ELEMENTAL FINGERPRINTING USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY AND CHEMOMETRICS: APPLICATION TO ENVIRONMENTAL SCIENCE AND PROVENANCE STUDIES

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ABSTRACT

This dissertation presents results for five research projects showcasing the versatility of ICPMS coupled with multivariate statistics for elemental fingerprinting in environmental, forensic, and provenance studies.

The first project evaluates the feasibility of elemental fingerprinting to distinguish "contaminated" drywall from "non-contaminated" drywall. Some drywall has been shown to outgas reduced sulfur species, which has caused health and other problems for homeowners. The elemental fingerprinting approach was compared with conventional methods, and was shown to distinguish the two groups. Moreover, it shows promise in determining the geographic source of gypsum, the primary constituent in drywall.

Another project describes the use of elemental fingerprints in determining the geographic source of farm-raised catfish. Elemental fingerprints were able to distinguish catfish based on country of origin, with Ba and Rb playing an important role in the discrimination. Differences in source water chemistry likely influenced the outcome. $^{87}Sr/^{86}Sr$ ratios were determined in the bones of fish from Macon and Stoneville, Mississippi, and results showed significant differences between them.

Next, a laboratory exercise, based on elemental fingerprinting of soil, was developed to promote an inquiry-based learning experience for forensic science majors. All eight-student groups classified their unknown soil among the different locations. Students learn, however, that applying the methodology in forensic investigations is more complicated and has potential pitfalls.

The ability to determine the authenticity of dietary supplements and its botanical source is important for public safety. In this study we show that elemental fingerprinting can be used to discriminate ephedra from U.S. and China, and to distinguish *E. przewalskii* from the other species tested. However, the approach did not have the discriminatory power to separate species other than *E. przewalskii*.

Finally, we evaluate the effect of Hg-free artisanal mining (Clean Tech mine) on heavy metals levels in local environment (soil) and in hair and fingernails of miners in Mozambique. Mercury levels in soil from Clean Tech are strikingly lower than the other mines which employ Hg. Clean Tech miners also tended to have lower levels of metals in their fingernails. Heavy metals in biological samples from miners were substantially higher than non-mining populations.

LIST OF ABBREVIATIONS

ASGM	Artisanal Small-scale Gold Mining
AC	Alternating current
DA	Discriminant Analysis
DC	Direct current
DI	Deionized Water
ESA	Electric sector analyzer
FTIR	Fourier Transform Infrared
HCA	Hierarchical Cluster Analysis
ICP	Inductively Coupled Plasma
ICP-QMS	Inductively Coupled Plasma-Quadrupole Mass Spectrometry
ICP-SFMS	Inductively Coupled Plasma-Sector Field Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
m/z	Mass to charge ratio
MANOVA	Multivariate Analysis of Variance
NCNPR	National Center for Natural Products Research
PCA	Principal Component Analysis
ppt	Parts per trillion
ppm	Part per million
TGA	Thermogravimetric analysis

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION AND ORGANIZATION OF THE DISSERTATION

One of the most versatile tools in elemental analysis is Inductively Coupled Plasma Mass Spectrometry (ICPMS). With technical advances and improvements over the years, elements can now be reliably quantified in a wide-range of samples at trace and ultra-trace levels. Because of its low detection limits, high sensitivity, large linear dynamic range, and high resolution (sector field version), ICPMS has been used for diverse and varied applications in areas such as environmental, geological, and biomedical studies. With its multi-element capability, it is also ideally suited for elemental fingerprinting which has become a powerful approach for provenance studies. This dissertation focuses on elemental fingerprinting using data obtained by ICPMS and analyzed by multivariate statistics. It presents five such research projects in which elements are quantified in soil, foodstuffs, botanicals, and other biological samples for environmental, forensic, and provenance-related studies. Each of these projects is presented in separate chapters.

The first chapter provides relevant background material and discusses the fundamental theory of the instrument and analytical methods used throughout this study. It discusses the need for analytical techniques for provenance of products, especially food. In addition, it introduces the statistical analysis used throughout this work. Specific research projects are discussed in the remaining five chapters, each which have their own abstract, introduction, objectives, materials and methods, results and discussion, and conclusions sections.

Chapter two evaluates the feasibility of using elemental fingerprints for distinguishing contaminated, sometimes called “Chinese” drywall, from non-contaminated drywall. Contaminated drywall is known to outgas volatile sulfur species, which cause a range of problems for homeowners. Results were compared to infrared spectroscopy and Sr content, the conventional way to distinguish between the two. Elemental fingerprinting also showed potential in determining the geographic source of gypsum. Gypsum from three different mines and a flue gas desulfurization plant were evaluated.

The third chapter describes the use of elemental fingerprints in determining the geographic source of farm-raised catfish. The ability to determine the source of food is necessary to enforce existing import laws, secure food supply, and protect consumers from fraud and deception. In the U.S., increasing competition and importation of catfish from other countries prompted implementation of Country of Origin Labeling (COOL), a law that requires certain commodities to give information about the country of origin in their label. In this study, we evaluated the capability of elemental fingerprinting to determining the provenance of catfish (*Ictalurus punctatus).*

The fourth chapter presents a laboratory exercise developed to promote an inquiry-based learning experience for forensic science majors. Students in an instrumental analysis course with a forensic emphasis were presented with a mock scenario in which soil was collected from a murder suspect’s car mat, from the crime scene, from adjacent areas, and from more distant locations. Students were then asked to conduct a comparative analysis using the soil’s elemental distribution fingerprints. Students learn sample preparation using microwave acid digestion, ICPMS, and multivariate statistics. Whereas results suggest that the elemental fingerprinting approach can be used to distinguish soils from different land-use areas and geographic locations,

applying the methodology in forensic investigations is more complicated and has potential pitfalls.

The fifth chapter evaluates the elemental profile of ephedra to determine the geographic source and to distinguish between species from the U.S. and China. The ability to determine the authenticity of the dietary supplement and its botanical source is important for public safety because mislabeling may potentially cause adverse effects. Results from the study showed that elemental profile could be used to discriminate ephedra from US and China, and to distinguish *E. przewalskii* from the rest of ephedra species.

In the final sixth chapter, the environmental and health consequences of using mercury in artisanal mining were investigated. Heavy metals were determined in hair and fingernails of artisanal small-scale gold miners in Manica District of Mozambique. The district has several mines, including Munhena, Tsetsera, and Clean Tech. The latter is unique among the mines in that it utilizes centrifugation and magnet technology instead of Hg to extract gold. Hg concentration in soil from Clean Tech is strikingly lower than the other mines, indicating that their operation is environmentally friendly. Clean Tech also tended to have lower levels of metals in fingernails. The heavy metal levels in fingernail and hair from miners were substantially higher than non-mining populations.

1.2 BACKGROUND OF THE STUDY

1.2.1 Provenance and authentication of food and food products

The expanding world economy, growing consumer demand, and growth of international trade have resulted in globalization of food and beverages. As foodstuffs become more globalized and shipped around the world, there is an increasing demand for assurance of the origin and authenticity of food. Consumers require the integrity, safety, and quality of food and supplies. Market demand relies on trust and confidence of consumers. There is growing concern about foodborne diseases and toxic contaminants in foods, and this has created public anxiety. Consumers require reliable information about the authenticity and sources of food to prevent food adulteration, fraud, and deception. To help protect consumer and producer, the European Union adopted two regulations in 1992, and the United States of America passed a similar farm bill in 2008.

The European Union adopted the regulations are Regulation (EEC) No 2081/92 and Regulation (EEC) No 2082/92. The purpose of the first regulation is to protect names while the second one is to protect traditional recipes and manufacturing processes. Categories of protected names such as designation of origin (PDO) and geographical indication (PGI) of agricultural products and foodstuffs are covered and distinguished in Regulation No 2081/92. The two categories are distinguished based on how closely the product is linked to the specific

geographical area whose name it bears. Regulation No 2082/92 specifies the character for agricultural products and foodstuffs.

In the U.S., mandatory Country of Origin Labeling (COOL) was implemented in September 2008. The 2002 Farm Bill, 2002 Supplemental Appropriations Act and the 2008 Farm Bill amended the Agricultural Marketing Act of 1946 that requires the label of the covered commodities to provide specific and accurate country of origin information to the consumers.

With a globalized economy, food authenticity and provenance has created a challenge to food control authorities. The ability to determine the geographic source of a product is important to enforce existing import laws and requirements, and implement antidumping duties [1], secure food supply [2], and protect consumer from overpayment and deception [3]. Different approaches and techniques have been studied to trace the geographical origin of a sample. These methods, discussed briefly below, include genetic-based approaches [4-7], isotope ratio analysis of light elements [8-12], isotope ratio analysis of heavy elements [13-15], and elemental profiling [1, 16-18].

1.2.1.1 Genetic Testing

Genetic-based authentication has been applied to identification of many types of fish and seafood species, including salmonids [19], scombroids [20], mollusk [21], flatfish [22], and eels [23]. DNA-based authentication methods are based on polymorphisms in the genetic codes of different species. This genetic variation results from naturally occurring mutations in the genetic code [24]. Genetic-based approaches use molecular biology techniques such as Polymerase Chain Reaction (PCR) method [4], Amplified Fragment Length Polymorphism (AFLP) analysis, Randomly Amplified Polymorphic DNA (RAPD) analysis, and Inter-Simple Sequence Repeat

(ISSR) analysis [4-7]. PCR, in vitro analysis of amplified portion of specific DNA sequence of an organism, is done using specific oligonucleotide primers and the thermostable DNA polymerase enzyme [25].

DNA analysis is best for unadulterated samples and it requires good quality DNA. Detection of species-specific genetic polymorphisms requires careful extraction of DNA with sufficient quality and quantity. Factors that can affect the quality of DNA includes heat, pH, hydrolysis, depurination, and nucleases which causes enzymatic degradation [26]. These factors could result in the shorter target sequence and reduced quality of DNA obtained from processed seafoods. The advantages and disadvantages of these techniques are summarized in Table 1.1. PCR methodologies such as AFLP analysis, RAPD analysis, and ISSR analysis do require prior knowledge of DNA sequence [4]. However, in PCR method, variation in DNA samples and thermal stability of DNA polymerase makes it difficult for standardization of the procedure [27]. The majority of these techniques are highly specific – identification is optimized for a specific species. Prior knowledge of the species is necessary in order to efficiently perform the analysis. Using a wrong genetic testing method could give a false positive or false negative result. This calls for a need of a standardized approach to DNA-based authentication of animal food products [28].

1.2.1.2 Stable Isotope Ratio Analysis

Stable Isotope Ratio Analysis (SIRA) of elements can be used to determine the geographical origin of samples. SIRA has been used to geo-locate source of cocaine [8], heroin [9], diazepam samples [10], animal products [11], and tablets [12]. The isotopic ratio of an element in a sample is not constant in nature because of isotope fractionation that can happen

during their involvement in various chemical, physical, and biological processes [29]. Table 1.2 summarizes the ways in which stable isotope ratio can be applied for authentication purposes. Hydrogen and oxygen isotope ratios are latitude dependent. Geographical isotope variation results from the meteorological cycle of evaporation, condensation, and precipitation [30]. Nitrogen and carbon isotope ratios are affected by an organism's diet, which may be related to trophic level and geography/habitat, and for human's cultural practices [31].

Table 1.1. Advantages and disadvantages of DNA-based authentication method [7]

Method	Applicable to degraded material	Simple Protocol	Low DNA requirement	Mixture Detection	Time-efficient	No Prior knowledge required	Reproducible between labs	Standardized across taxa
Hybridization	X			X				
Species-specific primer	X	X	X	X	X		X	
RFLP		X	X		X	X	X	
SSCP		X	X		X			
RAPD		X	X		X			
Traditional Sequencing	X*	X	X		X	X	X	
DNA barcoding	X*	X	X		X	X	X	X

X indicate that they exhibit the corresponding feature.
* Only applies to small fragments in the case of severely degraded samples.

Table 1.2. Applications and fractionations of isotope ratio [17]

Isotope Ratio	Fractionation	Information
$^{2}H/^{1}H$	Evaporation, condensation, precipitation	Geographical
$^{13}C/^{12}C$	C3 and C4 plants	Diet (geographical proxy)
$^{15}N/^{14}N$	Trophic level, marine and terrestrial plants, agricultural practice	Diet (geographical proxy)
$^{18}O/^{16}O$	Evaporation, condensation, precipitation	Geographical
$^{34}S/^{32}S$	Bacterial	Geographical (marine)
$^{87}Sr/^{86}Sr$	Age of the rock and Rb/Sr ratio	Underlying geology, geographical

Sample integrity and its ability to retain isotopic composition must be taken into account. Isotopic fractionation can occur during manufacturing processes. Microbes can have a profound effect on the isotopic composition of materials that are susceptible to microbial degradation. Isotopic homogeneity and heterogeneity must be considered in isotopic studies [32].

1.2.1.3 Strontium Isotope Ratio

Strontium is a divalent alkaline earth element that has four naturally occurring stable isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr, ^{88}Sr [33]. The relative abundance of the isotopes ^{84}Sr, ^{86}Sr, and ^{88}Sr in earth materials are mostly constant [34]. On the other hand, ^{87}Sr is a radiogenic isotope that is produced from the radioactive decay of ^{87}Rb thereby the amount of ^{87}Sr in a mineral rocks increases over time [33. 35]. This makes ^{87}Sr useful as a tracer in understanding the geological processes such as petrogenesis, weathering, atmospheric fluxes, and cation biocycling [33-34]. Studies that use ^{87}Sr as a geological tracer has been published in the literature [36-37].

Strontium has also been used for provenance and fingerprinting studies including fish [38-42]. Strontium, with a relatively high mass, does not fractionate to the extent C, H, N, O, and S do [43]. For soil and vegetation system, fractionation of $^{87}Sr/^{86}Sr$ is corrected for during measurement and is considered negligible [34]. As noted, due to radiogenic nature of ^{87}Sr, the amount of $^{87}Sr/^{86}Sr$ depends on geographical region. Thus, $^{87}Sr/^{86}Sr$ ratios can provide information about the geographical background and source of a variety of samples including food products [34. 43].

Strontium in bones and fish otoliths (ear bones) has been studied for provenance purposes [42, 44-46]. Strontium and calcium, both Group IIA in the periodic table, have relatively similar electron configuration, charge, and radius [34]. Sr^{2+} and Ca^{2+} have ionic radius of 1.13Å and

0.99Å respectively [33]. Because of this, strontium can be incorporated in bones through several processes: ionic exchange with Ca^{2+} in bone, surface adsorption of Sr in bones, or binding of Sr^{2+} by preosteoid protein [47]. Sr isotope ratio in human teeth and bones were used to study migration events in the past [48-49]. Sr in fish otoliths was used as estimate geographic origin and date of fish introduction of an exotic fish into a lake [45].

1.2.1.4 Elemental Profiling

Trace metal profiling has been cited in the literature since the 1980s to determine geographical origin of agricultural products. The evolution and improvement of analytical instrumentation have led to better analytical techniques for elemental profiling. ICP-MS, first introduced in the early 1980's, has become a major technique in elemental analysis [50]. ICP-MS has wide linear dynamic range, high sensitivity, high sample throughput, and multi-element and isotope ratio capabilities. The introduction of Sector Field (SF) high resolution ICP-MS has improved the sensitivity and detection limits, and eliminated many of isobaric interferences found in quadrupole-ICP-MS. The improved capabilities of ICP-MS were validated with older techniques such as Inductively-Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [50-51].

Numerous studies have used elemental profiling along with multivariate statistics to trace geographical origin of plant samples. Nikdel et al. used the method to determine the country of origin and detection of adulteration of orange juice [52]. Schwarts and Hecking determined geographic origin of agricultural products [53]. Anderson et al. used trace metal profiling along with multivariate statistics to determine geographic origin of potatoes [3]. Anderson and Smith

studied geographic growing origins of coffee [54]. Samsoe-Petersen determined the uptake of trace elements and PAHs by fruits and vegetables from contaminated soils [55].

Others have used elemental profiling with multivariate statistics to trace geographical origin of aquatic organisms. This is potentially more complicated than terrestrial plants, which are fixed in soil. Windom et al. compared the trace metal concentrations in the muscle tissue of a bottom dwelling benthopelagic fish (*Coryphanoides armatus*), which is found in Pacific and Atlantic oceans [56]. Favretto et al. differentiated mussels from different sites [57]. Jung et al. analyzed bioaccumulation of trace metals in brown shrimp (*Craggnon cragnon*) under laboratory-controlled environment [58].

Several factors potentially affect trace element concentrations and bioaccumulation in fish. These include the sources of metals/elements and metabolic pathways involving assimilation of these metals/elements [59]. Metabolic pathways include processes such as the uptake, elimination, storage, and transformation of food by the fish. These processes are affected by the differences in permeability, metabolic rate, quantities and types of metal-binding ligands present in the environment where the fish is grown. These processes vary from species to species and to some extent between individuals of the same species, which can contribute to the overall assimilation pattern and quantification of metal/elemental turnover in fish tissues [59]. Fish diets and the aquatic system from which fishes are grown can contribute to assimilation of these metals/elements [60-61]. Variations in elemental concentration in muscle tissue of farm-raised catfish can be attributed to various factors. These may include the fish size, diet, feeding rates, water quality, and aquatic environment from which includes the pond size, shape, design, and stocking density.

1.3 ANALYTICAL METHODS AND INSTRUMENTS

1.3.1 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is an analytical technique designed for elemental analysis. It became commercially available in the early 1980s [62]. Technical advances and improvements over the years make it suitable for varied and diverse applications in geological, environmental, biomedical, and forensic fields [63-65]. Its superior detection capability and high sensitivity enables analysis at ultra-trace levels (concentrations at parts per trillion or less). Its wide linear dynamic range allows quantification from ultra-trace levels to parts per million or higher [62]. ICP-MS has the capability to determine more than 60 elements from the periodic table in a matter of seconds [66]. The primary advantage of ICP-MS over other atomic analytical methods (e.g. Flame Atomic Absorption, Electrothermal Atomization) is its ability to determine multiple elements, with low detection limits, and high throughput [62].

ICP-MS combines ICP as ionization source and with mass spectrometer as detector. Samples are introduced through ICP, which desolvates, atomizes and ionizes the sample, usually introduced as an aerosol. Ions are then directed into the mass spectrometer, where they are separated based on their *m/z* ratio, and counted using a detector (electron multiplier or faraday). Common mass analyzers used in ICP-MS include, quadrupole, time-of-flight, and sector-field [62, 66]. Details about the two mass analyzers used for the present work (quadrupole and sector-field) is discussed below.

1.3.1.1 ICP as ionization source

Samples are first converted to an aerosol before introducing into the ICP [67]. Aerosol can be generated by laser ablation (for solid samples), or by introducing liquid samples through a nebulizer. The aerosol is desolvated, atomized, and then ionized in very hot plasma (~6,000 to 10,000K). A schematic diagram of the ICP is given in Figure 1.1. RF power is applied to the induction coil, causing the alternating current to oscillate within the coil at a frequency of the generator. This produces an oscillating electric field, which induces an oscillating magnetic field around the coil. Argon gas is then introduced through the torch and a spark is applied to the flowing argon, which ionizes the argon gas. The argon ions and electrons are then caught in the oscillating and magnetic field. The high-energy electrons and argon ions collide with each other forming very hot plasma (~6000-10,000K) [66].

