.4. Analyses by PCA and LDA were performed using the same discrimination element menus as described in Section 6.3.

8.3.2. LIBS

Two-dimensional PCA score plots for the pellet and tape methods are shown in Figure 48a and b, respectively. The pellet method provided slightly tighter clustering for HA (red) and CC6 (blue). The tape method provided slightly tighter clustering for KNT (green) and slightly better separation from CC6. Overall the PCA groupings of the three sites are nearly identical for pellets and tape, which is likely because of the similar precisions of the two methods.

Figure 48. PCA and LDA plots for HA (red), KNT (green), and CC6 (blue) generated from LIBS data of sieved pellets and tape-mounted soil.

The following discrimination element menu was used: Ca I 643.9/ Fe I 360.9, Cr I 360.5/ Sr II 421.6, Fe I 495.8/ Li I 670.8, and Ba II 493.4/ Fe I 360.9. Numbers and different shades denote the different sub-plots. a. PCA score plot for pellets, b. PCA score plot for tape-mounted specimens, c. LDA canonical plot for pellets, d. LDA canonical plot for tape-mounted specimens. Misclassified specimens are circled.

Two-dimensional LDA canonical plots for the pellet and tape methods are shown in Figure 48c and d, respectively. Again, the two sample types yielded very similar plots with the tape method providing slightly better separation between CC6 and KNT. The confusion matrix for the tape method is shown in Table 26. Five replicates were misclassified out of 184 replicates for a correct classification rate of 97.3 %. All five originated from KNT, but were classified as originating from CC6: two from sub-plot 6, two from sub-plot

5, and one from sub-plot 1 (circled on Figure 48d). In comparison to the pellet method (see Table 16 in Section 6.3.2), this is a slight improvement in discrimination.

Table 26. Confusion matrix for LDA with leave-one-out cross-validation on 184 LIBS replicates from HA, CC6 and KNT mounted on tape.

		Predicted Origin			Total
		CC6	HA	KNT	
True Origin	CC6	65	0	0	65
	HA	0	50	0	50
	KNT	5	0	64	69

8.3.3. LA-ICP-MS

Two-dimensional LDA canonical plots for the pellet and tape methods are shown in Figure 49a and b, respectively. Interestingly, the clustering by sub-plot with the tape method was not as tight as what was seen in previous PCA and LDA plots of LA-ICP-MS data using the pellet method. This is likely because of a slight reduction in the precision of the data obtained from the tape method. The confusion matrix for the tape method is shown in Table 27. Two replicates were misclassified out of 142 replicates for a correct classification rate of 98.6 %. Both originated from KNT (sub-plot 6), but were classified as originating from CC6 (circled in Figure 49b). In comparison to the pellet method (see Table 17 in Section 6.3.3), this is a slight improvement in discrimination.

Figure 49. LDA canonical plots for HA (red), KNT (green) and CC6 (blue) generated from LA-ICP-MS data of sieved pellets and tape-mounted soils.

The following discrimination element menu was used: ^{27}Al , ^{137}Ba , ^{42}Ca , ^7Li , ^{25}Mg , ^{88}Sr , $^{47,49}\text{Ti}$, ^{238}U , ^{51}V , and ^{91}Zr . Numbers and different shades denote the different sub-plots. Misclassified specimens are circled. a. data from pellets, b. data from tape-mounted specimens.

Table 27. Confusion matrix for LDA with leave-one-out cross-validation on 142 LA-ICP-MS replicates from HA, CC6 and KNT mounted on tape.

		Predicted Origin			Total
		CC6	HA	KNT	
True Origin	CC6	46	0	0	46
	HA	0	40	0	40
	KNT	2	0	54	56

8.3.4. Conclusion

Samples were prepared using only 10 ± 4 mg of sieved soil that was spiked but not milled, and only a portion of this was consumed in the LA-ICP-MS and LIBS analyses. Discrimination of the same set of specimens using the same discrimination element menu yielded very similar results for both the tape-mounting and pellet methods: 97.3 % and 94.5 % %, respectively, for LIBS and 98.6 % and 97.1 %, respectively, for LA-ICP-MS. Once again, LIBS and LA-ICP-MS provided similar information.

8.4. Conclusions

The concerns of the forensic community were addressed using the tape mounting method, where 10 ± 4 mg of sieved, spiked, non-milled soil were mounted onto a clear, low-background adhesive tape. The milling step, which is time-consuming and destructive, was eliminated. The entire sample preparation process could be performed in as little as one day. Tape-mounted soils can be stored and re-analyzed by LIBS, LA-ICP-MS, color, microscopy, and mineralogy. Analysis parameters for LIBS and LA-ICP-MS were optimized to minimize background contributions from the tape. Precision, LODs, and accuracy were comparable to the pellet method. Discrimination, by both PCA and LDA, was comparable to results achieved with pellets, at greater than 94.5 % correct classification rates. Similar information was provided using LIBS and LA-ICP-MS.

9. Environmental Application: Ruvu River Sediments

Suspended sediments offer a large surface area for adsorption of contaminants and excess nutrients in bodies of water. In a river system, these can be transported quite far, resulting in increased pollution and changes to the biota, which can cause algal blooms and other deleterious effects [97]. Overall the result is diminished water quality that affects everyone and everything living in and around the river basin. In order to decrease the sediment loading in the river system, it is necessary to locate the sources of the soil erosion and sediment release into the watershed [10]. The Ruvu river is one such system, originating as forest streams in the Uluguru Mountains, Morogoro Region, United Republic of Tanzania (“Tanzania”) that join together and travel through Pwani Region before ending in the Ruvu Estuary in the Zanzibar Channel near Bagamoyo (see Figure 50). The Ruvu River basin supplies Tanzania’s largest city, Dar es Salaam [97], and is being studied by Global Water for Sustainability (GLOWS) program in collaboration with Florida International University (FIU) [98].

Figure 50. Relief map of Tanzania showing the location of the Ruvu River. Modified from [99].

Because the a river basin is a large catchment system including the main river and all its tributaries, a targeted approach is required in order to focus on the largest sediment contributors. Sediment fingerprinting

is an effective tool for this purpose. This involves the collection of sediments from the river and soil specimens from potential sources and generating elemental profiles for each of them. Then the elemental profiles of the soils and sediments are compared in order to determine the contribution of each source to the overall sediment profile [10]. Analysis of filters with adhered sediment (“filter sediments”) can be challenging since the sediment is present in small quantities, cannot be separated from the filters, and is not uniformly distributed on the filters. Previous work on the Mara River in Kenya used microwave digestion of the whole filter followed by ICP-MS to generate the elemental profiles [10]. A solid sampling method was desired by GLOWS to avoid acid digestions. Methods of solid sample preparation and LA-ICP-MS analysis for sediment filter specimens were developed and are discussed in this Section. The actual sediment fingerprinting will be done by GLOWS at a later date [98].

9.1. Specimens

Eighty-eight soil specimens (S1 – S88) were provided. These were collected at various upstream locations in the Ruvu River basin using a plastic spade to a depth of 2 cm, dried and sieved to $< 63 \mu\text{m}$ and packaged in individual bags for shipment. Forty-five sediment filter specimens (F1 – F45) were provided. These were collected by filtration of river water in situ with cellulose nitrate membrane as described by Dutton et al [10]. Briefly, cellulose nitrate membrane filter circles of 47 mm diameter and $0.45 \mu\text{m}$ pore size (Whatman Ltd., Piscataway, NJ, USA) were cleaned, dried and weighed prior to use. Sediments were collected onto the filters using a DH-59 depth-integrating suspended sediment sampler. Filters with adhered sediment (“sediment filters”) were dried and weighed to determine the mass of sediment, and packaged in plastic Petri dishes for shipment. Cleaned, dried, and weighed blank filters were also supplied for method development.

9.2. Method Development: Preparation & Analysis of Sediment Filter Pellets

Initially a direct ablation of the sediment on the filter was considered. However, the sediment layer itself was thin and not uniformly distributed (see Figure 51). A direct ablation would require a large raster in order to account for the heterogeneity. Contributions from the filter would have to be subtracted, but the relative contribution of the filter would change depending on sediment layer thickness and laser-to-sample

coupling of the sediment in that particular location. Use of an internal standard to the sediment would help account for variable coupling, but adding it in a homogeneous manner was impractical. Therefore analysis using direct ablation would have been semi-quantitative at best.

Figure 51. Two examples of sediment filter specimens showing uneven sediment distribution.

Preparing pellets was considered next since milling would result in homogeneous sample and internal standard powder. In addition, any contamination imparted by the sample preparation process would be imparted equally to both the soils and sediment filters. However there were still some issues to be addressed. Firstly, the filters were quite thin and weighed only 86 mg each and the sediments were also minimal, with an average loading of 94 mg per filter (ranging from 8 to 300 mg). The overall mass would be too small to generate a 13 mm pellet of sufficient thickness to withstand the laser ablation. The minimum thickness required depends on the LA conditions and laser-to-sample coupling of the particular specimen, but 2mm has proved to be a good minimum, providing robust pellets that are thick enough that the laser does not bore right through them. Pellet dies for 6mm diameter pellets were obtained for use with smaller specimens. This reduction in diameter resulted in a 4.7 fold reduction in the amount of powder required for the same pellet thickness, which meant that instead of the 470 mg of soil required for the 13 mm diameter pellets, only 85 mg would be required.