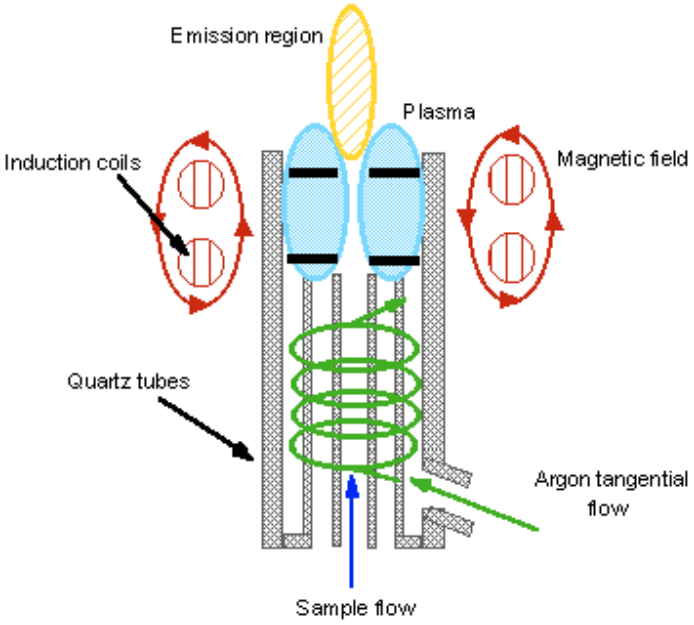

Figure 1.1. Schematic of ICP torch [68]

1.3.1.2 Quadrupole mass analyzer

The quadrupole mass analyzer utilizes electric field to separate ions based on their *m/z* ratio. The quadrupole field is created by applying a direct current (DC) and alternating current (AC) on opposite pairs of the four rods shown in Figure 1.2. The AC/DC ratio for each pair of rods can be changed and optimized so that only selected ions can pass through the flight path centered between the four rods. AC/DC ratio affects the trajectory of ions travelling the flight path. For given AC/DC ratio, only ions with certain *m/z* are allowed to pass through the flight path while the other ions are ejected from the quadrupole [62].

Quadrupole-based instruments do not have the sensitivity and resolution of double-focusing mass analyzer. Quadrupole mass analyzers, without collision cells, also suffer from its limited ability to resolve polyatomic spectral interferences. However, it is very fast that it can give a complete spectrum in less than 100ms. Quadrupole is rugged, compact and in relatively inexpensive compared to other mass analyzers [66].

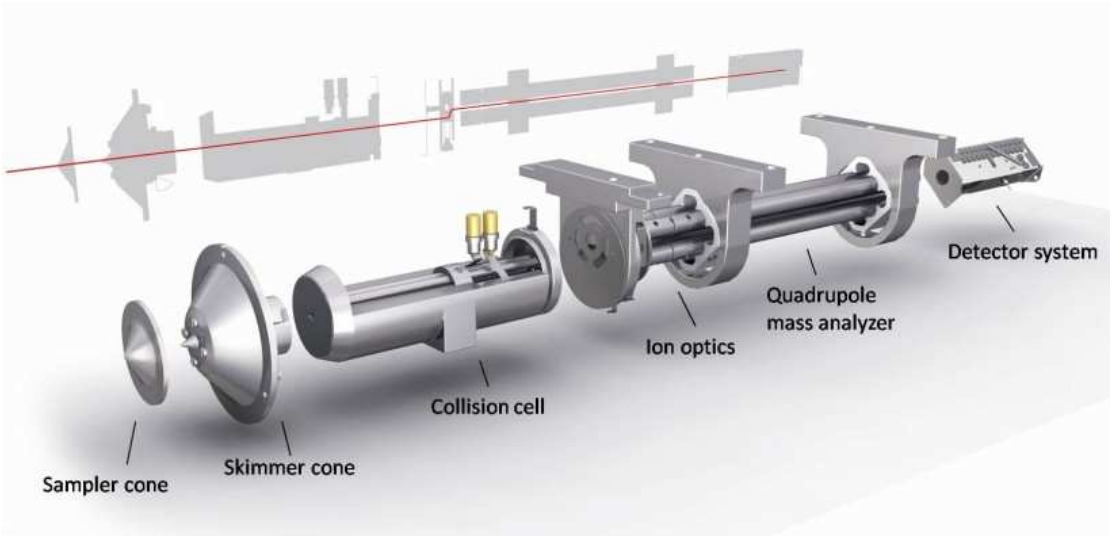

Figure 1.2. Schematic of a quadrupole mass analyzer (X-Series 2). Used with permission from ThermoFisher Inc.

1.3.1.3 Sector-field mass analyzer

Sector-field mass analyzer utilizes two major components in separating the ions: magnetic sector analyzer and electrostatic analyzer (ESA) shown in Figure 1.3. Magnetic sector is applied with magnetic field perpendicular to the motion of the ions. Ions that enter the magnetic sector are deflected and their degree of deflection is proportional to their *m/z*. On the other hand, ESA is used to focus ions based on their kinetic energy because the ions accelerated from the ICP to the magnetic sector will have varying kinetic energy. Because it only allows ions with certain kinetic energy to reach detector, ESA greatly improved the resolution of a magnetic sector-IC-PMS [62].

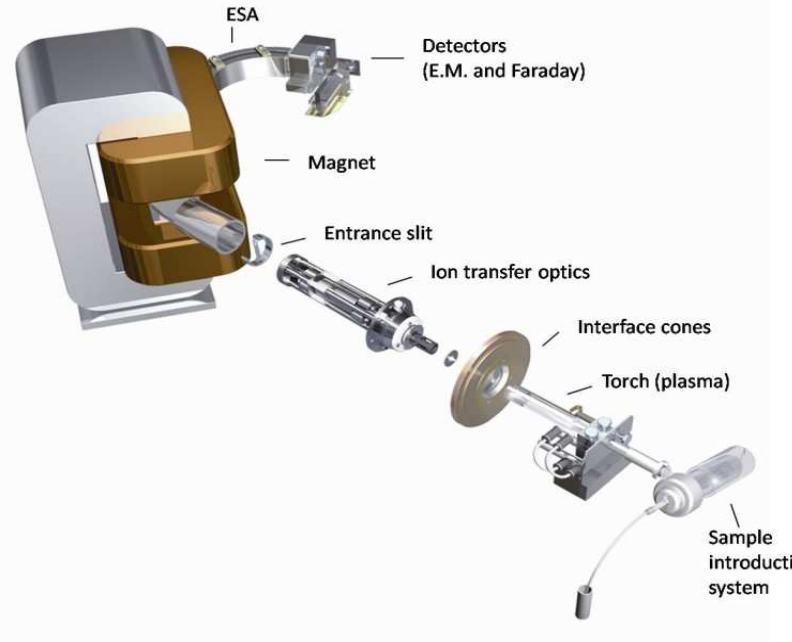

Figure 1.3. Schematic of a sector field ICPMS (Element-XR). Used with permission from ThermoFisher Inc.

1.4 STATISTICAL METHODS

1.4.1 Principal Component Analysis (PCA)

The purpose of principal component analysis (PCA) is to reduce a data set containing many variables to a smaller number of composite variables, which represents most of the information in the original data set. PCA expresses covariation in many variables in a smaller number of composite variables. This is accomplished by determining the strongest linear correlation structure among variables [69]. PCA determines the principal components (PCs) that are linear combination of the original variables [2]. PCA determines the variance explained by each axis (eigenvalues) and the coefficients in the linear equations (eigenvectors) that combine the original variables. The size of the eigenvalue determines how much variance is represented by each PC. The eigenvectors list the contribution of each response variable to a particular component [69].

In this study, we used PCA and plotted the samples with respect to the PCs in the ordination space that gives 2D or 3D views of how individual samples differ from one another. However, it is noted that PCA measures total sample variation and does not explicitly take into account the variation between groups [2]. To get the best possible evaluation of the group classification and discrimination, elemental profiles will be analyzed using DA (see below).

1.4.2 Discriminant Analysis (DA)

Discriminant analysis is an eigenanalysis technique, which maximizes the among-group differences relative to within-group variation. It requires pre-defined groups [69]. It helps the researcher in studying the differences between two or more groups of objects with respect to several variables simultaneously [70]. DA is used in different fields of study for: (a) summarizing the differences between groups, (b) multivariate testing to check whether or not two or more groups are significantly different from each other, (c) checking for misclassified items, (d) predicting the membership of a sample point in a group, (e) determining which variables contribute most to discriminating between groups [69].

DA determines the canonical axes or discriminant functions that best discriminates among groups. DA determines the optimal linear combination of variables such that the first function represents the most overall discrimination between the groups, the second represents the second most, and so on. The discriminant functions are then subjected to significance test. Classification of variables is performed once the functions are determined to be statistically significant. Standardized beta coefficients are then determined for each variable. The size of the standard beta coefficients determines the independent contribution of each variable in discriminating the groups [69].

Discriminant analysis is a specific type of Multivariate Analysis of Variance (MANOVA). However, the results are summarized differently and the emphasis is different [69]. In MANOVA, the groups are the independent variables while the continuous variables are the dependent variables. MANOVA finds the differences in the dependent variables among the groups. In DA, the continuous response variable are treated like predictor variables, similar to logistic multiple regression. The response variables are used to predict the membership in a

group in DA [69]. Another important feature of DA is that it allows one to rank continuous variables in order of their importance in discriminating groups, while accounting for correlations among these variables. For the purpose of my study, I will be able to determine which elements are most important in discriminating geographic origin of fish/ephedra.

The four multivariate measures, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley, and Roy's Max Root are used for significance testing in DA. The four measures differ from each in the way in which they combine the dependent variable to determine the variance in the data. Among the four multivariate measures, Wilk's lambda is the most frequently used one because the values of the other three measures become similar as the sample size increases [71]. I will use Wilk's lambda because it is intermediate in its conservativeness to the other tests.

Wilk's lambda takes into consideration both the homogeneity within the groups and the variance between groups [70]. Wilk's lambda determines the degree of group separation accounted for in the dependent variable by the independent variable [71]. Wilk's lambda is used to test mean of differences. It is the error sum of squares divided by the sum of squares and the error sum of squares. The value ranges from 0 to 1. The size of the lambda values is a measure of how separated the group is. The larger the value the smaller is the difference between the groups. The lambda value is not used for testing of significance. Significance test is performed by chi-square approximation derived from Wilk's lambda [69].

1.4.3 Multivariate Analysis of Variance (MANOVA)

MANOVA is a multivariate statistical test for differences among groups based on a set of dependent variables. Both MANOVA and ANOVA tests for statistical difference between groups. However, ANOVA tests for differences in means between two or more groups involving one response variable, whereas, MANOVA tests for the difference in two or more vectors of

means. Therefore, MANOVA is used when there is more than one response variable [72]. When the number of observations greatly exceeds the number of response variables, correlations among the response variables can be considered (as in DA) without the danger of overfitting. However, when the number of observations does not exceed the number of response variables, then the use of MANOVA requires the assumption of independence among response variables.

1.4.4. Hierarchical Clustering Analysis (HCA)

HCA is an unsupervised pattern recognition method designed to discover classifications within a complex data sets [73]. Hierarchical Clustering Analysis (HCA) uses measures to sequentially join an object into groups [74]. The procedure starts with each point as individual cluster. At each step, the two clusters that are most similar are combined into a single new cluster and the process continues until all points are combined into one cluster [75]. This procedure is agglomerative clustering approach, in which single objects are connected to each other to form a group [76]. The first step in an agglomerative Hierarchical Clustering Analysis (HCA) is to determine and evaluate the similarity or dissimilarity of objects [77]. There are four methods to determine similarity between objects: (1) Correlation coefficient, (2) Euclidean distance, (3) Manhattan distance, and (4) Mahalanobis distance. The second step in HCA is to link the objects. A number of linkage methods are available for clustering. These include Ward's method, complete linkage, or average linkage.

This study used Ward's method of clustering based on a Euclidean distance matrix. Euclidean distance, ED_{ih}, beween sample *i* and *h* is calculated as follows:

$$ED_{ih} = \sqrt{\sum_{j=1}^{p} (a_{ij} - a_{hj})^2} \qquad \text{Eq. 1 [16]}$$

where: p is the number of variables, a_{ij} is a matrix of variables *i* and *j*. The higher the similarity

between the samples, the lower is the Euclidean distance [77]. The linkage method, Ward's, uses the ANOVA sum of squares between the two clusters added over all the variables as the distance between the two clusters [75]. It involves clustering procedure seeking to form partition such that the error sum of squares is minimized with each grouping [78]. The error sum of squares is calculated as the sum of the squares of the distances from each individual to the centroid of its group [16]. Results from HCA are represented by a dendogram, a diagram that illustrates relationship between the cluster and sub-cluster. It also shows the fusion or divisions made at each successive stage of analysis [78].

1.4.5 Box-Whisker Plot

The plot shows the range, median, the first quartile, the third quartile of data set. Whiskers, lines extending from each end of the box, represent the range of the data points. A horizontal line within the box represents the median. The ends of the box represent the first and third quartiles, which correspond to 25 and 75 percentile of the data set, respectively.

1.5 LITERATURE CITED

[1] Smith, R.G., Watts, C.A. Determination of the Country of Origin of Farm-raised Shrimp (Family Penaeide) using trace metal profiling and multivariate statistics. *J. Agric. Food Chem.* **2009**, 57 pp. 8244-8249

[2] Anderson, K.A., and Smith, B.W. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. *J. Agric. Food Chem*. **2005**, 53 pp. 410-418

[3] Anderson, K.A. et al. Determining the geographic origin of potatoes with trace metal analysis using statistical and neural network classifiers. *J. Agric. Food Chem*. **1999**, 47 pp. 1568-1575

[4] Techen, N., Crockett, S.L., Khan, I.A., Scheffler, B.E. Authentication of Medicinal Plants Using Molecular Biology Techniques to Compliment Conventional Methods. *Current Medicinal Chemistry* **2004**, 11 pp. 1391-1401

[5] Sucher, N.J., Carles, M.C. Genome-based Approaches to the Authentication of Medicinal Plants. *Planta Med*. **2008**, 74 pp. 603-623

[6] Joshi V.C., Khan, I.A. Macroscopic and Microscopic Authentication of Chinese and North American Species of Ephedra. *Journal of AOAC International* **2005**, 88 pp. 707-713

[7] Techen, N., Khan, I.A., Pan, Z., Scheffler, B.E. The Use of Polymerase Chain Reaction (PCR) for the Identification of Ephedra DNA in Dietray Supplements. *Planta Med*. **2006**, 72 pp. 241-247

[8] Ehlringer, J.R., Casale, J.F., Lott, M.J., Ford, V.L. Tracing the Geographical Origin of Cocaine. *Nature*. **2000**, 408

[9] Besacier, F. et al. Isotopic Analysis of 13C as a Tool for Comparison and Origin Assignment of seized heroin samples. *J. Forensic Science*. **1997**, 42 (3) pp 429 – 423

[10] Bommer, P. et al Determination of the Origin of Drugs by Measuring Natural Iosotope

Contents: D/H and 13C/12C Ratios of Some Diazepan Samples. *Z. Natuforsch* **1976**, 31c. pp. 111 – 114

[11] Renou, J.P., et al. Characterization of Animal Products According to Geographic Origin and Feeding Diet Using Nuclear Magnetic Resonance and Isotope Ratio Mass Spectrometry. Part II. Beef Meat. *Food Chem*. **2004**, 86 pp 251 – 256

[12] Palhol, F., et al. 15N/14N Isotopic ratio and statistical analysis: an efficient way of linking seized ecstacy tablets. *Anal. Chim. Acta* **2004**, 510 pp 1 - 8

[13] McHard, J.A., Foulk, J.F., Winefordner, J.D. A Comparison of Trace Element Content of Florida and Brazil Orange Juice. *J. Agric. Food Chem*. **1979**, 27 1326 – 1328

[14] Horn, P., Schaf, P., Prangazzi, P. 87Sr/86Sr From rock and soil into vine and wine. Z. Lebensn. *Unters. Forsch* **1993** 196 pp. 407 – 409

[15] Oda, H., Kawasaki, A., Hirata, T. Determining the Geographic origin of brown-rice with isotope ratios of 11B/10B and 87Sr/86Sr. *Anal. Sci*. **2001**, 17 pp. 1627 - 1630

[16] Marcos, A., Fisher, A., Rea, G., Hill, J. Prelimniary Study Using Trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J. of Anal. Atomic Spectrometry*. **1998**, 13 pp. 521 – 525

[17] Anderson, K.A., et al. Determining geographic origin of potatoes with trace metal analysis using statistical and neural network classifiers. *J. Agric. Food Chem*. **1999**, 47 pp. 1568-1575

[18] Smith, R.G. Determination of the Country of Origin of Garlic (Allum sativum) using trace metal profiling. *J. Agric. Food Chem*. **2005**, Vol 53 pp. 4041-4045

[19] Dooley JJ, Sage HD, Brown HM, Garrett SD. Improved fish species identification by use of lab-on-a-chip technology. *Food Control* **2005**, 16:601–7.

[20] Hsieh HS, Chai TJ, Hwang DF. Using the PCR-RFLP method to identify the species of different processed products of billfish meats. *Food Control* **2007**, 18:369–74.

[21] Rego I, Martinez A, Gonzalez-Tizon A, Vieites J, Leira F, Mendez J. PCR technique for the identification of mussel species. *J. Agric Food Chem* **2002**, 50(7):1780–1784.

[22] Sanjuan A, Comesana AS. Molecular identification of nine commercial flaffish species by polymerase chain reaction-restriction fragment length polymorphism analysis of a segment of the cytochrome b region. *J Food Prot* **2005**, 65(6):1016–1023.

[23] Lin YS, Poh YP, Lin SM, Tzeng CS. Molecular techniques to identify freshwater eels: RFLP analyses of PCR-amplified DNA fragments and allele specific PCR from mitochondrial DNA. *Zool Stud* **2002**, 41(4):421–30.

[24] Liu ZJ, Cordes JF. DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* **2004**, 238:1–37.

[25] Joshi, K. et al. Molecular Markers in Drug Herbal Technology. *Current Science* **2004**, 87 pp. 159-165

[26] Marmiroli N, Peano C, Maestri E. Advanced PCR techniques in identifying food components. In: Lees M, editor. Food authenticity and traceability. Cambridge, U.K.:Woodhead Publishing Ltd. **2003**, p 3–33.

[27] Meunier, J.R. and Grimont, P.A.D. *Research in Microbiology* **1993**, 144 pp. 373-379

[28] Wong, E. and Hanner, R.H. DNA barcoding detects market substitution in North American seafood. *Food Research International* **2008**, 41 pp. 828–837

[29] Lewis, J. and Hird, S. Determining the geographical origin of foods: considerations when designing experimental protocols and choosing analytical approaches, in: Caroli (Ed), John Wiley and Sons Inc., U.S.A. 2007, pp. 115 - 131

[30] Yuntseover, Y., & Gat, J. R. Atmospheric waters. IAEA technical reports series 210—stable isotope hydrology, **1981**, (pp. 103–142).