The second issue was how the membrane filters would affect pellet consistency. Before receiving the blank filters, cellulose nitrate filter papers found in the laboratory were used to test milling conditions. These filters were thick, flexible, and fibrous, and had to be cut into small pieces that had to be milled separately to avoid overloading the mill. Fortunately, the GLOWS blank filters were thin, brittle, and

membranous, so overloading was not an issue. However, when one was milled alone, it resulted in only a small amount of dust, with the rest adhering to the sides of the milling jars. When a blank filter was milled with soil, a good quality fine powder was achieved, and a robust pellet was produced using the 6 mm dies pressed at 1.3 metric tonnes for five minutes under vacuum. Milling times of five and ten minutes were tested with and without cutting or crumpling up the filter into smaller pieces prior to milling. Under all conditions, sediments were well homogenized, but homogenization of the filters required more care. Without crumpling, five minutes was not sufficient to achieve complete homogenization of the filters, and small pieces of intact filter were observed in the powder. Ten minutes was enough in most cases. When filters were pre-crumpled, five minutes was sufficient to achieve complete homogenization of filters and sediment. However, it was difficult to crumple the sediment filters completely without losing some of the sediment. Therefore, careful pre-crumpling and ten minutes of milling at 25 Hz was used to ensure effective homogenization.

The third issue was how to account for the elements present in the filters themselves. Dutton et al in a Kenyan sediment fingerprinting study [10] performed ICP-MS after digesting blank filters, and found that concentrations of most elements were below MDL, except for iron, which was only twice the MDL. Since iron is a major element, the contribution from the filters was deemed low enough to be negligible compared to the high concentrations seen in sediments [10]. No data was provided for the internal standards scandium or indium, so the filters were analyzed using LA-ICP-MS to determine if any significant contributions from Sc or In could be detected (see Figure 44). No scandium was detected. Indium was detected, but in very small amounts.

The fourth issue was how to obtain quantitative results since the sediments would be diluted with the filters. Normally, RM pellets are used as calibrators, but these are not diluted with the filter. Normally when a binder is used to dilute specimens prior to pelletizing, the same amount is used for all specimens and standards. Therefore, RM filter pellets were prepared to test their effectiveness as calibrators. The average mass of sediment on the filter specimens was approximately equal to the average mass of the filters alone (94 mg and 86 mg, respectively). For each RM, a blank filter was selected, and a mass of the RM equal to the mass of the blank filter was added with the appropriate amount of the internal standards

calculated on the basis of the mass of sediment, for a final concentration of 349.5 µg/g scandium and 299.8 µg/g indium. Each spiked RM was milled and homogenized with the crumpled blank filter, and pellets of 6 mm diameter were pressed as above.

LA-ICP-MS was performed using regular RM pellets and RM filter pellets as described in Section 3.2.1.1. Data analysis using GLITTER was performed as described in Section 3.2.1.2 using NIST SRM 2704 as the calibrator. Both the regular RM pellet and RM filter pellet were tested as calibrators, as well as using the actual certified concentrations or using the final diluted concentrations. Scandium and indium were investigated as internal standards. Using the RM filter pellets with the actual certified concentrations provided good performance in terms of bias and reproducibility. Scandium and In performed equally well in method development, so Sc was chosen for its absence in the blank filters.

9.3. Preparation & Analysis of Sediment Filter Pellets

Sediment filter specimens were spiked with internal standard, as described in Section 3.1.4. The volume of scandium and indium added was calculated on the basis of each individual sediment weight supplied, for a final concentration of 349.5 µg/g scandium and 299.8 µg/g indium added to the sediment portion. Spiked sediment filter specimens were dried at 50 °C for 1-2 hours. Each spiked sediment filter was milled and homogenized (including filter) using a high speed ball mill mixer with a tungsten carbide jar and ball for ten minutes at 25 Hz, and then the powder was pressed into one or more pellets of 6 mm diameter and 2 mm thickness using stainless steel dies (Carver, Wabash, IN, USA) for five minutes at 1.2 metric tonnes. Pellets were mounted onto an adhesive backing, packaged in weighing paper and labeled.