[31] Kelly, S. et al. Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis Trends in Food Science & Technology 2005, 16 pp. 555–567

[32] Coleman, M. Stable Isotope Characterization – How it works, why it works and how good it is. In: Netwrok developing forensic applications of stable isotope ratio mass spectrometery conference. 2002

[33] Faure, G.; and J.L Powell. The Geochemistry of Rubidium and Strontium. In *Strontium Isotope Geology*; Springer-Verlag: Berlin, Germany, 1972; pp. 1-8

[34] Stewart, B.W.; R.C. Capo; O.A Chadwick. Quantitative strontium isotope models for weathering, pedogenesis and biogeochemical cycling. *Geoderma* **1998**, 82, 173-195

[35] Knudson, K.J.; T.D. Price; J.E. Buikstra; and D.E. Blom. The Use of Strontium Isotope Analysis to Investigate Tiwanaku Migration and Mortuary Ritual In Bolivia and Peru. *Archaeometry* **2004**, 46, 5-18

[36] Gilli, A.; D.A. Hodell; G.D. Kamenov; and M. Brenner. Geological and archaeological implications of strontium isotope analysis of exposed bedrock in the Chicxulub crater basin, northwestern Yucatán, Mexico. *Geology* **2009**, 37, 723-726

[37] Martin, J.B.; P.J. Moore. Sr concentrations and isotoperatios as tracers of ground-water circulation in carbonate platforms: Examples from San Salvador Island and Long Island, Bahamas. *Chemical Geology* **2008**, 249, 52-65

[38] Almeida, C.M.R.; and M.T.S.D. Vasconcelos. Multi-Element Composition And 87Sr/86Sr Of Wines And Their Potentialities As Fingerprints Of Wine Provenance. *Ciência Téc. Vitiv*. **2003**, 18, 15-27

[39] Kawasaki, A.; H. Oda; and T. Hirata. Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Sci. Plant Nutri*. **2002**, 48, 635-640

[40] Rodrigues, C.; and C. Máguas. Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authenticity of coffee. *Eur. Food Res. Technol*. **2011**, 232, 361-373

[41] Wolff, B.A.; B.M. Johnson; A.R. Breton; P.J.Martinez; D.L. Winkelman. Origins of invasive piscivores determined from the strontium isotop ratio (87Sr/86Sr) of otoliths. *Can. J. Fish. Aquat. Sci*. **2012**, 69, 724-739

[42] Kennedy, B.P.; C.P. Chamberlain; J.D. Blum; K.H. Nislow; and C.L Folt. Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **2005**, 62, 48-57

[43] Capo, R.C.; B.W. Stewart; and O.A. Chadwick. Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* **1998**, 82, 197-225

[44] Price, T.D.; L. Manzanilla; and W.D. Middleton. Immigration and the Ancient City of Teotihuacan in Mexico: a Study Using Strontium Isotope Ratios in Human Bone and Teeth. *Journal of Archaeological Science* **2000**, 27, 903–913

[45] Munro, A.R.; T.E. McMahon; J.R. Ruzycki. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yelloswtone Lake. *Can. J. Fish. Aquat. Sci*. **2005**, 62, 79-87

[46] Schweissing, M.M.; G. Grupe. Stable strontium isotopes in human teeth and bone: a key to migration events of the late Roman period in Bavaria. *Journal of Archaeological Science* **2003**, 30, 1373–1383

[47] Macdonald, N.S.; R.E. Nusbaum; R. Stearns, F. Ezmirlian, C. McArthur; and P. Spain. The skeletal Deposition of non-radioactive strontium. *The Journal of biological chemistry* **1951**, 188, 137-43

[48] Schweissing, M.M.; G. Grupe. Stable strontium isotopes in human teeth and bone: a key to migration events of the late Roman period in Bavaria. *Journal of Archaeological Science* **2003**, 30, 1373-1383

[49] Price, T.D. Immigration and the Ancient City of Teotihuacan in Mexico: a Study Using Strontium Isotope Ratios in Human Bone and Teeth. *Journal of Archaeological Science* **2000**, 27, 903–913

[50] Smith, R.G. Determination of the country of origin of garlic (Allum sativum) using trace metal profiling. *J. Agric. Food Chem*. **2005**, 53 (4041-4045)

[51] Cubadda, F. Applications of inductively coupled plasma mass spectrometry to trace element reaserch and control, in: Caroli (Ed), John Wiley and Sons Inc., U.S.A. **2007**, pp. 225-295

[52] Nikdel, S. et al. Trace metals: Defining geographical origin and detecting adulteration of orange juice. In Adulteration of Fruit Beverages; Nagy, S., Attaway, J.A., Rhodes, M. Eds.; Marcel Dekker: New York, **1988**, pp 81-105

[53] Schwartz, R.S., Hecking, L.T. Determination of geographic origin of agricultural products by multivariate analysis of trace element composition. *J. Anal. At. Spectrosc*. **1991**, 6 pp 637-642

[54] Anderson, K.A., and Smith, B.W. Chemical profiling to differentiate geographic growing origin of coffee. *J. Agric. Food Chem*. **1999**, 50 (7) pp 2068-2075

[55] Samsoe-Petersen, L. et al. Uptake of trace elements and PAHs by fruits and vegetables from contaminated soils. *Environ. Sci. Technol*. **2002**, 3 (14) pp 3057-3063

[56] Window, H. et al. Comparison of trace metal concentration in the muscle tissue of benthopelagic fish (Coryphanoides armatus). *Deep-Sea Res*. **1987**, 34 pp 213-220

[57] Favretto, L. et al. Principal component analysis: a chemometric aid for classification of polluted and unpolluted mussels. *Anal. Chim. Acta* **1989**, 220 pp 135-144

[58] Jung, K. and Zauke, G.P. Bioaccumulation of trace metals in the brown shrimp Craggnon cragnon (Linnaeus, 1758) from German Wadden Sea. *Aquat. Toxicol*. **2008**, 88 pp 243-249

[59] Langston, W.J. and Pence, S.K. Biological Factors involved in metal concentrations observed in aquatic organisms. In Metal Speciation and Bioavailability in Aquatic systems; Tessier, A. and Turner, D.R. Eds.: John Wiley and Sons Ltd. England **1995**, pp 407-478

[60] Phillips, D.J.H. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments – a review. *Environ. Pollut*. **1977**, 13 (282-317)

[61] Powell, J.H. and Powell, R.E. Trace elements in fish overlying subaqueous tailings in the Tropical West Pacific. *Water, Air, and Soil Pollution* **2001**, 125 pp 81-104

[62] Thomas, R. Pratical Guide to ICP-MS: A tutorial for beginners, **2008**, 2nd Edition. CRC Press, Boca Raton, FL, USA

[63] Pearce, N.J.G, J.A. Westgate, W.T. Perkins, S.J. Preece.The application of ICP-MS methods to tephrochronological problems. *Applied Geochemistry* **2004**, 19, 289–322

[64] Husted, S., D.P. Persson, K.H. Laursen, T.H. Hansen, P. Pedas, M. Schiller, J.N. Hegelund, and J.K. Schjoerring Review: The role of atomic spectrometry in plant science. *J. Anal. At. Spectrom.*, **2011**, 26, 52

[65] Gonzalvez, A., S. Armenta, M. de la GuardiaTrace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. *Trends in Analytical Chemistry*, **2009**, 28 (11), 1295-1311

[66] Robinson, J.W., E.M.S Frame, and G.M. Frame II. Undergraduate Instrumental Analysis, 6th Edition **2005**, Marcel Decker, New York, NY, USA

[67] Wolf, R.E. What is ICP-MS?and more importantly, what can it do? **2005** http://crustal.usgs.gov/laboratories/icpms/intro.html (accessed April 13, 2014)

[68] New Mexico State University. Introduction to ICP Instrumentation. http://web.nmsu.edu/~kburke/Instrumentation/NMSU_Optima2100.html (Accessed April 13, 2014)

[69] McCune, B. and Grace, J.B. Analysis of Ecological Communities. MGM Software Design. U.S.A. **2002**, pp 205 – 210

[70] Klecka, W.R. Discriminant Analysis **1980**, Sage Publications, Inc. USA

[71] Foster, J. et al. Understanding and Using Advanced Statistics **2006**, The Cromwell Press Ltd. Great Britain

[72] A. Lehman, N. O'Rourke, L. Hatcher, and E.J Stepanski. JMP for Basic Univariate and Multivariate Statistics: Methods for Researchers and Social Scientists, 2nd Edition. 2**013** SAS Institute Inc, Cary, NC, USA

[73] P. A. Gore, Jr. Cluster Analysis. In: Handbook of Applied Multivariate Statistics and Mathematical Modeling. Ed: H.E.A. Tinsley and S.D. Brown. **2000**, Academic Press. San Diego, California, USA

[74] Hunter, J.C. and McCoy, R.A. Applying randomization tests to cluster analysis. *Journal of Vegetation Science* **2004** 15 pp 135-138

[75] SAS Institute Inc. JMP 11 Basic Analysis. SAS Institute Inc. **2013**

[76] Brereton, R.G. Chemometrics: Data Analysis for the Laboratory and Chemical Plant **2003** John Wiley and Sons, Ltd. England

[77] Giacomino, A., et al. The role of chemometrics in single and sequential extraction assays: A Review, Part II. Cluster analysis, multiple linear regression, mixture resolution, experimental design and other techniques. *Analytica Chimica Acta* **2011** 688 pp. 122-139

[78] B.S. Everitt. Cluster Analysis, 3rd Edition. Halsted Press, New York, NY, USA **1993**

CHAPTER TWO

ELEMENTAL FINGERPRINTING OF GYPSUM DRYWALL USING SECTOR FIELD ICP-MS AND MULTIVARIATE STATISTICS

2.1 ABSTRACT

Outgassing of volatile sulfur compounds from gypsum drywall from some sources has resulted in odors, corrosion of wiring and metals, and health problems for homeowners. Infrared spectroscopy has been the primary analytical tool to distinguish between “problematic” drywall and “non-problematic” drywall. In this study, elemental fingerprinting using inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics was shown to be an effective alternative. The approach also showed potential in determining the geographic source of gypsum. Nineteen elements (Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr, Tl, U, V, and Zn) were measured. Half of the twenty drywall samples were classified as positive for contamination by FT-IR spectroscopy. Gypsum from three different mines and a flue gas desulfurization plant were also analyzed. Principal component analysis (PCA) and multivariate analysis of variance (MANOVA) of the elemental data showed significant differences between the problematic and non-problematic drywall, and between sources of gypsum. Strontium averaged 1793±508 µg/g in problematic drywall compared to 378±106 µg/g in non-problematic drywall ($p<0.0001$).

2.2. INTRODUCTION

Drywall is a common construction material for framing interior walls and ceilings of residential homes and buildings. It consists primarily of gypsum ($CaSO_4 \bullet 2H_2O$) that was either mined from natural resources or obtained as a by-product from flue-gas desulfurization at coal-fired power plants. The gypsum is then partially dehydrated in a kiln to produce plaster of Paris. Additives and water are blended in and the material is spread between sheets of paper and dried [1,2]. Drywall became popular in the 1950's and 1960's because it is cheaper and faster than plaster alone. About 90% or 21 million tons of the gypsum consumption in the United States is related to drywall and plaster products, with a drywall manufacturing production capacity of 27 billion ft^2 per year [3].

Importation of drywall to the United States from China increased dramatically between 2004 and 2007, particularly in Florida, Mississippi, and Louisiana, due to a construction boom and repairs following hurricanes Katrina and Wilma. During this period some homeowners with new drywall installations became sick, experiencing a multitude of symptoms, including respiratory and sinus problems [4]. Many of the same homeowners also observed that copper pipes in air conditioning systems and other areas were corroding, and silver jewelry were turning black. These problems were more prevalent in homes with drywall originating from China, and consequently problematic drywall is now commonly referred to as "Chinese Drywall" even though it may not have actually originated from China. Subsequent studies show that volatile

sulfur compounds including carbon disulfide, carbonyl sulfide, and hydrogen sulfide are responsible for the damage [4,5]. Hereafter, we refer to drywall that has a tendency to outgas sulfur species at levels that become problematic as "problematic" drywall.

In order to eliminate the use of problematic drywall in construction and to identify homes with the suspect materials, it is necessary to identify the "problematic" drywall. To that end, features of the infrared vibrational spectra and levels of strontium have been found to be reliable identifiers of problematic drywall [6-8]. Thus, Fourier Transform-Infrared Spectroscopy (FT-IR) and X-Ray Fluorescence (XRF) have been used to identify problematic drywall.

2.2.1 Identifying problematic drywall using infrared spectroscopy and strontium content

A characteristic FT-IR spectrum of gypsum is shown in Figure 2.1. There is a strong peak at 1104 cm^{-1}, due to the asymmetric stretch of SO_4^{-2}; medium-intensity peaks at 3401 cm^{-1} and 3528 cm^{-1} attributed to symmetric and asymmetric stretch of H_2O, respectively; peaks at 1620 cm^{-1} (medium-intensity) and 1683 cm^{-1} (weak) due to H_2O bending. All the aforementioned peaks are present for samples of drywall. However, for samples of problematic drywall there are two additional peaks at 876 and 1442 cm^{-1} associated with a CO_3^{-2} stretch [8]. These peaks have been matched with reference spectra of strontianite ($SrCO_3$), and thermogravimetric IR analysis confirmed the presence of carbonates [8]. Because of this both the presence of carbonate peaks in the IR spectra and the concentration of strontium in the sample has been used as indicators of problematic drywall. Indeed, imported drywall from China, known to be problematic, has been shown to contain higher amounts of strontium than non-problematic drywall [7,8]. Thus, on site elemental analysis based on strontium levels is feasible using portable XRF instruments calibrated using certified reference materials [7].

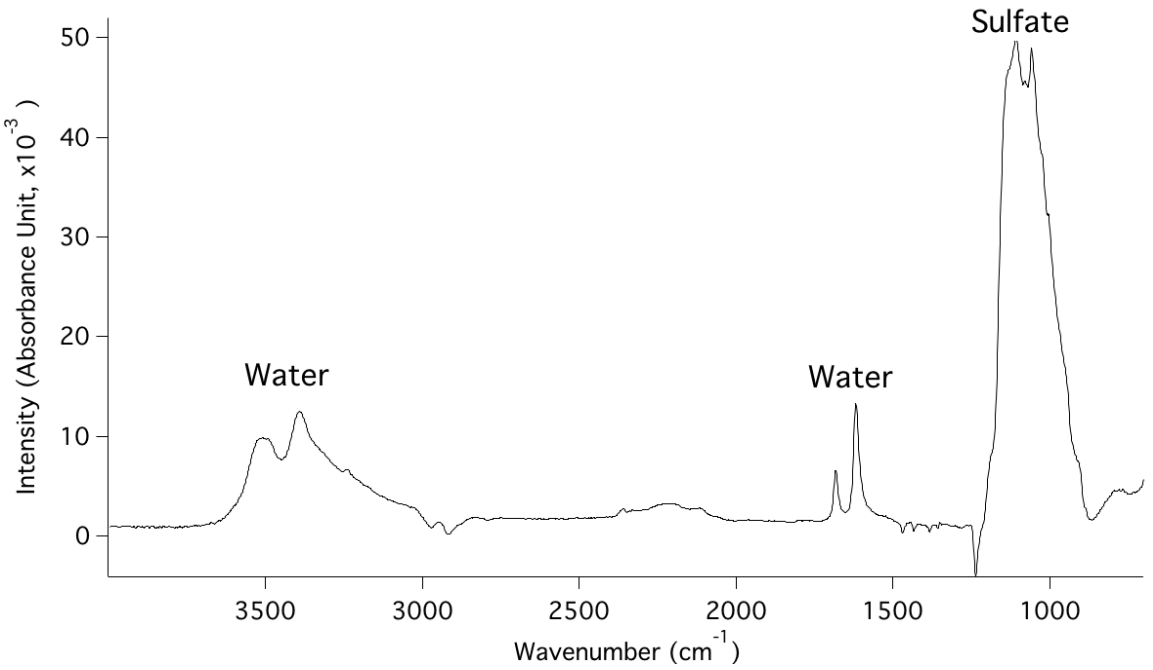

Figure 2.1. FT-IR spectrum of a gypsum reference material, FGD-2.

2.2.2 Basis of elemental fingerprinting for provenance studies

Elemental fingerprinting has been widely cited in the literature, especially in forensic-related studies [9] and for the provenance of agricultural and food products [10-13]. It is well known that geologic materials (e.g. soils, minerals) have site-specific composition due to differences in parent source material, age and weathering of deposits, and other factors [9,14]. For gypsum, the characteristic trace element distribution may be present in the raw material or modified during processing into the drywall products. Given sufficient and reproducible differences in the elemental pattern between drywall from different sources it may be feasible to not only identify problematic drywall but to provenance the product.

The purpose of the current study was to evaluate elemental fingerprinting using inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics as a means

to: 1) distinguish between problematic and non-problematic drywall, and 2) discriminate gypsum samples based on their natural or synthetic sources.

2.3 EXPERIMENTAL

2.3.1 Samples

Twenty samples of ground drywall were obtained from an independent testing laboratory (Assured Bio Labs, TN, USA). The samples originated mostly from homes in the southeast United States (Figure 2). The sources of gypsum for this drywalls are unknown. Only a few grams per sample were available for analysis. The testing lab used FT-IR to classify the samples as positive or negative for contamination and we confirmed these results using FT-IR in our laboratory (discussed below).

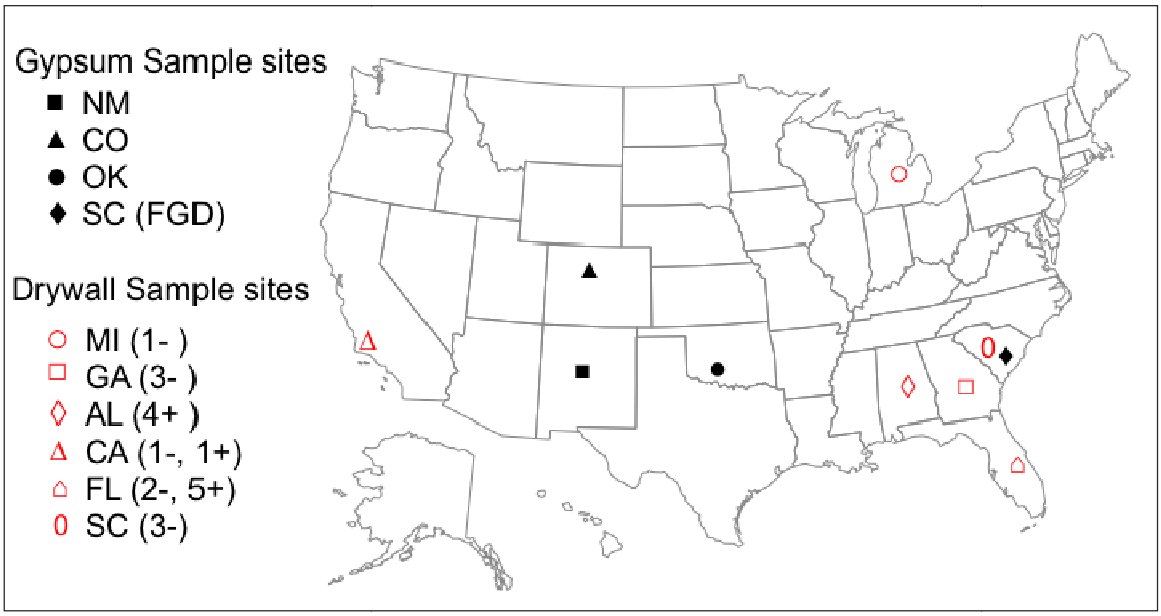

Figure 2.2. Locations where gypsum and drywall were obtained. (+ = problematic drywall; - = non-problematic drywall)

Powdered gypsum was provided by American Gypsum Company and originated from three mines located in New Mexico, Colorado, and Oklahoma, and from flue gas desulfurization (FGD) associated with the Winyah (SC 1), Cross (SC 2), and Belowes Creek (SC 3) coal-fired power generating stations located in South Carolina (Figure 2.2). Natural gypsum was obtained from White Mesa Mine in San Ysidro, New Mexico (NM), a mine in Duke, Oklahoma (OK), and from two different mills at a natural surface mine in Gypsum, Colorado (CO 1 and CO 2).

2.3.2 Infrared spectroscopy

FT-IR spectra of pulverized gypsum and drywalls were obtained using Bruker Tensor 27 equipped with Pike MIRacle Attenuated Total Reflectance (ATR) accessory. A diamond ATR crystal was used for drywalls while a ZnSe crystal was used for gypsum samples. A small amount of sample (approximately 10 mg) was placed on the crystal. A clamp was used to press the sample on the crystal. IR spectra of the sample were collected from 550 – 4000 cm^{-1} for 64 scans at a resolution of 4 cm^{-1}. Background spectra were collected prior to sample analysis and subtracted from the sample spectra.