LA-ICP-MS analysis was requested for specimens F1 – F35. Parameters were as described in Section 3.2.1 with the following exceptions: a UP213 laser ablation system (New Wave Research, Fremont, CA, USA) was used in spot mode with a 190 µm spot size. One pellet was randomly selected as a duplicate, and an additional four replicate measurements were performed at the end of the analysis. The NIST SRM 2710 and PACS-2 RM filter pellets were used as controls. Four replicate measurements were acquired for each RM filter pellet at the beginning and end of each day, and sets of two replicate measurements were acquired periodically throughout the analysis. The NIST SRM 2704 RM filter pellet was used as the calibrator (except where noted below, see Section 9.5.2), with scandium as the internal standard.

9.4. Preparation & Analysis of Soil Pellets

Soil pellets of 13 mm diameter were prepared using the bulk pellet method (see Sections 3.1.4 and 3.1.6) with a final concentration of 349.5 µg/g scandium and 299.8 µg/g indium in each soil sample. Analysis by LA-ICP-MS was performed on the soil pellets as described in Section 3.2.1 using NIST SRM 2704 as the calibrator (except where noted below, see Section 9.5.1), and scandium as the internal standard. The NIST SRM 2710 and PACS-2 RM pellets were used as controls. Four soil pellets were randomly selected as duplicates, and an additional four replicate measurements of each were performed at the end of the analysis.

9.5. Results & Discussion

Elemental data were reported in µg/g (ppm) for elements that passed the quality control. Mean values and standard deviations were estimated for the four replicate measurements of each pellet. Before performing any calculations, any < MDL values were assigned the MDL. Calibration standard pellets, containing known amounts of a sub-set of the elements in the element menu, were also analyzed to confirm linearity of the measurement response.

Recoveries (% Rec.) for each isotope of each of the control RMs were reported as a percent of the certified concentration values. This is another way of expressing bias that is used in environmental reporting, where recoveries between 75 and 125 % were considered acceptable [10]. As mentioned above, NIST SRM 2710 and PACS-2 were used as controls. For isotopes where NIST SRM 2710 was used as the calibrator, NIST SRM 2704 was used as the control. Recoveries were not calculated for the calibrator. Reproducibility for each duplicate run was reported as a percent difference (Dup. % Diff.) in the measured concentration of each set of four replicates, for each isotope, with 10 % or less being acceptable. The overall MDL (MDL Avg) for each isotope was calculated by averaging the MDL over all replicates. Variability (Var.) for each isotope was assessed by calculating the relative standard deviation (RSD) of the four replicate measurements on each pellet, then averaging the RSDs of all pellets, with 10 % or less being acceptable.

9.5.1. Soils

The following isotopes passed the quality control for the analysis of soil using the bulk pellet method: ^7Li , ^{25}Mg , ^{31}P , ^{39}K , ^{51}V , $^{52,53}\text{Cr}$, ^{57}Fe , ^{60}Ni , ^{63}Cu , ^{66}Zn , ^{71}Ga , ^{75}As , ^{85}Rb , $^{98}\text{Mo}^b$, ^{118}Sn , $^{123}\text{Sb}^b$, ^{137}Ba , ^{205}Tl , $^{207,208}\text{Pb}$, $^{238}\text{U}^b$. Quality data are summarized in Table 28. Instead of NIST SRM 2704, NIST SRM 2710 was used to calculate specimen concentrations for the following elements: Molybdenum (the NIST SRM 2704 certificate did not list a concentration for Mo), Sb (recovery was unacceptable when NIST SRM 2704 was used), and U (duplicate runs were more than 10 % different when NIST SRM 2704 was used). Selenium, Cd, and Au were not used because they were frequently below MDL. Only one replicate measurement was below MDL for As and for Sb. W and Co were not used because of possible contamination from the milling process. Sodium, Si, Ti, Se, Sr, Zr, Ag, Cd, Lu, Au, Hg, and Th were not used because duplicate runs were frequently > 10 %. Sodium, Al, Si, Ca, Mn, Se, Sr, Y, Cd, Au, and Hg were not used because recovery was unacceptable (< 75% or > 125 %).

Table 28. Quality data for soils from the Ruvu River basin.

Calibrator: NIST SRM 2704 (except Mo, Sb, U). Blank cells indicate that a recovery could not be calculated. This was because either a certified value was not available or the RM was used as the calibrator.

*: NIST SRM 2710 was used as a calibrator for these isotopes.

Isotope	PACS-2 % Rec. (n=10)	2710 % Rec. (n=36)	2704 % Rec. (n=34)	S10 Dup. % Diff.	S12 Dup. % Diff.	S30 Dup. % Diff.	S83 Dup. % Diff.	MDL Avg (ppm)	Var. (% RSD)
⁷ Li	107			1	5	1	10	0.054	8
²⁵ Mg	84	104		9	3	3	4	0.32	5
³¹ P	87	86		8	4	8	3	1.7	5
³⁹ K	84	89		5	3	3	4	0.38	4
⁵¹ V	86	94		9	7	1	10	0.076	4
⁵² Cr	82	84		8	6	6	6	0.24	7
⁵³ Cr	80	82		7	7	6	8	0.31	8
⁵⁷ Fe	88	102		4	1	2	5	1.2	5
⁶⁰ Ni	90	101		5	6	7	7	0.092	7
⁶³ Cu	90	93		5	2	6	7	0.071	6
⁶⁶ Zn	90	93		4	1	2	3	0.16	7
⁷¹ Ga		92		0	2	10	7	0.023	5
⁷⁵ As	88	97		3	7	7	5	0.13	11
⁸⁵ Rb		93		3	5	3	8	0.024	5
⁹⁸ Mo*	100			8	6	4	10	0.016	11
¹¹⁸ Sn	106			3	4	7	4	0.019	6
¹²³ Sb*	80		122	5	3	5	8	0.015	13
¹³⁷ Ba		92		10	5	0	9	0.047	4
²⁰⁵ Tl	101	90		2	4	5	5	0.0025	7
²⁰⁷ Pb	106	100		5	3	4	4	0.018	7
²⁰⁸ Pb	110	99		3	8	5	7	0.007	6
²³⁸ U*	75		95	0	4	0	0	0.002	8

9.5.2. Sediment Filters

Sediment mass loadings ranged from 8 to 300 mg. All but one produced enough powder after milling for at least one pellet of 6mm diameter and 2 mm thickness; many produced enough for two or more pellets. Specimen F5, with just over 41 mg of sediment on the filter, produced a pellet only 1 mm thick. This may have been because of increased density or loss through adhesion to the walls of the milling jar. Therefore, although as little as 8 mg of sediment with an 86 mg filter provided enough powder for a pellet 2 mm thick, the actual amount may depend on the nature of the specimen. Of the specimens producing only one pellet, sediment masses ranged from 8 – 148 mg, with an average and standard deviation of 47 ± 34 mg. Of the specimens producing two pellets, sediment masses ranged from 73 – 149 mg with an average and standard deviation of 93 ± 22 mg, which also worked out to nearly 47 mg per pellet.

The following isotopes passed the quality control: ^7Li , ^{23}Na , ^{25}Mg , ^{31}P , ^{39}K , ^{51}V , $^{52,53}\text{Cr}$, ^{57}Fe , ^{63}Cu , ^{66}Zn , ^{71}Ga , ^{75}As , ^{85}Rb , ^{98}Mo , $^{118,120}\text{Sn}$, ^{137}Ba , ^{176}Lu , ^{205}Tl , $^{206,207,208}\text{Pb}$, and ^{232}Th . Quality data are summarized in Table 29. Since sediment filter specimen F5 did not provide enough powder for a pellet thick enough to withstand the ablation, analysis was performed using modified conditions (laser repetition rate reduced to five Hz, energy reduced to 80 %), and results were provided for informational purposes, but should be used with caution. Instead of NIST SRM 2704, NIST SRM 2710 was used to calculate concentrations for the following elements: Molybdenum (the NIST SRM 2704 certificate did not list a concentration for Mo), Na and Mg (recoveries were unacceptable when NIST SRM 2704 was used), and P, Fe, and As (duplicate runs were > 10 % different when NIST SRM 2704 was used). Tungsten and Co were not used because of possible contamination from the milling process. Selenium and Au were not used because they were frequently below MDL. Si, Zr, Sb, and U were not used because duplicate runs were frequently > 10 %. Aluminum, Si, Ca, Ti, Mn, Ni, Sr, Y, Ag, and Sb were not used because recovery was unacceptable (< 75 % or > 125 %). Gallium-69, Se, Zr, Ag, Cd, Sb, Au, and Hg were not used because variability was frequently > 10 %. The following isotopes present in the soil element menu did not pass QC for sediment filters: ^{60}Ni , ^{123}Sb , ^{238}U . The following isotopes not present in the soil element menu successfully passed QC for sediment filters: ^{23}Na , ^{120}Sn , ^{176}Lu , ^{206}Pb , ^{232}Th .