2.3.3 Thermogravimetric analysis coupled with infrared spectroscopy

Thermogravimetric Analysis (TGA) was carried out using an EXSTAR 6000 SII coupled with a Cary 660 FT-IR (Agilent Technologies) to determine the presence of carbonates and identify other gases that may be evolved when the samples of drywall are heated. Briefly, approximately 16 mg of drywall was placed in a ceramic cup and heated from 30^0C to 1100^0C at a rate of 10^0C per minute. Evolved gases were carried by high-purity nitrogen through silicone

tubing to a gas cell located in the FT-IR beam path. FT-IR spectra were collected every 20 seconds (average of 8 scans) from 400 cm^{-1} to 6000 cm^{-1} at 4 cm^{-1} interval.

2.3.4 Sample digestion for ICP-MS analysis

Approximately 0.1 g samples were digested in 9 mL of concentrated HNO_3 and 2 mL concentrated HCl, and 0.05 mL of HF in an Ethos microwave digestion system (Milestone Inc. Shelton, CT, USA) using a 41-vessel (PFA) multi-prep router. After addition of acids, the samples were cold-digested for an hour prior to microwave digestion. The samples were heated to 180˚C in 60 min and held at that temperature for an additional 20 min. The digests were diluted to 50 mL with deionized water, followed by a second, ten-fold dilution, making the final acid concentration ~2%. A diluent containing 2 ng/g of Rh as the internal standard was used to make the second dilution.

2.3.5 Determination of elements using sector field ICP-MS

The ICP-MS used was a Thermo-Fisher Element-XR, which is equipped with a double-focusing magnetic sector mass spectrometer. A PFA micro-flow nebulizer with a HF resistant sample introduction system (ESI, Omaha, NE, USA) was used to introduce samples into the ICP-MS. The instrument was optimized prior to analysis for sensitivity, precision and oxide levels. Instrumental and data acquisition parameters are given in Table 2.1. Approximately 1.5 million counts per second and <4% relative standard deviation (RSD) was achieved for 1 ng/g of ^{115}In in low resolution mode. External calibration was used to quantify the elements. A series of multi-element standards (0.01-100 ng/g) were prepared such that it contains the same acid make-up and Rh concentration with that of the sample solution.

The method was validated using certified reference material FGD-2 (Analytical Group of Domtar Inc., Senneville, Quebec, Canada). Recoveries for those elements with reference values (Mn, Co, V, Zn, Cr, U) were within ±20% except for Zn (60%) and V (145%). Method detection limits were estimated by replicate analyses of reagent blanks carried through the entire analytical process; results ranged from ~0.002 ng/g for Cd to ~10 ng/g ng/g for Ca. The following nineteen elements were used for fingerprinting of drywall and gypsum samples: Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr, Tl, U, V, and Zn. Some elements (Co, Cu, Ni V, and Zn for drywall; Cu, Cs and Tl for gypsum) were excluded from statistical analysis (see below) because levels were near or below detection limits.

Table 2.1: ICP-MS instrument settings.

Plasma	
Auxiliary gas flow	1.00 L min^{-1}
Sample gas flow	1.16 L min^{-1}
Cool gas flow	16.0 L min^{-1}
RF power	1450 W
Data acquisition	
Isotopes monitored in LR[a]	^{7}Li, ^{9}Be, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{133}Cs, ^{137}Ba, ^{205}Tl, ^{208}Pb, ^{238}U,
Isotopes monitored in MR[a]	^{24}Mg, ^{27}Al, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{69}Ga
Mass window	20% for LR, 150% for MR
Integration time (ms)	10 for LR, 50 ms for MR
Runs/passes	3/2
Scan type	E-scan

[a] LR = Low Resolution; MR = Medium Resolution

2.3.6 Statistical analysis

Statistical evaluation was done using JMP software ®10.0 (SAS; Cary, NC, USA). To compensate for the varying ranges of elemental concentration, data were normalized using Z-scores prior to statistical evaluation. Differences between problematic and non-problematic drywalls were evaluated using univariate and multivariate analysis. Univariate testing was

carried out using a two-tailed T-test to determine the t-ratio for each element. If the groups are significantly different, the higher the t-ratio, the greater is the influence of that element on the difference between the groups. Multivariate statistics, including principle component analysis (PCA), Hierarchical Cluster Analysis (HCA), and Multivariate Analysis of Variance (MANOVA) were used to evaluate the elemental concentrations and compare problematic and non-problematic drywall. The same statistical analyses were also used to distinguish gypsum based on their geographic origin. These statistical analyses are commonly used for datasets with numerous measurements on individual samples; here they are used to evaluate the differences between samples and sample groups.

PCA is an exploratory data evaluation method to assess the grouping tendency of the samples. It reduces a complex data set into a smaller number of composite variables (Principal Components (PCs), which are determined by obtaining the longest linear correlation structure among the variables [15-16]. The PCs are then plotted on a score plot to evaluate clustering of samples. For a two-dimensional plot, density ellipses can be determined for each group in the score plot. Density ellipse is calculated from the bivariate normal distribution fit to the two principal components. The bivariate normal density is a function of the means and standard deviations of the two components and the correlation between them [17].

PCA also determines the variance explained by each axis (eigenvalues) and the linear equations (eigenvectors) that combine the original variables. The size of the eigenvalue determines how much variance each PC represents. The eigenvectors list the contribution of each response variable to a particular component [16]. The eigenvectors are plotted on a loading plot, which shows the correlation between the variables and correlation of the variables with the principal components.

Elemental profiles were evaluated using Hierarchical Cluster Analysis (HCA) to strengthen and validate the results from PCA. HCA is an unsupervised pattern recognition method designed to discover classifications within a complex data sets [18]. Hierarchical Clustering Analysis (HCA) uses measures to sequentially join an object into groups [19]. The procedure starts with each point as individual cluster. At each step, the two clusters that are most similar are combined into a single new cluster and the process continues until all points are combined into one cluster [17]. This procedure is agglomerative clustering approach, in which single objects are connected to each other to form a group [20]. The first step in an agglomerative Hierarchical Clustering Analysis (HCA) is to determine and evaluate the similarity or dissimilarity of objects [21]. There are four methods to determine similarity between objects: (1) Correlation coefficient, (2) Euclidean distance, (3) Manhattan distance, and (4) Mahalanobis distance. The second step in HCA is to link the objects. A number of linkage methods are available for clustering. These include Ward's method, complete linkage, or average linkage.

This study used Ward's method of clustering based on a Euclidean distance matrix. Euclidean distance, ED_{ih}, beween sample *i* and *h* is calculated as follows:

$$ED_{ih} = \sqrt{\sum_{j=1}^{p} (a_{ij} - a_{hj})^2} \qquad \text{Eq. 1 [16]}$$

where: p is the number of variables, a_{ij} is a matrix of variables *i* and *j*. The higher the similarity between the samples, the lower is the Euclidean distance [21]. The linkage method, Ward's, uses the ANOVA sum of squares between the two clusters added over all the variables as the distance between the two clusters [17]. It involves clustering procedure seeking to form partition such that the error sum of squares is minimized with each grouping [22]. The error sum of squares is calculated as the sum of the squares of the distances from each individual to the centroid of its

group [16]. Results from HCA are represented by a dendogram, a diagram that illustrates relationship between the cluster and sub-cluster. It also shows the fusion or divisions made at each successive stage of analysis [22].

While PCA and HCA is used to show patterns, Multivariate Analysis of Variance (MANOVA) is used to test if the groups differ significantly with respect to elemental composition. MANOVA is a multivariate statistical test for differences among groups based on a set of dependent variables. Both MANOVA and ANOVA tests for statistical difference between groups. However, ANOVA tests for differences in means between two or more groups involving one response variable, whereas, MANOVA tests for the difference in two or more vectors of means and is used when there is more than one response variable [23]. MANOVA determines a liner combination of the original (dependent) variables that maximizes the difference between groups. MANOVA uses this new variable to tests whether the mean differences between the groups are significant [16]. When the differences between the groups are significant, the relative contribution of each response variable to the differences between the groups can be evaluated. This is accomplished by assessing the differences between the least square means for each elements.

Elements that showed to have high influence on significant difference between the groups were plotted using Box-and-Whisker plot. The plot shows the range, median, the first quartile, the third quartile of data set. Whiskers, lines extending from each end of the box, represent the range of the data points. A horizontal line within the box represents the median. The ends of the box represent the first and third quartiles, which correspond to 25 and 75 percentile of the data set, respectively.

2.4 RESULTS AND DISCUSSION

2.4.1 Infrared spectroscopy and thermogravimetric analysis

Infrared spectra were collected for each sample of pulverized drywall. Using an IR-based classification scheme [8], ten samples showed peaks at 1445 cm^{-1} and 875 cm^{-1} and were classified as "problematic" and ten samples showed no such peaks and classified as "non-problematic" (Figure 2.3). As discussed earlier, these two peaks have been shown to be prevalent for drywall that outgasses reduced sulfur species and correspond to the vibrational frequency from carbonate (CO_3^{2-}) mineral [8]. The spectrum traced in black on Figure 3 represents Sample 20. The peak at 1450 cm^{-1} is the "weakest" of the samples classified as problematic. Interestingly, this sample is the only one that was classified differently by Sr levels, where it fell with non-problematic samples (discussed later). The two peaks characteristic of contamination were not observed in any of the US gypsum samples (Figure 2.4). The figure also reflects the major type of mineral phase present. The two peaks between ~3400 cm^{-1} and ~3600 cm^{-1} are associated with water stretches, and their position varies with mineral phase [24]. For the FGD and New Mexico samples, vibrations occurred at a shorter wavenumber (3402 and 3522 cm^{-1}) corresponding to the dihydrate ($CaSO_4 \cdot 2H_2O$). In contrast, the mined samples (except for New Mexico) had absorptions at a longer wavenumber (3568 and 3614 cm^{-1}) corresponding to the hemihydrate ($CaSO_4 \cdot 0.5H_2O$). This was confirmed by thermogravimetric analysis (TGA), which

showed greater weight loss for the FGD and NM samples (~17%) compared to mined samples (~6%) (Figure 2.5).

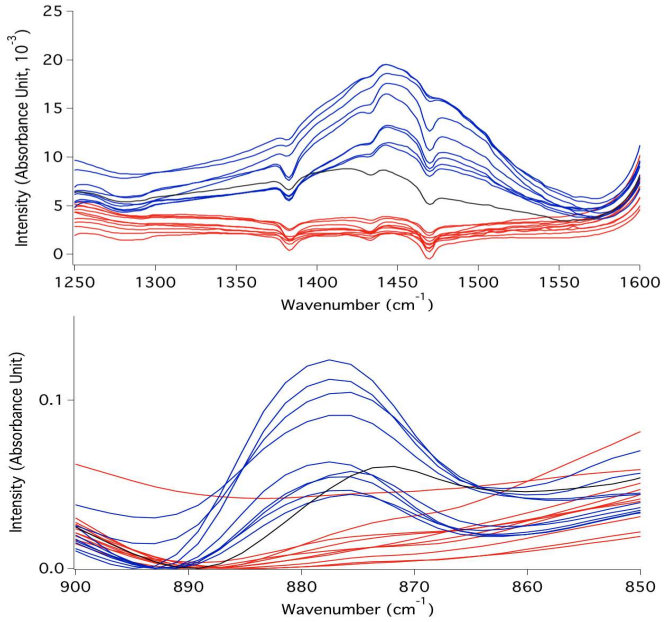

Figure 2.3. Zoomed-in IR spectra of drywall samples showing peaks at 1445 and 875 cm^{-1} (Blue and Black = problematic; Red = non-problematic drywall)

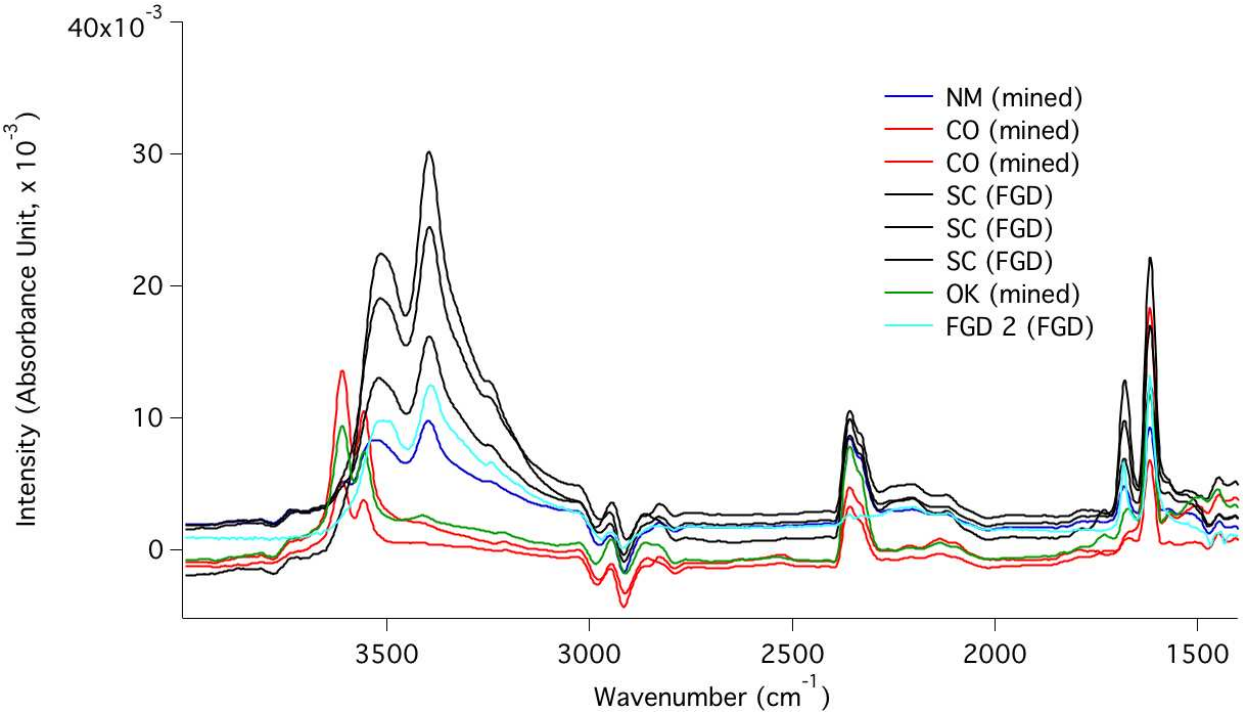

Figure 2.4. FT-IR spectrum of gypsum samples from the USA.

TGA analysis of the drywall samples showed weight losses that peak at approximately 150°C, 700°C, and 885°C (Figure 2.6). Coupling the TGA with IR analyses revealed that the gases released at these temperatures are H_2O, CO_2 and SO_2, respectively (Figure 2.7). No other gases (e.g. reduced sulfur species) were identified during the analyses, possibly because of the limited amount of sample available for analysis. From the TGA results it is apparent that problematic samples have substantially higher levels of carbonate, evolving more CO_2 between approximately 625°C and 775°C than non-problematic samples (Figure 6). Problematic samples also evolve more SO_2 at around 885°C, though the difference is not as great. From the weight loss we estimate that the levels of carbonate in the problematic samples were ~7.4% compared to ~1.6% in non-problematic samples. Similarly the levels of sulfur in the problematic samples were higher (~0.83%) compared to non-problematic (~0.26%). The source of the evolved SO_2 is not clear but is likely from sulfur containing species other than sulfate because a spectrum (Figure 2.5) of the FGD 2, which is a reference material for gypsum, calcium sulfate, does not yield the same peak.

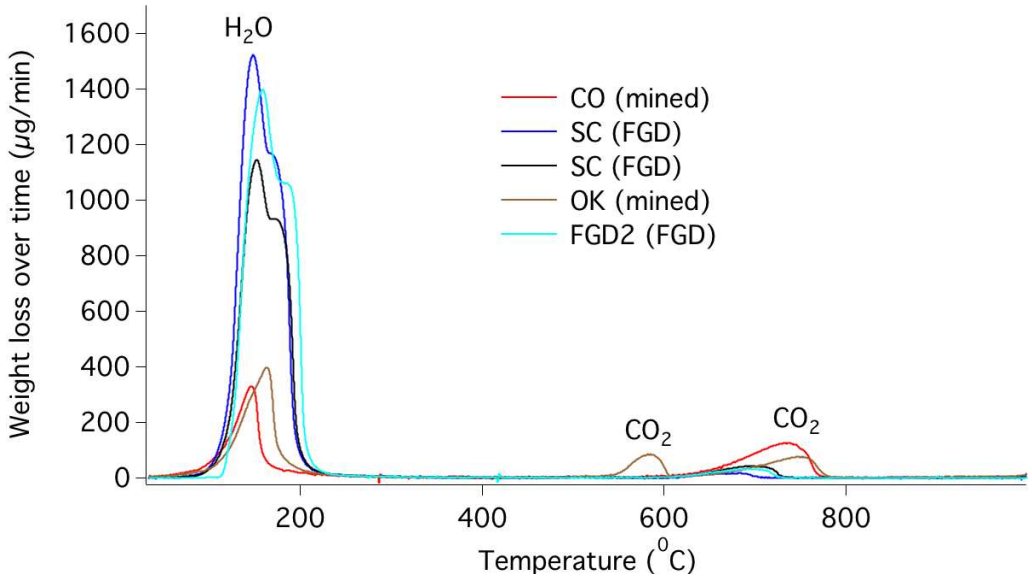

Figure 2.5. Weight loss per minute (µg/min) versus temperature (°C) of gypsum samples analyzed using TGA.

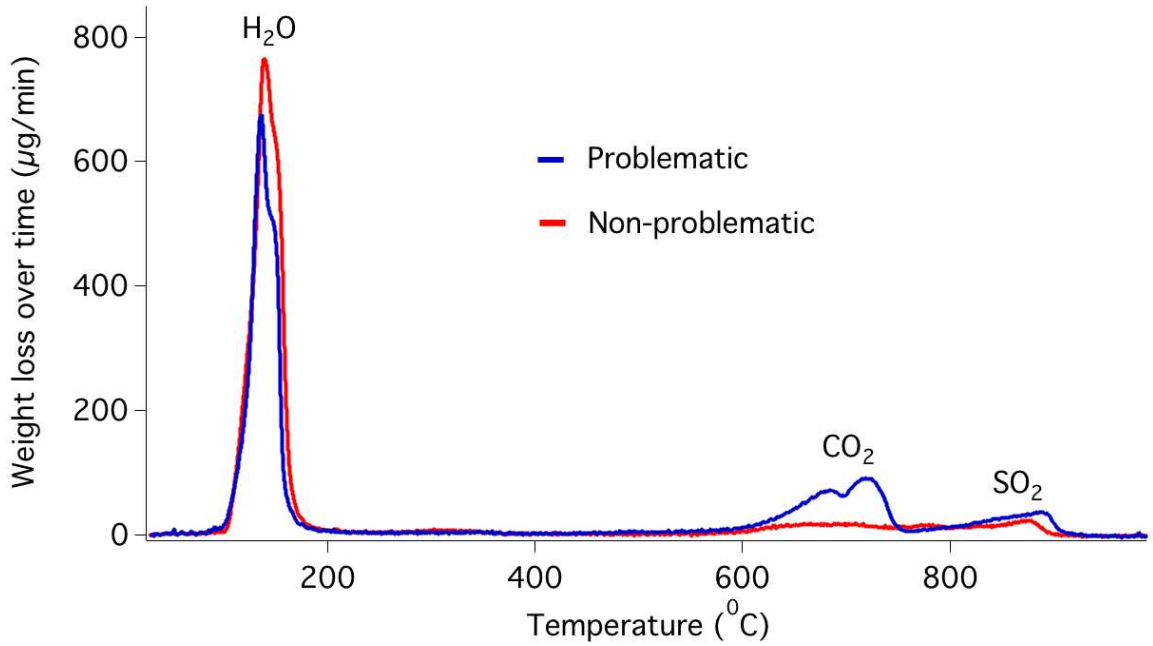

Figure 2.6. Weight loss per minute (µg/min) versus temperature

TGA analysis of the gypsum also showed that the samples lost weight around 150^0C and 700^0C, which corresponds to loss of water and CO_2, respectively. Unlike drywall, gypsum samples did not evolve SO_2 during heating (Figure 2.5). Gypsum from Oklahoma lost an additional weight at around 580^0C, which correspond to an evolution of CO_2 gas as confirmed by an FTIR spectrum of the gas evolved at that temperature. CO_2 gases were evolved from Oklahoma gypsum at 580^0C and at 700^0C, suggesting that these CO_2 gases came from different sources within the sample. It can be seen from the TGA results that gypsum from natural mines have higher levels of carbonate than synthetic gypsum.