Table 29. Quality data for sediment filters from the Ruvu River.
 Calibrator: NIST SRM 2704 (except Na, Mg, P, Fe, As, Mo). Blank cells indicate that a recovery could not be calculated. This was because either a certified value was not available or the RM was used as the calibrator. *: NIST SRM 2710 was used as a calibrator for these isotopes.

Isotope	PACS-2 % Rec. (n=5)	2710 % Rec. (n=7)	2704 % Rec. (n=19)	F12 Dup. % Diff.	MDL Avg (ppm)	Var. (% RSD)
⁷ Li	95			0.8	0.041	2
²³ Na*	87		82	2	0.26	5
²⁵ Mg*	80		111	5	1.2	2
³¹ P*	108		121	6	1.5	8
³⁹ K	79	86		2	0.42	2
⁵¹ V	93	95		0.1	0.036	2
⁵² Cr	88	101		2	0.27	4
⁵³ Cr	87	85		0.8	0.31	5
⁵⁷ Fe*	96		102	3	5.0	7
⁶³ Cu	91	91		0.5	0.10	7
⁶⁶ Zn	90	89		4	0.21	7
⁷¹ Ga		99		0.8	0.027	9
⁷⁵ As*	90		108	6	0.12	9
⁸⁵ Rb		93		0.4	0.029	7
⁹⁸ Mo*	98				0.033	10
¹¹⁸ Sn	99			5	0.026	7
¹²⁰ Sn	100			5	0.023	8
¹³⁷ Ba		85		0.7	0.23	5
¹⁷⁶ Lu				6	0.007	7
²⁰⁵ Tl	92	94		4	0.007	10
²⁰⁶ Pb	82	92		7	0.10	7
²⁰⁷ Pb	86	96		7	0.11	7
²⁰⁸ Pb	84	96		5	0.062	7
²³² Th		124		8	0.014	7

9.6. Conclusions

Performance of both the sediment filter method and bulk soils method were excellent. An element menu of 20 elements was obtained with recoveries between 75 and 125 %, precision of 10% or less (RSD), variability of 10 % or less (for duplicates), and limits of detection in the ppb to low ppm range. Isotopes common to both methods included: ⁷Li, ²⁵Mg, ³¹P, ³⁹K, ⁵¹V, ^{52,53}Cr, ⁵⁷Fe, ⁶³Cu, ⁶⁶Zn, ⁷¹Ga, ⁷⁵As, ⁸⁵Rb, ⁹⁸Mo, ¹¹⁸Sn, ¹³⁷Ba, ²⁰⁵Tl, and ^{207,208}Pb. Additional isotopes for soil included: ⁶⁰Ni, ¹²³Sb, ²³⁸U. Additional isotopes for sediment filters included: ²³Na, ¹²⁰Sn, ¹⁷⁶Lu, ²⁰⁶Pb, ²³²Th. These methods were capable of generating quantitative multi-element data of sufficient quality for use in sediment fingerprinting applications with sediment masses as low as 47 ± 34 mg on an 86 mg cellulose nitrate membrane filter.

10. Conclusions

10.1. Method Development & Validation

Methods for preparation of homogeneous pellets from large sieved and bulk soils have been developed and optimized. Quantitative multi-element methods of analysis have been developed for both LIBS and LA-ICP-MS. This work represents the first use of a 266 nm laser for LIBS analysis of soil. Detection limits on the order of 10 ppm were obtained for LIBS and 10 ppb for LA-ICP-MS. Precisions between 4 and 15 % RSD were obtained for LIBS and between 2 and 9 % for most elements in LA-ICP-MS.

An inter-laboratory study permitted a direct comparison of the analytical performance between different elemental analysis techniques and between participant laboratories using the same technique. Quantitative analysis was possible with all three techniques through external calibration. Direct analysis of soil produced limits of detection of 0.01-23 ppm for LA-ICP-MS, 0.25-574 ppm for LIBS, 16-4400 ppm for μ XRF for elements useful in forensic discrimination. Intra-laboratory precision for most elements was 6 % RSD or less for LA-ICP-MS, 8 % or less for μ XRF, and 17 % or less for LIBS. Inter-laboratory precision for most elements were 19 % or less for LA-ICP-MS and 25 % or less for μ XRF, which was encouraging for a first inter-laboratory exercise using an analytical protocol that was not yet fully standardized. The statistics, particularly for μ XRF and LIBS, would have benefited from more participants. Still, the results obtained in this study allowed each participant to identify potential sources of uncertainty and fine-tune their methods to work towards the optimization and development of standardized methods of analysis.

10.2. Effects of Spatial and Lithological Factors on Discrimination

Discrimination methods were developed for elemental profiles generated by LIBS and LA-ICP-MS. Discrimination of specimens from different locations was possible using either LA-ICP-MS or LIBS with univariate statistical tools, such as ANOVA, and with multivariate statistical tools, such as PCA and LDA. Although the precision of LIBS was not quite as good as that of LA-ICP-MS, and correct classification rates were lower, similar information was obtained with both techniques. Note that the variability within a

site was much greater for soil than it would be for sources of other types of trace evidence (for example, a pane of glass). This means that simple univariate techniques like ANOVA and t-test were not well-suited to soil analysis. Multivariate techniques provided a much more powerful method of discrimination on the basis of a large number of small differences.

PCA was useful in visualizing the natural variation in the specimens and is recommended when the location of a specimen is unknown or when exploring trends in the elemental profiles. Spatial heterogeneity, in both elemental profile and color, was determined to be site-specific; some sites showed very tight groupings while others showed a larger spread, with tighter groupings at the within-sub-plot and within-sample level, highlighting the importance of proper characterization of each site to determine the within-site variation.

LDA was a useful tool for forensic discrimination of soils from different locations within a region. The discrimination element menu was important in achieving the best possible discrimination, and various approaches were investigated for determining the optimal combination of elements. In a real forensic case, optimization of the element menu should be performed on the known specimens only (i.e., generating the model using the training set). Once a good separation of the known is achieved, only then should the model be tested using the questioned specimen(s) to avoid biasing the discrimination results. Correct classification rates of 94.5 % or better were achieved in a simulated forensic discrimination of three similar sites.

In general, elemental profile differences were greatest for specimens from different regions separated by large distances, even within the same lithological type. Within a region containing only one lithological type, local effects generally outweighed the underlying lithology in their contributions to the elemental profile. It is therefore unlikely that a calcareous soil specimen from Miami would be mistaken for one from Washington. In addition, with the availability of lithological and elemental maps and databases for many regions, it will be possible to eliminate certain areas from consideration as a possible source of an unknown sample and therefore narrow down a search area. Some examples of scenarios in which this information could be useful when the crime scene location is unknown include: comparison of soil found on a victim with the soil at the burial location to determine if the body has been moved; determination of the recent origin of an individual at an immigration checkpoint; determination of the recent origin of a human

trafficking victim or exotic plant or animal. Determining the locations from which the soil did not originate can be almost as interesting as determining the actual origin.

10.3. Applicability to the Forensic Community

Soil is ubiquitous, and yet often ignored. Many laboratories do not perform soil analysis at all, because of the specialized training and expertise required for many of the above analyses. However if they already have access to elemental analysis instruments used for glass, paint, metals and gunshot residue, perhaps they would be more likely to use them for soil analysis if an established method were made available. Elemental analysis will not likely replace conventional techniques, but rather act as a source of complimentary information or as a confirmatory method. The elemental analysis methods presented here are an additional tool for the forensic examination of soil. Soil is a difficult matrix. Forensic (or other) scientists will find the information in this work useful in choosing the sample preparation and elemental analysis methods most suited to their needs, and to identify important considerations for method development, validation, and interpretation.

For forensic analysis, precision, cost per analysis, and specimen consumption of an analysis are important. Of the three methods evaluated here, LA-ICP-MS provided the most precise and accurate data but is the most expensive. While μ XRF is non-destructive, LA-ICP-MS and LIBS consume only microgram amounts of mass (quasi-non-destructive) still allowing for future reanalysis of pellets or tape-mounted specimens. Depending on the type and location of the soil samples, the optimal element menu may vary, especially if anthropogenic contributions, such as pollution, are suspected. The LIBS spectra contain a richness of information in that they contain information on all elements without the requirement for prior knowledge of the element menu. In addition, LIBS has good sensitivity in the low atomic mass range, while LA-ICP-MS is subject to interference in this range. LIBS & μ XRF are less expensive and easier to operate and maintain than LA-ICP-MS, and field-portable units are now commercially available for both. All three techniques can be used for analysis of additional matrices (glass, paint, metals, and inks). Since the LIBS and LA-ICP-MS methods used a 266 nm laser, they can also be used for transparent materials, such as glass.