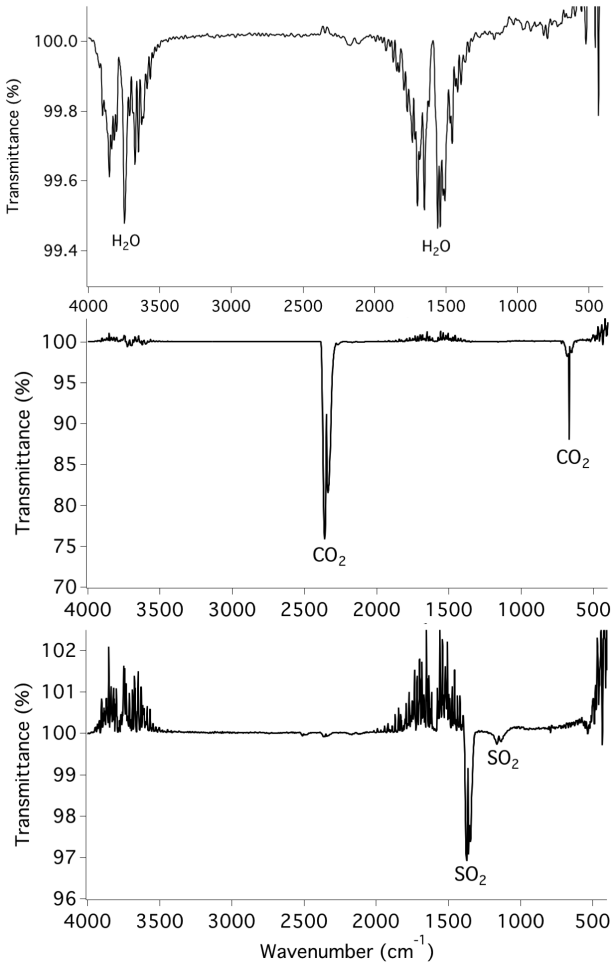

Figure 2.7. FT-IR spectra of gases evolved from TGA of drywall showing peaks characteristic for H_2O, CO_2 and SO_2. Top spectra collected when drywall was ~180°C, center at ~720°C, and bottom at ~890°C.

2.4.2 Elemental concentrations and fingerprinting

2.4.2.1 Problematic versus non-problematic drywall

Elemental concentrations of drywalls are presented in Table 2.2. Concentrations ranged from 0.205 µg/g for Cd to ~10% for Ca. Sample grouping tendency was evaluated using PCA and HCA. The PCA score plot (Figure 2.8) shows a two-dimensional plot of the 20 scores (samples) using the first two Principal Components. The first and second principal component explained 62.5% and 12.9% of the total variation in the elemental composition. The two

principal components, together, explain 75.4% of the total variance in the elemental composition. The PCA score plot shows clustering of problematic and non-problematic groups (Figure 2.8). Problematic drywalls are clustered together in the lower right quadrant of the plot. The PCA loading plot shows the elements Ba, Cs, Mg, Mn, Rb, Sr, and Tl are pointed towards this same quadrant (Figure 2.8). This indicates that these elements in drywall have higher concentrations in the problematic drywalls than the non-problematic ones. Non-problematic drywalls were clustered together mostly in the upper left corner of the plot with the PCA loading plot showing that Cd, Cr, and U tend to be higher in those samples. PCA loading plot showed that the elements, Rb, Mn, Cs, Sr, and Tl are highly correlated with Principal Component 1, which indicate that these elements play an important role in distinguishing contaminated drywall from non-contaminated ones. Coincidentally, these five elements were elevated in problematic drywall.

The PCA score plot also shows the 95% density ellipse of the two principal components (Figure 2.8). Sample 20 is shown to be on the outside of the density ellipse of contaminated drywall group. This suggests that Sample 20 may not belong to that group. A dendogram (Figure 2.9) from Cluster Analysis also suggests that the similarity in the elemental composition of Sample 20 is closer to that of the non-contaminated drywall than that of the contaminated drywall. The Sr concentration of Sample 20 is consistent with the Sr concentration of non-contaminated drywall (Table 2.2). Even though Sample 20 was considered to be a contaminated sample by FTIR, elemental fingerprints and Sr concentration suggests otherwise. In addition, even though sample 20 showed FTIR peaks indicating contamination, peaks were fairly weak compared to the rest of the contaminated drywalls.

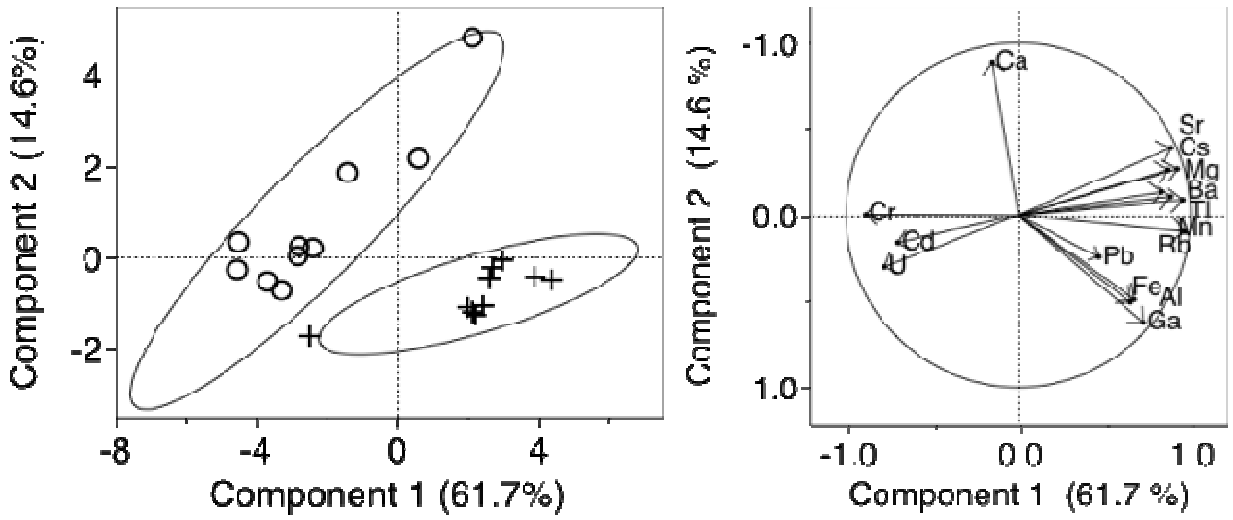

Figure 2.8. PCA score plot (left) and loading plot (right) for elemental fingerprints of drywall. (O=non-contaminated; +=contaminated)

To test for statistical significance between contaminated and non-contaminated drywalls, data were evaluated using MANOVA on summed responses. MANOVA revealed significant differences between problematic and non-problematic drywall ($p = 0.0009$) and that Sr differed the most between the two groups. As noted, previous studies have used Sr to identify problematic versus non-problematic drywall samples [7,8]. We also found that Sr was significantly different ($p<0.0001$) in problematic drywall (1820 ± 510 µg/g) compared to non-problematic drywall (390 ± 110 µg/g) as shown in Figure 2.10. MANOVA also revealed that the elements, Sr, U, Cs, Mg, and Mn (in decreasing order of importance) have a big impact on the discrimination between these two groups. Results from t-test (Table 2.2) also showed that these elements are the top five elements with the highest t-ratio.

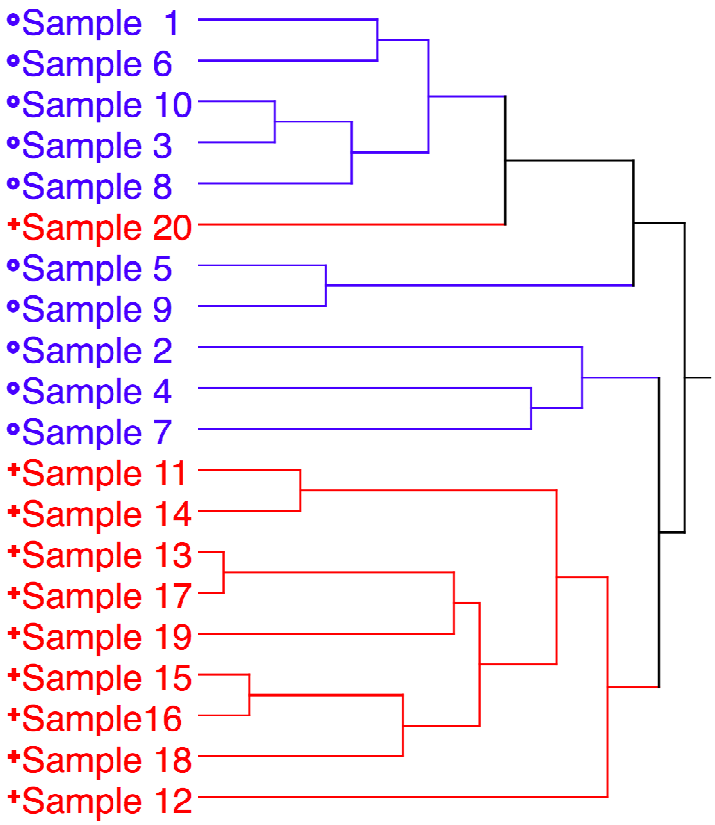

Figure 2.9. Dendogram obtained from HCA for elemental fingerprints of drywall.

MANOVA, t-test, and PCA demonstrated that elemental profiles could be used to distinguish contaminated drywall from non-contaminated ones. However, the three statistical tests gave different results with respect to the relative influence of elements in the discrimination between groups. MANOVA and t-test revealed the same top five elements (Sr, U, Cs, Mg, and Mn) while PCA revealed a different set of top five elements (Rb, Mn, Cs, Sr, and Tl) to be influential in the discrimination between the two groups.

Table 2.2. Concentration (µg/g) of 16 elements in non-problematic drywall (samples 1-10) and problematic drywall (samples 11-20)

Sample	*Al*	*Ba*	*Ca*	*Cd*	*Cr*	*Cs*	*Fe*	*Ga*	*Mg*	*Mn*	*Pb*	*Rb*	*Sr*	*Tl*	*U*
1	1860	12.2	97200	0.253	20.4	0.240	815	0.191	1220	13.9	1.10	1.89	317	0.096	1.55
2	1810	22.1	91100	0.230	5.54	0.282	3590	0.362	2900	12.5	1.87	2.41	272	0.168	1.69
3	2550	12.8	93200	0.224	19.9	0.286	982	0.217	1660	20.4	1.30	2.83	340	0.102	1.32
4	5120	42.3	90600	0.278	5.58	0.503	2170	0.496	3340	68.7	4.03	6.90	661	0.151	0.764
5	679	3.43	89500	0.300	29.0	0.172	1100	0.164	121	4.99	1.22	0.57	326	0.119	1.51
6	2530	11.8	101000	0.260	27.9	0.288	985	0.254	1360	16.3	1.39	2.52	336	0.111	1.21
7	6190	53.9	78200	0.215	7.78	0.731	2850	0.772	3880	59.5	2.99	11.2	483	0.163	0.961
8	3200	14.3	96000	0.254	23.8	0.328	1060	0.307	1770	22.0	1.40	3.18	378	0.108	1.34
9	468	2.88	94900	0.313	29.4	0.197	1130	0.192	689	7.68	1.22	0.65	323	0.120	1.46
10	3420	13.5	96300	0.228	19.8	0.296	1060	0.335	1890	22.1	1.34	2.90	457	0.106	1.30
Average:	2780	18.9	92800	0.256	18.9	0.332	1570	0.329	1880	24.8	1.79	3.50	389	0.124	1.31
11	3310	101	93700	0.206	3.84	1.90	2520	0.484	18100	101	1.83	12.6	2260	0.210	0.846
12	3760	199	97100	0.232	3.12	1.19	1750	0.379	8310	55.7	7.19	7.36	1620	0.193	0.699
13	2610	87.7	95500	0.207	2.63	1.49	1730	0.316	8820	64.9	2.55	8.07	1690	0.239	0.556
14	3910	112	95200	0.223	3.96	2.00	2640	0.491	20000	107	1.95	13.1	2480	0.226	0.884
15	3770	77.7	96600	0.220	3.01	1.38	1940	0.350	13700	82.3	1.69	8.75	2230	0.188	0.651
16	3650	77.5	93500	0.216	3.07	1.36	1880	0.443	14700	82.6	1.74	8.99	2090	0.190	0.693
17	2750	82.7	97100	0.205	2.81	1.52	1750	0.340	8880	65.0	2.64	8.25	1730	0.234	0.554
18	4150	52.0	100000	0.206	4.14	1.36	1860	0.405	8380	73.7	1.31	8.36	1860	0.184	0.516
19	3490	95.3	91900	0.215	10.8	1.96	2150	0.442	8920	74.8	2.77	10.7	1750	0.255	0.622
20	1330	16.4	101000	0.219	11.7	0.268	644	0.234	1460	25.6	1.02	1.87	454	0.093	0.643
Average:	3270	90.1	96200	0.215	4.91	1.44	1890	0.388	11100	73.3	2.45	8.81	1820	0.201	0.667
t-ratio:	0.779	4.53	1.56	-3.74	-4.42	6.65	0.896	0.929	5.21	4.85	1.08	3.74	7.91	4.64	-6.72

However, all three statistical analyses agreed that the elements Sr, Cs and Mn are the three most important elements in distinguishing the two groups. A box plot of these three elements is shown in Figure 2.10. We note that factors that can influence the elemental composition of drywall include the geographic source of the gypsum, whether it's natural or synthetic, and the type and quantity of additives used to make drywall. The geographic source of the drywall in this study is not known.

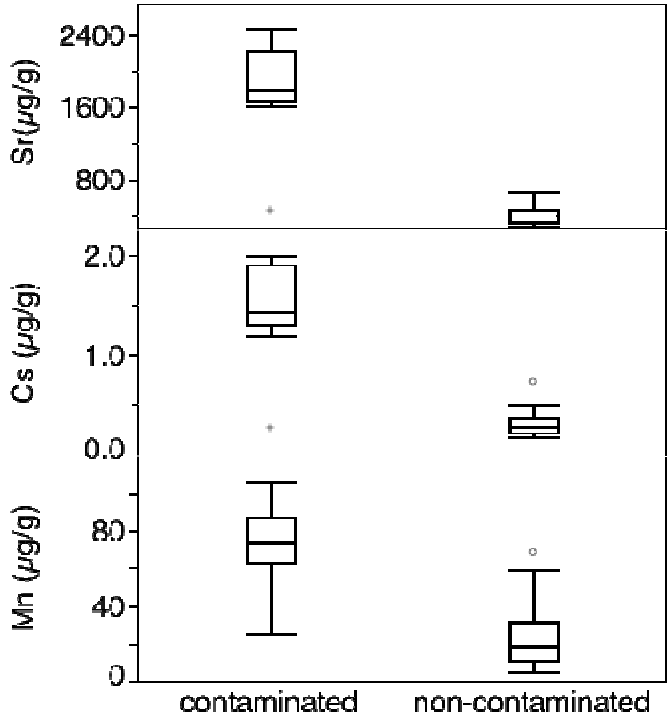

Figure 2.10. Box-Whisker plot showing the concentration (µg/g) of Cs, Mn, and Sr in contaminated and non-contaminated drywall samples. These elements were shown to have an important role in the discrimination between problematic and non-problematic drywalls.

2.4.2.2 Gypsum provenance

Elemental concentration in gypsum ranged from 0.11 µg/g for Cd to ~15% for Ca. Discrimination of gypsum sources based on elemental profiles were evaluated using PCA, HCA, and MANOVA. Clustering of groups is shown in the PCA score plot (Figure 11). Gypsum from

two Colorado mills is clustered together and overlaps each other. Gypsum samples from three FGD sources from South Carolina are clustered near each other on the upper left of the score plot. Gypsum from New Mexico is clustered in the lower left of the quadrant near the Colorado group. The PCA loading plot shows which group has the highest elemental concentration (Figure 2.11). Oklahoma has the highest concentration of Al, V, Ba, Rb, Zn, Pb, Cd, Co, and Mn. Gypsum from Colorado has the highest concentration of U, Mg, and Sr. Gypsum from SC1 has the highest concentration of Fe, Ni and Cr. The three FGD sources from South Carolina have the highest concentration of Ca. The elements Rb, Mn, Cd, Co, Ba, and Al have the highest eigenvectors for Component 1. Rb and Mn has eigenvector of 0.310 and 0.306 respectively, whereas Cd, Co, Ba and V have the same eigenvector value of 0.304. These elements are highly correlated with component 1 and are responsible for the variance explained in the first principal component.

HCA dendogram confirmed and strengthened the clusters observed from the PCA score plot (Figure 2.12). Gypsum samples were clustered based on their sources. Oklahoma is clustered completely separate from the all the other groups indicating that elemental composition of gypsum from Oklahoma is substantially different from the rest of the groups (gypsum source). Gypsum from New Mexico, SC1, SC2, and SC3 are clustered separately based on their source. The HCA dendogram showed that both Colorado gypsum samples are joined together as one cluster indicating similarity of elemental composition. This observation is also apparent in the PCA score plot. Moreover, MANOVA of elemental composition of gypsum from CO1 and CO2 gave a $p=0.155$ suggesting that the two groups are not statistically different. That the samples are indistinguishable, despite being processed by different mills, suggests that the milling units do

not greatly influence elemental composition or that the level of contamination from the mill is at least uniform.

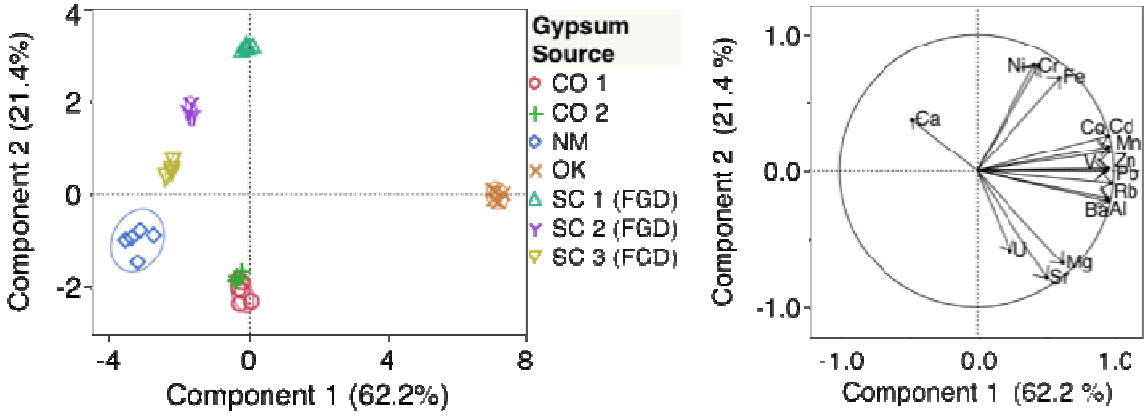

Figure 2.11. PCA score plot (left) and loading plot (right) of elemental profile of gypsum collected from USA.

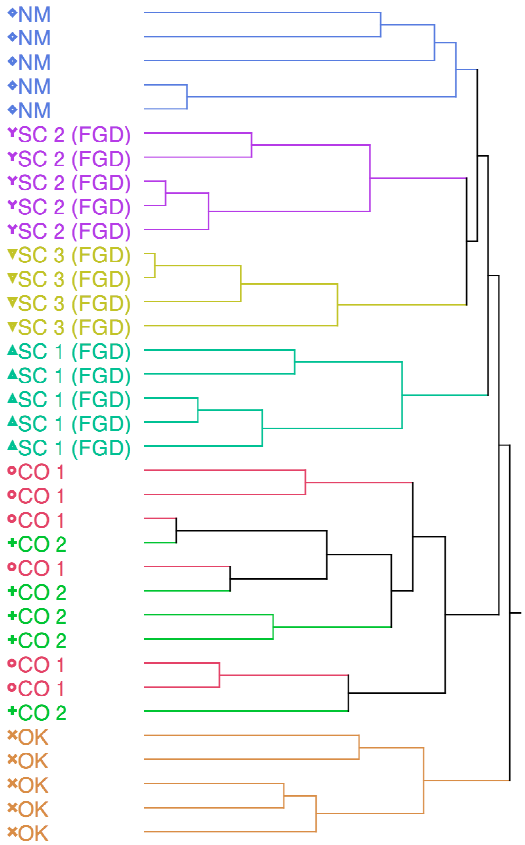

Figure 2.12. Dendogram obtained from HCA for elemental fingerprints of gypsum

MANOVA of the sum of responses gave a $p<0.0001$ indicates that at least one of the groups is statistically different from the other groups. MANOVA also showed that the elemental composition of gypsum from Oklahoma and New Mexico differed the most (Table 2.3). Evaluation of the group's least square means suggests that the elements, V, Rb, Al, Co and Mn are responsible for the discrimination between the groups (Table 2.3).

Table 2.3. Least square means obtained from MANOVA for each element from different gypsum sources

	CO 1	*CO 2*	*NM*	*OK*	*SC 1 (FGD)*	*SC 2 (FGD)*	*SC 3 (FGD)*
Al	0.107	0.061	-0.972	2.23	-0.528	-0.744	-0.213
Ba	0.034	-0.007	-0.593	2.30	-0.531	-0.767	-0.559
Ca	-0.142	-0.070	-0.717	-1.19	0.326	0.969	1.064
Cd	-0.299	-0.382	-0.746	2.22	0.375	-0.416	-0.865
Co	-0.446	-0.435	-0.940	2.25	0.328	-0.207	-0.569
Cr	-0.514	-0.329	-1.615	0.654	1.654	0.595	-0.428
Fe	-0.359	-0.421	-1.691	1.05	1.372	0.588	-0.579
Mg	1.09	0.991	-0.966	1.13	-0.922	-0.944	-0.743
Mn	-0.425	-0.431	-0.619	2.35	0.112	-0.420	-0.603
Ni	-0.608	-0.452	-1.087	0.636	1.870	0.213	-0.564
Pb	-0.396	-0.393	-0.415	2.41	-0.400	-0.409	-0.398
Rb	-0.059	-0.065	-0.986	2.29	-0.370	-0.597	-0.254
Sr	1.19	0.978	-0.411	0.935	-1.030	-1.023	-1.094
U	1.45	1.256	-1.24	-0.0670	-0.429	-0.518	-0.920
V	0.183	0.171	-1.52	1.95	0.165	-0.407	-0.712
Zn	-0.363	-0.398	-0.493	2.41	-0.335	-0.386	-0.451

Overall, our data suggest that elemental profiles have potential to be used to distinguish gypsum based on geographic source. PCA and MANOVA gave different results with respect to the relative influence of elements with respect to the discrimination between groups. PCA suggests that Rb>Mn>(V, Cd, Co, Ba, and Al) while MANOVA suggests that it's the elements V, Rb, Al, Co, and Mn (in decreasing order of importance) that are responsible for discrimination of gypsum based on sources. This indicates that some factors in addition to

geographic origin are influencing the concentration of elements in gypsum. However, both MANOVA and PCA agreed V, Rb, Al, Co and Mn, this could mean that geographic origin has a huge impact on the elemental composition of gypsum.

Whereas elemental fingerprinting is more time-consuming and costly than FT-IR analysis, there are potential advantages to the former approach, particularly if a database of elemental patterns could be established for gypsum from major sources and for drywall from major manufacturers (since the impact of additives and processing needs to be evaluated). Comparison of sample data to such a database could provide provenance information that may be particularly useful in certain cases where the source of the source of material is in question rather than just if the sample is problematic or not.

2.5. CONCLUSIONS

Some sources of drywall have a tendency to outgas volatile sulfur compounds that can lead to a multitude of problems. Multivariate statistical analysis of elemental profiles obtained from acid-digested drywall by ICP-MS revealed significant differences between problematic and non-problematic groups. Thus elemental fingerprinting may serve as an alternative or confirmatory approach to help identify and eliminate problematic materials from use. Elemental fingerprinting was also shown to be feasible in discriminating gypsum, which is an important component in making drywall.

2.6 ACKNOWLEDGMENTS

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2.7 LITERATURE CITED

[1] R. Maddalena, M. Russell, M. Melody, M.G. Apte. Lawrence Berkeley National Laboratory, *Small-Chamber Measurements of Chemical-Specific Emission Factors for Drywall,* Lawrence Livermore National Laboratory, LBNL-3986E (2010). Available at http://www.cpsc.gov/PageFiles/114649/lblreport.pdf.

[2] A. Burdack-Freitag, F. Mayer, and K. Breuer. Identification of Odor-Active Organic Sulfur Compounds in Gypsum Products. Clean – Soil, Air, Water, 37, 459 (2009).

[3] USGS (United States Geological Survey), Mineral commodity summaries 2012, 198, 70 (2012).

[4] CDC (Center for Disease Control and Prevention) Imported Drywall and Your Home, http://www.cdc.gov/nceh/drywall/docs/importeddrywallandyourhome.pdf

[5] J.G. Allen, D.L. MacIntosh, L.E. Saltzman, B.J. Baker, J.M. Matheson, J.R. Recht, T. Minegishi, M.A. Fragal, T.A. Myatt, J.D. Spengler, J.H. Stewart, J.F. McCarthy, Sci. Total Environ. 426, 113 (2012)

[6] CSPC (Consumer Safety Product Commission) Identification of problematic drywall: Source markers and detection methods, EH&E Report 16512 (2010). Available at: http://www.cpsc.gov/library/foia/foia10/os/EHESourceMarkers.pdf

[7] K. Steiner, J. Mater. Civ. Eng. 23, 1050 (2011)

[8] J. Hirsch, S.R. Lowry, M. Dowd. Spectroscopy 25, 30 (2010)

[9] G. Concheri, D. Bertoldi, E. Polone, S. Otto, R. Larcher, A. Squartini, PLoS One 6, 1 (2011)

[10] K. Anderson, B.A. Magnuson, M.L. Tschirgi, and B.J. Smith, J. Agric. Food Chem. 47, 1568 (1999)

[11] Bettina Franke, Haldimann, M., Reimann, J., Baumer, B., Gremaud, G., Hadorn, R., Bosset, J., Kreuzer, M. Eur. Food Res. Technol. 225, 501 (2007)

[12] A. Marcos, A. Fisher, G. Rea, Hill, J. Anal. Atomic Spec. 13, 521 (1998)

[13] R. Smith. J. Agric. Food Chem. 53, 4041 (2005)

[14] K. Pye, S.J. Blott, D.J. Croft, S. J. Witton, Forensic Sci. Intern. 167, 30 (2007)

[15] SAS Institute Inc. JMP® 10 Basic Analysis and Graphing, Second Edition. Cary, NC: SAS Institute Inc. (2012)

[16] B. McCune and J.B. Grace, Analysis of Ecological Communities. MGM Software Design. U.S.A. 205 (2002)

[17] SAS Institute Inc. JMP 11 Basic Analysis. SAS Institute Inc. (2013)

[18] P. A. Gore, Jr. Cluster Analysis. In: Handbook of Applied Multivariate Statistics and Mathematical Modeling. Ed: H.E.A. Tinsley and S.D. Brown. Academic Press. San Diego, California, USA (2000)

[19] Hunter, J.C. and McCoy, R.A. Applying randomization tests to cluster analysis. Journal of Vegetation Science 2004 15 pp 135-138

[20] Brereton, R.G. Chemometrics: Data Analysis for the Laboratory and Chemical Plant 2003 John Wiley and Sons, Ltd. England

[21] Giacomino, A., et al. The role of chemometrics in single and sequential extraction assays: A Review, Part II. Cluster analysis, multiple linear regression, mixture resolution, experimental design and other techniques. Analytica Chimica Acta 2011 688 pp. 122-139

[22] B.S. Everitt. Cluster Analysis, 3rd Edition. Halsted Press, New York, NY, USA (1993)

[23] A. Lehman, N. O'Rourke, L. Hatcher, and E.J Stepanski. JMP for Basic Univariate and Multivariate Statistics: Methods for Researchers and Social Scientists, 2nd Edition. SAS Institute Inc, Cary, NC, USA (2013)

[24] Valimbe, P.S., V.M. Malhotra, and D.D. Banerjee. Structural characteristics and thermal stability of FGD scrubber sludge. Pages 1123-1127

CHAPTER THREE

FINGERPRINTING OF FARM-RAISED CATFISH (*ICTALURUS PUNCTATUS*) USING TRACE ELEMENTS AND $^{87}Sr/^{86}Sr$ RATIOS DETERMINED BY ICP-MS

3.1 ABSTRACT

The market for seafood is expanding worldwide. In a globalized economy, the ability to determine the source of food is necessary to enforce existing import laws, secure food supply, and protect consumers from fraud and deception. In the U.S., increasing competition and importation of catfish from other countries prompted implementation of Country of Origin Labeling (COOL), a law which requires certain commodities to give information about the country of origin in their label. In this study, we evaluated the capability of elemental fingerprinting to determining the provenance of catfish (*Ictalurus punctatus)*. Farm-raised catfish from the U.S. (Mississippi and Alabama), China and Vietnam were studied. Fish-muscle tissue was microwave-digested and 15 elements (^{27}Al, ^{137}Ba, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{133}Cs, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{85}Rb, ^{88}Sr, ^{51}V, and ^{66}Zn) were determined using sector field-inductively coupled plasma mass spectrometry (SF-ICP-MS). In addition, ^{87}Sr/^{86}Sr ratios were determined in the bones of fish from Macon and Stoneville, Mississippi, using multi-collector (MC)-ICP-MS. Data were evaluated using multivariate statistics. Water samples were analyzed from Macon and Stoneville in Mississippi; catfish from these two sites were fed similar diets but reside in different source waters. Results show that elemental profiles in catfish significantly differ based on country of origin, with Ba and Rb playing an important role in the discrimination. Differences in source water chemistry likely influenced the accumulation of these metals.

Results also indicate significant differences ($p<0.0001$) between $^{87}Sr/^{86}Sr$ in bones from ponds in Macon (0.70859±0.00041), and Stoneville, MS (0.70945±0.00038).

3.2 INTRODUCTION

3.2.1 Catfish Aquaculture, Legislation, and the Need for Determining Geographic Origin of Catfish

The largest aquaculture industry in the U.S. is farming and cultivation of catfish, with an annual production value of $450 million [1]. As of January 1, 2010, a total of 115 thousand acres were used for catfish production, with Mississippi (64,000 acres), Alabama (19,800 acres), and Arkansas (19,200 acres) accounting for 90% of all U.S. catfish acreage. In 2009, a total of 115 thousand pond water acres in the U.S. produces 36.2 million pounds of catfish, which sold for 373 million dollars. The top four catfish producers, Mississippi, Alabama, Arkansas, and Texas, accounts for 93% of the total U.S. sales, 57% of which come from Mississippi [2].

Growth of catfish industry started in the 1980s when catfish farming more than doubled its size (see Figures 3.1 and 3.2) [3]. In 1990, approximately 50% of the value of all aquaculture products harvested in the U.S. was attributed to catfish production [4]. In the 1990s, farmers were enjoying strong farm prices and low feed prices brought about by low corn and soybean prices, which lead to an increase in pond acreage – 157,000 acres in 1990 to 185,000 acres in 2001. However in 1998, when many countries were facing uncertain economic conditions, a number of major trends affected the U.S. aquaculture industry. The industry faced strong competition from other meat industry and most importantly from other foreign aquaculture industry. Increased competition in the U.S. market was due to (1) decrease in export demands for

U.S. products; (2) increase in importation of products due to currency devaluations relative to U.S. dollars; (3) relatively low prices for other protein sources such as pork and chicken [5].

Increase in imports (see Figure 3.2) and high price of catfish in the U.S. attracted attention from sellers of potential substitute products. Basa (*Pangasius bocourti*) and Tra (*Pangasius hypophthalmus*), the majority of which come from Vietnam, found their way to the U.S. market. Basa and Tra were not popular when imported under such names as "White Roughy" and "River Cobbler". However, when they started marketing their product as "catfish", the sales increased dramatically [1].

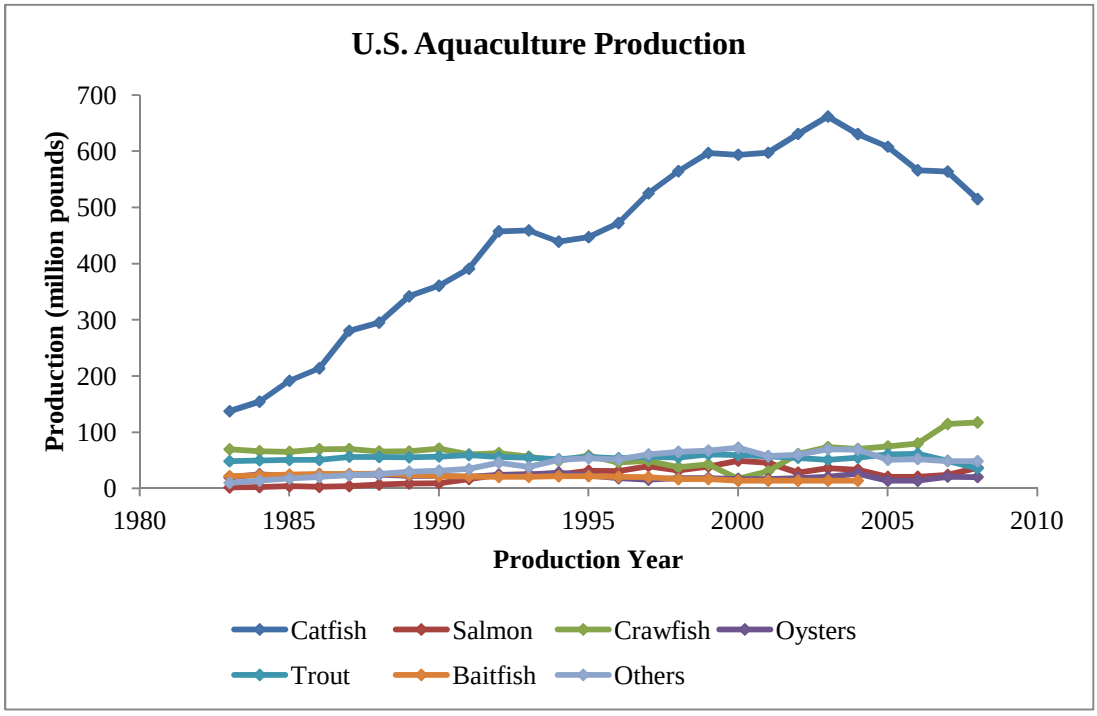

Figure 3.1. US aquaculture production in million pounds [3]

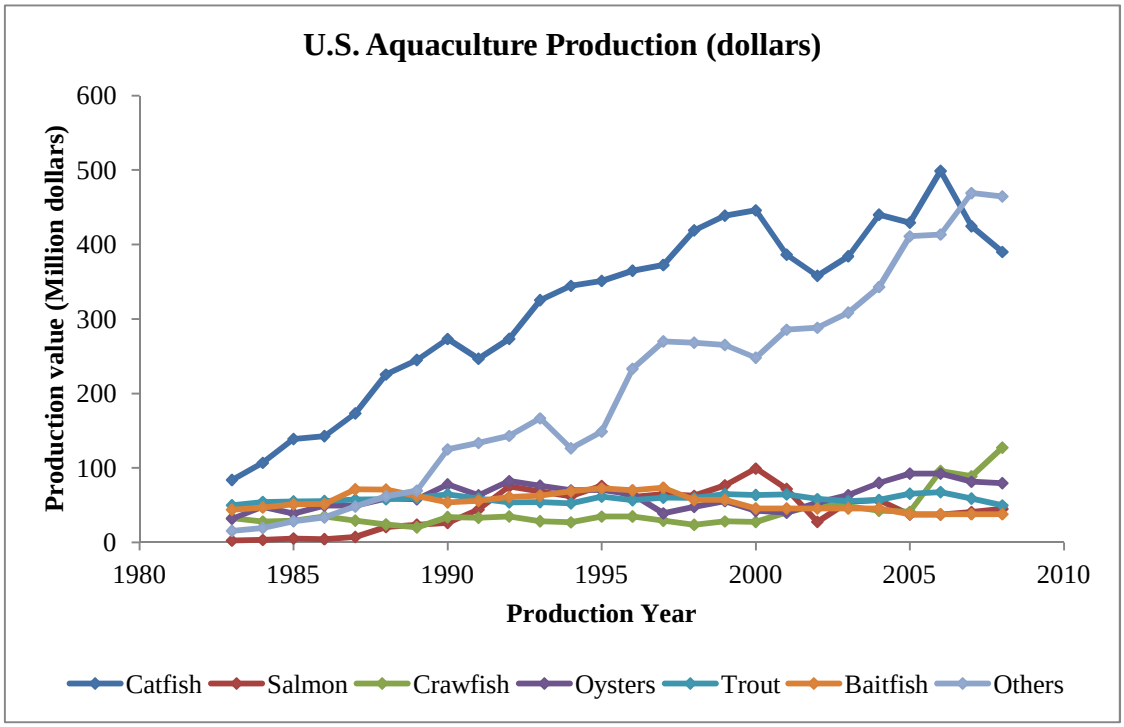

Figure 3.2. US aquaculture production in value (million dollars) [3]

In October 2001, catfish imports (primarily frozen fillets), 99% of which come from Vietnam, increased to 1.61 million pounds from 92,000 pounds imports in October of 1998 [6-7]. These fillets replaced 23% of the market share previously held by the U.S. catfish farmers [1]. Being the largest aquaculture industry in the U.S., these prompted the Catfish Farmer Association (CFA) to take some actions to protect its market share. On May 13, 2002, Farm Security and Rural Investment Act of 2002 (H.R. 2646) became P.L. 107-171. Section 10806 of the legislation states that the word "catfish" can be only be used to fishes belonging to the Ictaluridae family, where the channel catfish, which is native to North America, belongs. As a result, basa and tra, which belongs to Pangasidae family, should be labeled as such and not as "catfish".