Analysis parameters for LIBS and LA-ICP-MS were optimized to minimize background contributions from the tape. Precision, LODs, and accuracy were comparable to the pellet method. Discrimination, by both PCA and LDA, was comparable to results achieved with pellets, at greater than 94.5 % correct classification rates for both. Elemental analysis and discrimination methods developed in this work have been effective at discriminating specimens originating from different sources, including some that were not discriminated by color.

The pellet method has already been adopted by one of the inter-laboratory comparison participants for use with LA-ICP-MS in creation of a soil database. The concerns of the forensic community about the amount of specimen and time required were addressed using the tape-mounting method, where 10 ± 4 mg of sieved, spiked, non-milled soil were mounted onto a clear, low-background adhesive tape. The entire sample preparation process could be performed in as little as one day. Tape-mounted soils can be stored and re-analyzed by LIBS, LA-ICP-MS, color, microscopy, and mineralogy.

10.4. Applicability Outside the Forensic Community

Soil elemental profiles have many uses beyond the forensic community, including mineral exploration, environmental monitoring, nutrient analysis, and sediment fingerprinting. Methods for sample preparation and analysis for filter-bound sediments were developed for the latter application. Performance of both the sediment filter method and bulk soils method were excellent. An element menu of 20 elements was obtained with recoveries between 75 and 125 %, precision of 10% or less (RSD), variability of 10 % or less (for duplicates), and limits of detection in the ppb to low ppm range. These methods were capable of generating quantitative multi-element data of sufficient quality for use in sediment fingerprinting applications with sediment masses as low as 47 ± 34 mg on an 86 mg cellulose nitrate membrane filter.

10.5. Summary of Sample Preparation Techniques Developed

Methods of sample preparation were developed for various types of soil specimens, from milling and pelletizing for large bulk specimens and small filter-bound specimens to tape-mounting for small transfer specimens. The amount of specimen required to prepare each sample type, and the amount removed per analysis are summarized in Table 30.

Table 30. Summary of the amount of specimen required to prepare each sample type and the amount of soil removed with each analysis method.

Sample Type		Amount Required to Prepare Sample	Amount Removed per Analysis	
		Average $\pm$ SD	LIBS (5 Reps) ^a	LA (4 Reps) ^b
Pellets ($\varnothing=13\text{mm}$)	Mass (mg):	470 ± 50	0.10 ± 0.03 (n = 75)	0.45 ± 0.16 (n = 38)
	Volume (mm^3):	265^d	0.023 ± 0.009 (n = 75)	0.023 ± 0.009 (n = 38)
Pellets ($\varnothing = 6\text{mm}$) ^c	Mass (mg):	47 ± 34		
	Volume (mm^3):	57^d		
Tape	Mass (mg):	10 ± 4	0.8 ± 0.2 (n = 90)	1.7 ± 0.4 (n = 50)
	Area (mm^2):	190^e	38.9 ± 7.1 (n = 90)	11.0 ± 3.3 (n = 50)

a. 266 nm, 100 % energy (29 mJ), line mode, 175 $\mu\text{m/s}$, 0.67 Hz, 75 shots/rep 266 nm, focused 1.4 mm behind surface, 137.5 μm spot

b. 266 nm, 30 % energy (0.49 mJ), line mode, 150 $\mu\text{m/s}$, 50 s ablation @ 5 Hz, 500 shots/rep, 200 μm spot

c. Sediment milled with filter paper (86 $\pm$ 4 mg, cellulose nitrate). Mass of sediment in addition to filter mass

d. Approximate pellet volume, given a 2 mm height

e. Approximate tape-mounted area, given a 19 x 10 mm exposed surface

10.6. Recommendations for Future Research

Further research is necessary to fully standardize the method for use across forensic laboratories. This would involve larger inter-laboratory comparison exercises similar to the one reported in this work, and additional exercises involving discrimination of simulated evidence to determine acceptable match criteria, similar to what has been done with glass [89]. ASTM guidelines should be written in order to provide standardized methods to the forensic community. This work provides the beginnings of a database of elemental profiles for various regions in the USA. A centralized database containing soil elemental profiles

from the many smaller databases could be created to aid investigators in provenancing soils from unknown locations.

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APPENDICES

Appendix 1. Munsell Color Results.

Images consist of two or more pages from the Munsell Soil Color Book. Each page is a particular hue, as noted on the tab (Y is yellow, YR is a yellow-red, and GLEY is grey). Color determinations were made as described in 3.1.3. Each image represents the results from one Miami-Dade County site (see Figure 9). Orange numbers were added to the color chips to indicate the color determination of a specimen from a particular sub-plot, with intermediate colors being represented by numbers between chips.

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