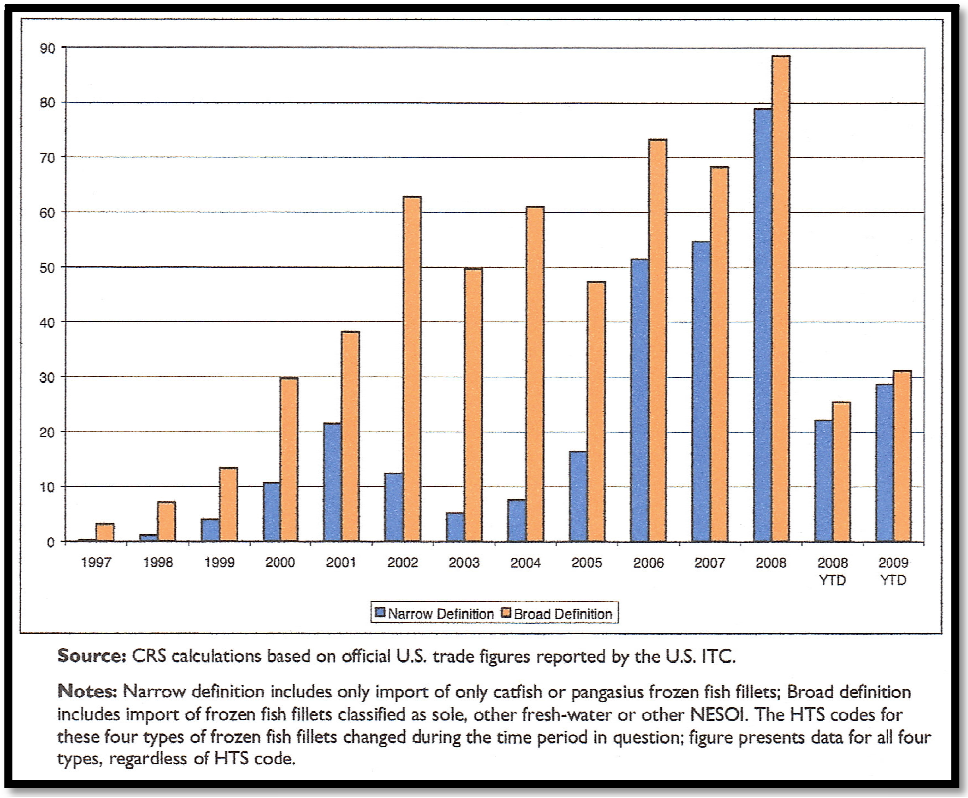

Figure 3.3. U.S. imports of selected frozen fillets from Vietnam (US million dollars) [8]

In September 2008, mandatory Country of Origin Labeling (COOL) was implemented. The 2002 Farm Bill, 2002 Supplemental Appropriations Act and the 2008 Farm Bill amended the Agricultural Marketing Act of 1946 that requires the label of the covered commodities to provide specific and accurate country of origin information to the consumers. Included in covered commodities are the catfish. Catfish producers must include in their product label the country of origin of the fish and whether their fish is farm-raised or wild-caught [9].

Determining the geographic source of and authenticity of a product is important to secure food supply [10], protect consumer from overpayment and deception [11], enforce existing import laws and requirements, and implement antidumping duties [12]. Different approaches and techniques have been studied to trace the geographical origin of foodstuffs. Analytical methods include isotope ratio analysis of “light” elements [13-20], isotope ratio analysis of “heavy”

elements including strontium [21-26], genetic-based approaches [27-30], and elemental profiling [12, 31-34].

3.2.2 Aquaculture of Channel Catfish

The channel catfish, *Ictalurus punctatus*, is the most commonly cultured catfish species in the United States for commercial purposes [35-36]. Channel catfish meets the desirable qualities for commercial farming and aquaculture. These qualities include feeding habits and body morphology. The fish exhibits very satisfactory growth and survives in a wide range of environments. It can tolerate commonly used culture systems such as ponds, cages, and raceways, which at times may become crowded. In addition, channel catfish accepts prepared, artificial diet, which turned them into a surface-eater instead of a bottom-feeder. In addition, the fish retain its flavor and the flesh even after undergoing a variety of processing methods [35].

Most channel catfish are cultured in ponds [37]. Water from these ponds may come from surface water, ground water, and rain water. Water for these ponds may come from several sources such as surface water and ground water [38]. Levee ponds are normally constructed for flat areas and are filled with ground water or surface water [39]. Watershed ponds are usually built for hilly terrains, where the main source of water could come from a runoff from rainfall on the watershed [39]. Runoff water is stored in dams that may be constructed across valleys. However, an outside water source such as wells, streams or rivers may be used to supply water to the watershed ponds when the water gets too low for fish production [39].

Water from these ponds is maintained under essentially static conditions. Significant water exchange may occur during high precipitation (dilution) and water evaporation (concentration) [38]. Management of water quality involves the regulation of chemical, physical,

and biological environment to optimize fish production [40]. The quality of water supply are affected by several factors such as feeding rate, metabolic wastes, plankton, and the interactions between the water and mud [38]. Changes in water quality are indirect or direct results of feeding rate [40]. High feeding rate would results to an increase in nutrients, which come from the metabolic waste in fish. These nutrients favor the growth of aquatic plants, usually phytoplankton, which require tremendous oxygen demands. The abundance of phytoplankton is indicated by the concentration of chlorophyll a.

3.2.3 Elemental Profiling of Aquatic Organisms

Elemental profiling along with multivariate statistics has been used for provenance and authenticity of aquatic organisms. Anderson et al. (2010) used elemental profiles to classify salmon based on production methods: wild or farm-raised [41]. Smith and Watts (2009) were able to provide evidence on the validity of elemental profiling to determine the country of origin of farm-raised shrimp (Family *Penaeide*) [12]. Arribére et al. (2006) used elemental profiles to indicate the site of provenance of some native and exotic fish species of northern Patagonian lakes [42]. Favretto et al. (1989) were able to differentiate polluted from unpolluted mussels [43]. With regards to seafood, these studies suggest that: (1) bioaccumulation of metals in fish tissues is related to the environmental condition where the organism spends its life cycle, (2) elemental profiles can be used determine the authenticity, geographic source, and fish production methods.

3.2.4 Accumulation of Metals in Fish Tissue

Fish take up essential and toxic metals through dietary and waterborne sources [44-46]. The principal source of accumulation for some elements is through dietary food while some elements are accumulated from waterborne sources. Cu and Zn in fish tissue are accumulated from dietary sources, while Pb comes from waterborne sources [47]. Accumulation of metals in tissue is affected by several factors. Elemental composition in fish tissue is species-dependent [48-49]. Living and feeding habits may contribute to the interspecies differences in elemental composition [44, 48-50].

The concentration, uptake route, and bioavailability of metals in the environment can affect metal accumulation in metals in fish tissues [44, 51]. Field and laboratory studies show correlation between the metal concentration in water and tissues [52-53]. Metal uptake in fish can be made through respiration (gills), adsorption, and ingestion [45, 54]. Depending on the uptake route and affinity to organs, metal concentration varies from tissues in different organs in fish [45]. Liver tends to have high concentration of Cu while gonads are high in Zn [44].

Environmental condition also affects metal concentration in fish tissue. Water temperature affects the uptake rate and deposition of metals in different organs in fish. Higher temperature tends to increase uptake rate of metals [55]. Accumulation of metals to be higher especially on burdened organs such as liver and kidney when there is an increase in temperature [56]. The increase in uptake and deposition rate may result from an increase in metabolic rate at higher temperatures [44].

3.2.5 Strontium Isotope Ratio

Strontium is a divalent alkaline earth element that has four naturally occurring stable

isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr, ^{88}Sr [57]. The relative abundance of the isotopes ^{84}Sr, ^{86}Sr, and ^{88}Sr in earth materials are mostly constant [58]. In contrast, ^{87}Sr is a radiogenic isotope that is produced from the radioactive decay of ^{87}Rb thereby the amount of ^{87}Sr in a mineral rocks increases over time [57, 59]. This makes ^{87}Sr useful as a tracer in understanding the geological processes such as petrogenesis, weathering, atmospheric fluxes, and cation biocycling [57-58]. Numerous studies that use ^{87}Sr as a geological tracer have been published in the literature [60-61].

Strontium has also been used for provenance and fingerprinting studies including fish [62-66]. Strontium, with a relatively high mass, does not fractionate to the extent C, H, N, O, and S do [67]. For soil and vegetation system, fractionation of $^{87}Sr/^{86}Sr$ is corrected for during measurement and is considered negligible [58]. As noted, due to radiogenic nature of ^{87}Sr, the amount of $^{87}Sr/^{86}Sr$ depends on geographical region. Thus, $^{87}Sr/^{86}Sr$ ratios can provide information about the geographical background and source of a variety of samples including food products [58, 67].

Strontium in bones and fish otoliths (ear bones) has been studied for provenance purposes [66, 68-70]. Strontium and calcium, both Group IIA in the periodic table, have relatively similar electron configuration, charge, and radius [58]. Sr^{2+} and Ca^{2+} have ionic radius of 1.13Å and 0.99Å respectively [57]. Because of this, strontium can be incorporated in bones through several processes: ionic exchange with Ca^{2+} in bone, surface adsorption of Sr in bones, or binding of Sr^{2+} by preosteoid protein [71]. Sr isotope ratios in human teeth and bones have been used to study past migration events in the past [72-73]. Sr in fish otoliths has been used to estimate geographic origin and the timing of fish introduction of an exotic fish into a lake [69].

3.2.6 Purpose of the study

In this study, fingerprinting of farm-raised catfish, *Ictalurus punctatus,* was conducted using elemental profiling and strontium isotope ratio analysis determined by ICP-MS and chemometrics. The purpose was to: (1) evaluate the feasibility of using elemental fingerprints of fish muscle tissues and strontium isotope ratio of bone samples for provenancing of catfish, and (2) determine the role of source-water chemistry on those fingerprints.

3.3 MATERIALS AND METHODS

3.3.1 Sample Sites

Catfish were obtained from commercial ponds located in Whenzhou and Huzhou in the Zhejiang province of China (Figure 3.4), Mississippi and Alabama in the U.S. (Figure 3.5), and from an unknown location in Vietnam. The U.S. catfish were collected from different commercial farms located in western Alabama (hereafter called "West" Alabama) and Macon, Mississippi. Catfish were also obtained from ponds maintained by USDA located in Auburn, Alabama, and from the National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi.

Catfish from the NWAC came from ~1 acre experimental levee ponds which are supplied by well water from the Mississippi river alluvial aquifer. The ponds at Macon, MS are supplied primarily from surface runoff, though wells supply water when needed. Both Stoneville and Macon catfish were fed with the same diet (28% protein, Delta Western Feed Mill) but resided in water from different sources in contact with different types of soil. We do not have detailed information about diet and water for catfish from China and Vietnam.

Figure 3.4. Location of ponds where catfish were obtained in China

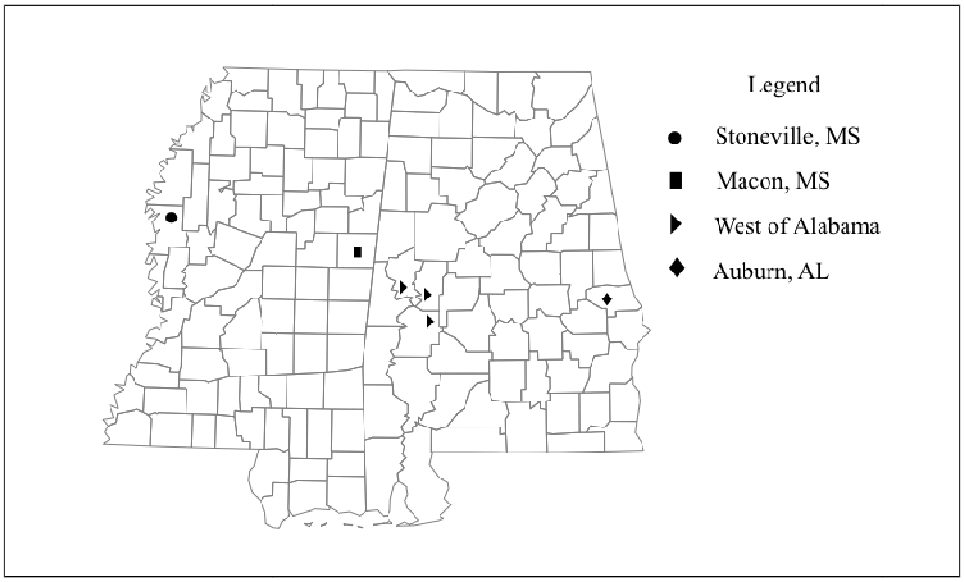

Figure 3.5. Location of ponds in the US where catfish were collected

3.3.2 Sample Collection and Processing

Twenty catfish from China were obtained live the fish-farm and sacrificed. Fillets from both sides were cut into small pieces for freeze-drying. The lyophilized tissue was sent to the University of Mississippi Department Chemistry and Biochemistry for analysis.

Twenty three catfish were collected from different commercial ponds located in western Alabama, and eight fish from a single pond located in Auburn. The fish were collected using a seine and filleted using a commercial fillet machine with stainless steel blades.

About 10 catfish were collected from a single pond in Stoneville, MS in June 2010. In November 2011, a total of 15 additional fish were collected from 3 different ponds in Macon, MS as well as 15 fish from 3 different ponds in Stoneville, MS. Catfish from Mississippi were collected using hooks. The age of these fish was estimated to be 2 – 3 years. The fish weighed an average of 890±217g. These fish were hand-filleted using stainless steel knife. Catfish fillets and bones were placed in a ziplock bag. Samples were shipped to the Department of Chemistry and Biochemistry at the University of Mississippi, where they were kept frozen until analysis. For the Stoneville and Macon sites, water samples were collected in acid-washed polyethylene bottles and preserved to 1%HNO_3.

3.3.3. Analysis of Fish Muscle Tissue

Fillets were thawed and rinsed with deionized water (DI) ≥18.2MΩ (Barnstead Nanopure Diamond ultrapure water system). About 2 cm^2 of muscle tissue was removed from the center of the fillet with a titanium knife. The muscle tissue was freeze-dried (Freezone 4.5; Labconco Corp., Kansas City, MO, USA). Fish tissue had an average moisture content of 78±3%.

Approximately 0.2-gram of the dried muscle tissues was weighed into an acid-washed Teflon (pfa) vessel and 5 mL of high purity concentrated HNO_3 (Trace Metal Grade, Fisher Scientific), 1 mL of 30% hydrogen peroxide (Certified ACS Grade, Fisher Scientific), and 2 mL DI water were added. The mixture was allowed to sit (cold-digest) for an hour. Digestion was completed using a closed-vessel microwave digestion system (Ethos; Milestone Inc., Shelton CT, USA) equipped with a multi-prep rotor (41 pfa vessels). The digestion program consisted of a 25-min ramp to 120^0C, 35-minute ramp to 160^0C, then 35 min ramp to 180^0C, where the temperature was held for 20 minutes. The resulting digest was transferred to an acid-washed polypropylene tube and diluted to 50-mL with DI water. This solution was further diluted, 2-fold with DI water, so that the resultant solution was 5% HNO_3.

The resulting solution was introduced into a high-resolution SF-ICPMS (Element-XR; Thermo Scientific) for multi-element analysis using concentric nebulizer with glass cyclonic spray chamber. An internal standard containing 1 ppb ^{103}Rh was added inline using a T-junction. The instrument was tuned to optimize sensitivity and stability prior to analysis. Instrument parameters are given in Table 3.1. Elements, Ba, Cd, Cs, Pb, Rb, and Sr, were analyzed in low resolution. Elements, Al, Co, Cr, Cu, Fe, Mn, Ni, V, and Zn, were analyzed in medium resolution.

Table 3.1. ICP-MS data acquisition and instrument parameters

Plasma	
Auxiliary gas flow	1.15 L min^{-1}
Sample gas flow	1.270 L min^{-1}
Cool gas flow	16.00 L min^{-1}
RF power	1450 W
Data acquisition	
Isotopes monitored in LR	^{37}Ba, ^{111}Cd, ^{133}Cs, ^{208}Pb, ^{85}Rb, ^{88}Sr
Isotopes monitored in MR	^{27}Al, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{60}Ni, ^{51}V, ^{66}Zn,
Integration time	10ms for LR 30ms for MR
Sample per peak	50 for LR 20 for MR

LR = low resolution; MR = medium resolution

External calibration was used to quantify elements. A series of multi-element standards (0.01 ppb, 0.05 ppb 0.1 ppb, 0.5 ppb, 1 ppb, 5 ppb, and 10 ppb) were prepared in 5% HNO_3. A stock solution of multi-element standard was purchased from SpexCertiPrep. Results were validated using a fish-muscle certified reference material, DORM-3 (NRC, Canada). Recoveries of the elements are given in Table 3.2.

Table 3.2. Recovery for DORM 3 reference material (n = 15)

Element	**Certified (ppm)**	**Found (ppm)**	**Recovery (%)**
Al	1700	1500 ± 57	88.2
Cd	0.29 ± 0.02	0.32 ± 0.01	112
Cr	1.89 ± 0.17	1.89 ± 0.17	99.9
Cu	15.5 ± 0.63	15.9 ± 0.6	102
Fe	347 ± 20	345 ± 16	99.9
Pb	0.395 ± 0.050	0.32 ± 0.16	81.1
Mn	4.6	3.10 ± 0.16	67.4
Ni	1.28 ± 0.24	1.34 ± 0.05	105
Zn	51.3 ± 3.1	55.8 ± 2.2	109

3.3.4 Analysis of Water Samples

Samples were filtered through 0.45 µm quartz fiber filters. The filtrate was collected into an acid-washed polypropylene tube and preserved to 1% HNO_3 (v/v). ICPMS analysis of water was as before (fish muscles) except that a multi-element internal standard containing 1 ppb ^{45}Sc, ^{89}Y, and ^{159}Tb was used. ^{45}Sc, was used for elements ^{27}Al, ^{52}Cr, ^{51}V, ^{55}Mn, ^{56}Fe, and ^{59}Co; ^{89}Y was used for elements ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{85}Rb, and ^{88}Sr; and ^{159}Tb was used for ^{137}Ba, ^{111}Cd, ^{133}Cs, ^{208}Pb and . Standards were prepared in 1% HNO_3 and external calibration was used to quantify elements. Results were validated using a standard reference material, NIST 1643e (Table 3.3).

3.3.5 Determination of $^{87}Sr/^{86}Sr$ in Fish bones

Fish backbone was rinsed with DI water and air-dried in a clean laminar flow hood. To remove extraneous soft tissues, bones were soaked in 10% H_2O_2 for 1 hour and then sonicating for 10 min. The samples were then rinsed with DI water, and the method repeated using methanol. Finally, samples were rinsed with DI water and air-dried in a clean laminar flow hood. The bones were then shipped to the Department of Chemistry and Biochemistry at Northern Arizona University (NAU) for Sr isotope ratio analysis.

At NAU, a nominal 0.25 g aliquot of sample was weighed into a Pyrex vial and the material was dry-ashed overnight (~ 16 hours) at 500 ^{0}C. The dry-ashed residue was transferred to a pre-cleaned 50 mL polypropylene centrifuge tube and 25 mL of 8 M HNO3 was added. The material dissolved upon standing for one hour at ambient temperature. Three blanks, two duplicates, and three preparations of a modern marine coral (having an $^{87}Sr/^{86}Sr$ of seawater) were also included with the dissolutions of the unknown samples.

Table 3.3 Recovery for elements in NIST 1643e

Elements	Certified (ppb)	Found (ppb)	Recovery (%)
Al	138.33 ± 8.4	128	92.5
Ba	531.0 ± 5.6	478	90.0
Be	13.64 ± 0.16	9.52	69.8
Cd	6.408 ± 0.071	5.56	86.8
Ca	31500 ± 1100	17800	56.4
Cr	19.90 ± 0.23	18.3	91.9
Co	26.4 ± 0.32	23.7	89.7
Cu	22.20 ± 0.31	17.5	78.8
Fe	95.7 ± 1.4	79.0	82.5
Pb	19.15 ± 0.20	17.0	88.7
Li	17.0 ± 1.7	9.52	56.0
Mg	7841 ± 96	4560	58.1
Mn	38.02 ± 0.44	32.6	85.7

Sr-Spec resin (EIChrom SR-B50-S) columns were prepared in a 7-mL polyethylene transfer pipets (Samco Type 205) equipped with a glass wool plug. Columns were cleaned prior to resin filling using dilute nitric acid solution. 100 mg of resin was slurry-packed into each column. About 12-13mL of sample solution were passed through each column. Columns were rinsed 8x with 1 mL of 8 M HNO3 to remove un-retained matrix elements. Strontium was eluted with 3 mL of water. $^{87}Sr/^{86}Sr$ ratios were then determined using a VG Axiom MC ICPMS equipped with a Scott double pass spray chamber and a self-aspirating concentric FEP nebulizer (~ 0.5 mL/min uptake rate).

3.3.6 Mass bias correction for Sr isotope ratios

Raw ratios were internally mass-bias corrected using $^{87}Sr/^{86}Sr = 0.1194$. NIST 987 ($SrCO_3$) solution was used as a control to further normalize the data using the accepted $^{87}Sr/^{86}Sr = 0.71025 \pm 0.00001$. The NIST 987 was analyzed intermittently throughout the course of the analysis and the obtained ratios were used to develop a normalizing factor applied to the unknown samples using a bracketing approach. These bias correction factors ranged from

1.000092 to 1.000176. The NIST 987 values were consistently biased negative and ranged from 0.71009 to 0.71023.

For each unknown sample, one or more blocks of 20 five-second ratio measurements were performed. The internal precision is expressed as a relative standard error ($S/N^{1/2}$); these ranged from 9 to 56 ppm with the great majority being between 15 and 25 ppm. Samples were diluted as needed to obtain ^{86}Sr signal of between 4 and $5x10^7$ cps, giving a ^{88}Sr signal of about 4 x 10^8 cps. The upper limit of the ^{88}Sr signal is 6 x 10^8 cps (the Faraday maximum output is 10 V), and it was ensured that saturation of the ^{88}Sr collector was not reached.

The total propagated uncertainty for $^{87}Sr/^{86}Sr$ in all unknown samples is approximately 0.00015 (k=2). The contributors to this are: 1) the uncertainties in the mass bias correction factor; and 2) the external precision based upon sequential repetitive measurements of several different unknown samples.

3.3.7 Statistical Evaluation of Data

Data were evaluated using JMP software ®10.0.2 (SAS; Cary, NC, USA). Data were standardized using Z-score prior to statistical evaluation to ensure that measurement scales do not affect the analysis and to compensate for the varying elemental concentration. Tests for statistical significance were defined at $\alpha = 0.05$. Correlations between variables were explored using simple Pearson's correlation.

The $^{87}Sr/^{86}Sr$ data were evaluated using a two-tailed T-test to test the significance between the two groups. Distribution of $^{87}Sr/^{86}Sr$ ratios within the group is demonstrated using Box plot. The line within the box corresponds to the median value while the lines at the ends of the box represents the 1st and 3rd quartile. Data points shown outside the box are the outlier

points. The lines extending from each end of the box are the whiskers line, which correspond to the range of the data set [74].

Elemental concentrations were evaluated using multivariate statistical analysis, Principal Component Analysis (PCA) and Discriminant Analysis (DA). However, it should be noted that PCA measures total sample variation and does not explicitly take into account the variation between groups [10]. To get the best possible evaluation of the group classification and discrimination, elemental profiles was analyzed using DA.

PCA is used as exploratory method to evaluate the grouping tendency of the samples. PCA reduces a data set with multiple variables into a smaller number of composite variables (Principal Components). This is accomplished by determining the strongest linear correlation structure among variables [75]. PCA determines the principal components (PCs) that are linear combination of the original variables [75]. PCA determines the variance explained by each axis (eigenvalues) and the linear equations (eigenvectors) that combine the original variables. The size of the eigenvalue determines how much variance each PC represents. The eigenvectors list the contribution of each response variable to a particular component [75].

Discriminant analysis is an eigenanalysis technique, which maximizes the among-group differences relative to within-group variation. DA determines the canonical axes or discriminant functions that best discriminate among groups. DA is used for: (a) summarizing the differences between groups, (b) multivariate testing to check whether or not two or more groups are significantly different from each other, (c) determining which variables contribute most to discriminating between groups [75].

3.4 RESULTS AND DISCUSSION

3.4.1 Elemental concentrations and fingerprinting of farm-raised catfish by country of origin

For the measured elements, concentrations in fish-muscle ranged from 0.00121 µg/g for Cd to 64.7 µg/g for Rb. A box-whisker summary plot of the elemental data is shown in Figure 3.6. Concentrations of Cd were relatively high in the fish from China and Vietnam compared to fish from the U.S. The Vietnam catfish were also relatively high in Ba and Rb. Samples from Huzhou China were relatively high in V and samples from Wenzhou China were relatively high in Cs. The other elements generally had similar concentrations between sites.

The data set consisting of all samples (U.S., China and Vietnam) was evaluated using PCA and DA. PCA is an exploratory classification technique that visually evaluates clustering of sample points. Sample grouping tendency was evaluated using PCA. Significant differences among groups were determined using DA. Clustering of groups by country of origin was observed in the PCA score plot of Components 1 and 2 (Figure 3.7). The plot shows that catfish from Vietnam are clustered separately from the rest of the groups. Catfish from Wenzhou and

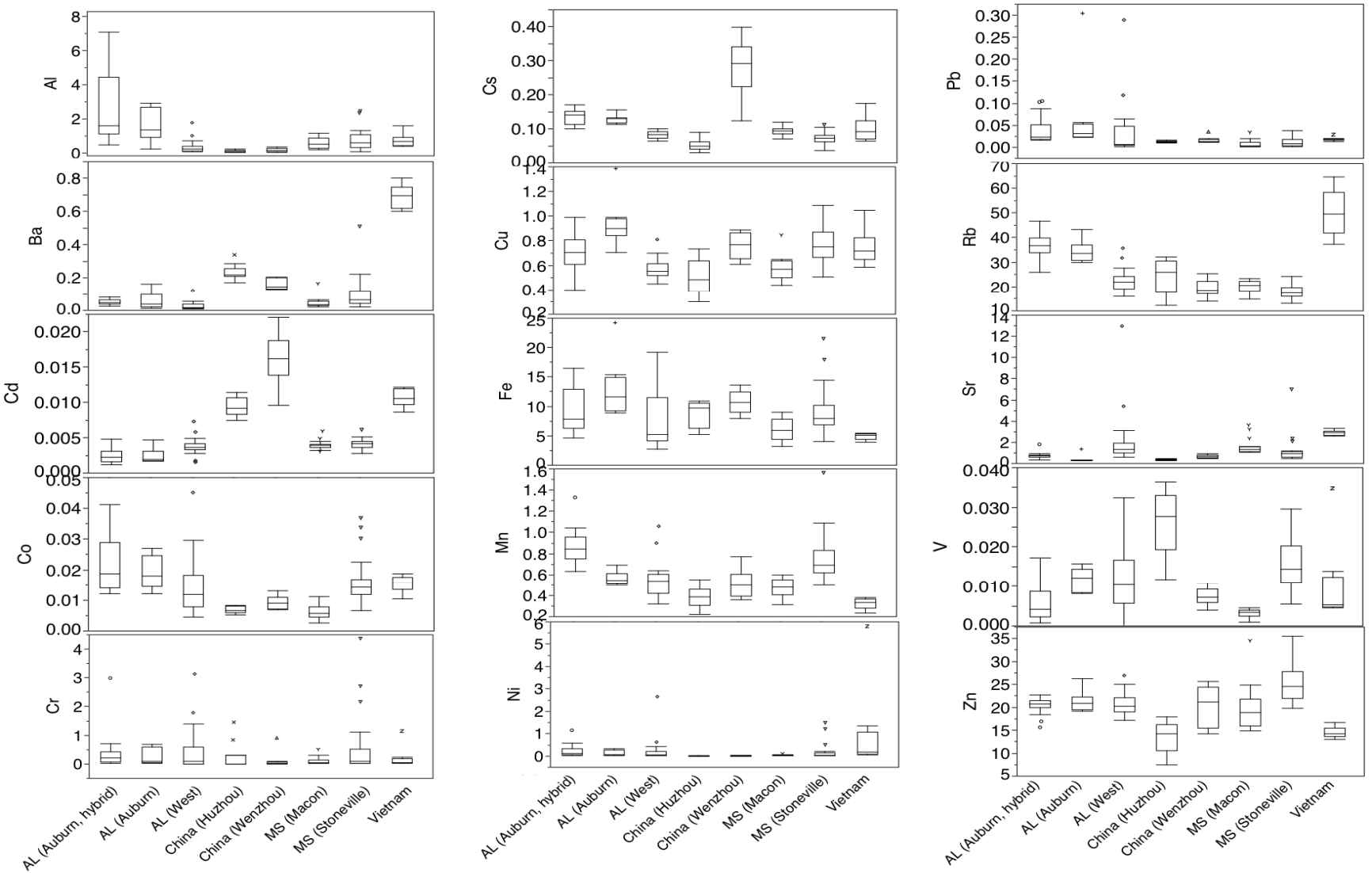

Figure 3.6. Box-Whisker plot of the element concentration (µg/g) in muscle tissue of catfish obtained from different ponds in the US, China, and Vietnam

Huzhou are clustered together and overlap each other. Catfish from various ponds in the US are also clustered together and can't be distinguished from each other.

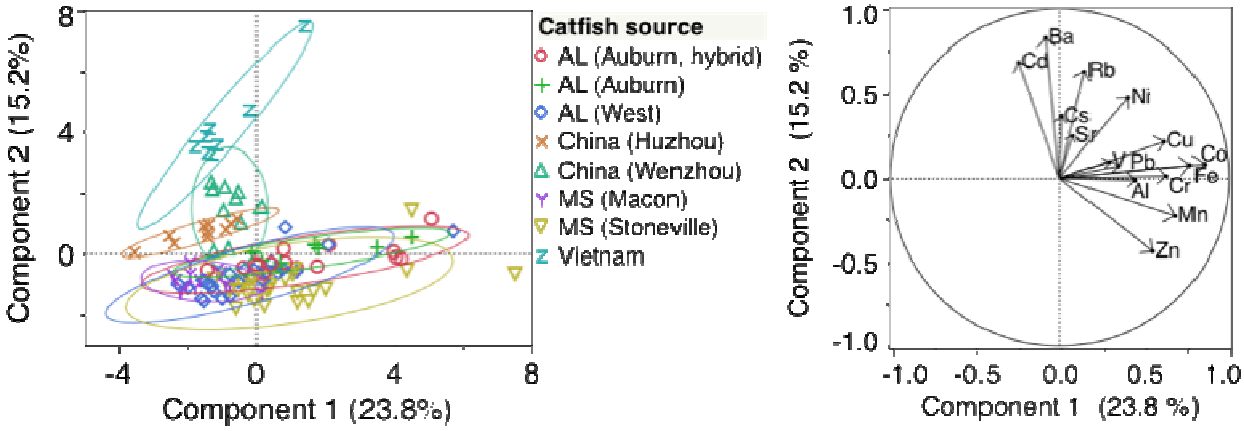

Figure 3.7. PCA score plot showing 95% density ellipse (left) and loading plot (right) of elemental profiles in catfish from U.S., China, and Vietnam

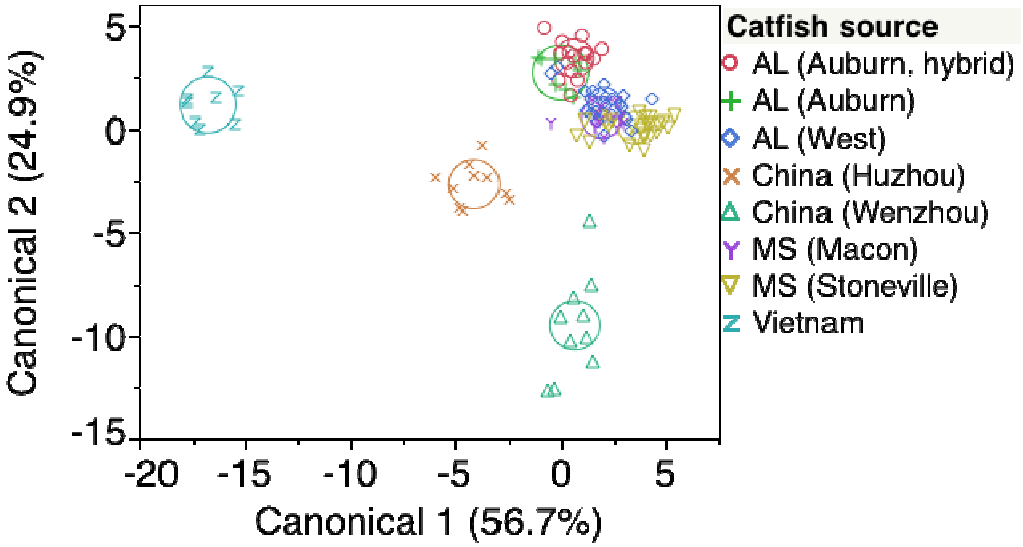

Figure 3.8. DA canonical plot of elemental profiles of catfish from U.S., China and Vietnam.

The DA results indicate that at least one group is statistically different from the other groups (Wilk's Lambda, $p<0.0001$) and are even more striking when plotted, showing clear separation between catfish from Vietnam, China and the U.S. (Figure 3.8). The Chinese samples

from Wenzhou and Huzhou, cities which are separated by ~250 miles, are themselves clustered separately from each other. The U.S. samples are clustered together and have some overlap, though it appears Auburn and Stoneville, geographically separated by ~370 miles, are separated.

3.4.2 Elemental concentrations and fingerprinting farm-raised catfish within the U.S.

For PCA analysis of the samples from the U.S. only (Mississippi and Alabama) showed that samples were clustered into three possible groups (Figure 3.9): (1) those from east Alabama (Auburn), which consist of both channel catfish and a hybrid species (a cross between channel catfish, *Ictalurus punctatus,* and blue catfish, *Ictalurus furcatus*), which lie in the upper quadrants of the score plot; (2) those from West Alabama and Macon Mississippi, which are in relative proximity to each other geographically (within 60 miles), and whose data overlap each other mostly in the lower left quadrant; and (3) those from Stoneville, Mississippi, which tended to lie in the lower right quadrant. Because the fish from Macon and Stoneville were fed the same diet and were the same size, yet resided in different source waters, we compared them further (discussed below).

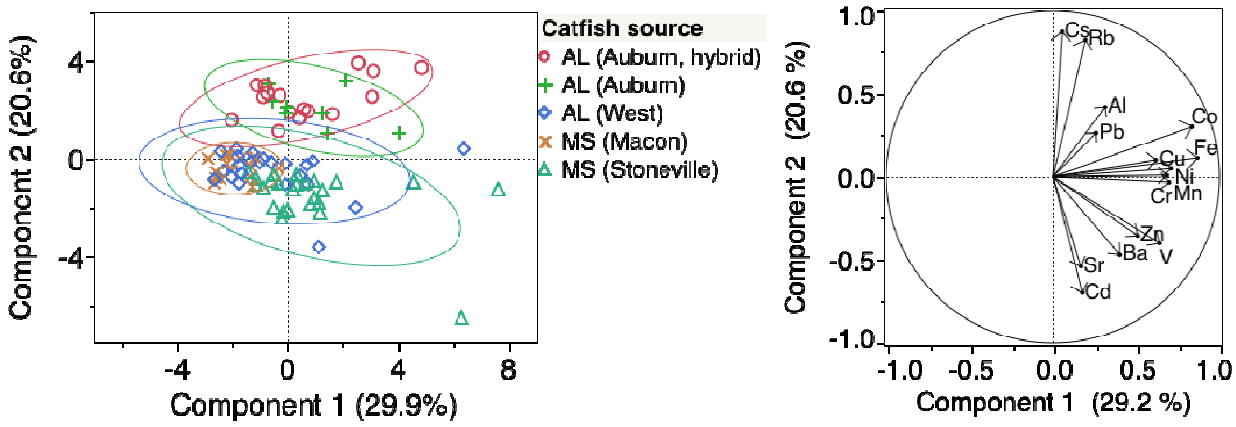

Figure 3.9. PCA score plot showing 95% density ellipse (left) and loading plot (right) of elemental profiles of catfish from the U.S.

It is important to note that channel catfish and hybrid catfish from the same general location (Auburn, AL) are clustered together in the PCA score plot, suggesting that the subtle differences in these two species do not have a great influence on the accumulation of elements in fish-muscle tissue, and thus can likely be grouped together in future elemental fingerprinting studies.

The loading plot for the U.S. data set (Figure 3.9) revealed that Co, Fe, Mn, Cr, and Cu are the elements responsible for the variance explained by Component 1, and Ba, Cd, Rb, Ni, and Zn are important in the variance explained by Component 2. Since the groups are better discriminated along Component 2, the top three elements that are responsible for distinguishing the groups are Ba, Cd, and Rb.

Regarding the DA analysis, canonical 1 and 2 were plotted in an ordination space to evaluate clustering of groups (Figure 3.10). Canonical 1 and 2 explained 72.7% and 16.3% of the fitted variation, respectively. This is a greater total % than for the PCA analysis, where components 1 and 2 explain 23.8% and 15.2% of the total variance, respectively, and may explain, in part, why the DA more clearly separated the country of origin on the plot.

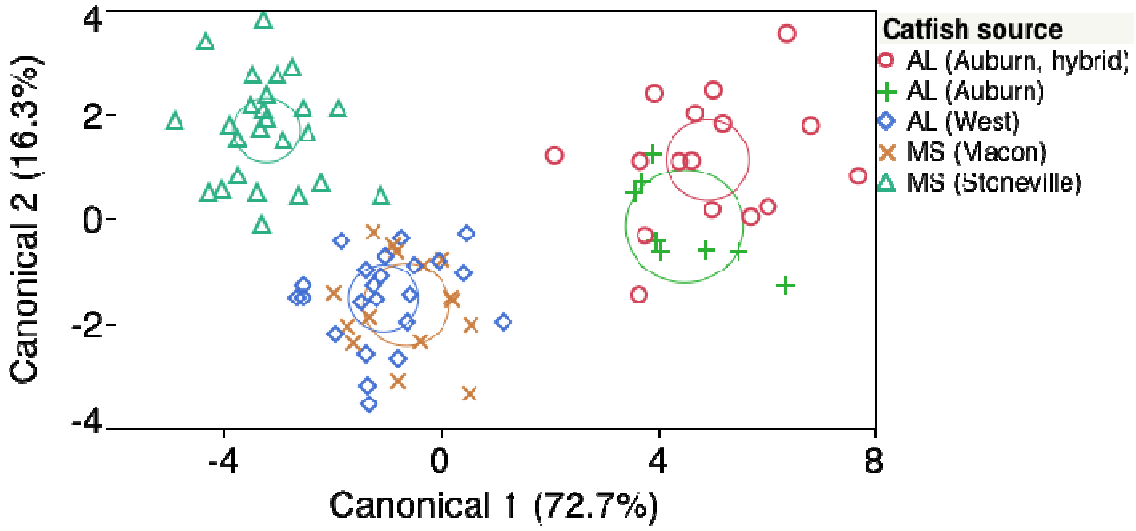

Figure 3.10. Canonical plot of elemental fingerprints of US catfish

For DA, the top five elements that have the highest standard scoring coefficient for Canonical 1 are Ba, Rb, Mn, Sr, and Cs. Elements Cd, Rb, Cs, Co, and Sr have the highest standard scoring coefficient for Canonical 2. Assessment of the standardized scoring coefficient obtained from DA revealed that the elements Ba, Rb and Mn have the biggest impact on the discrimination between groups. Both PCA and DA agree that Ba and Rb have a big influence on the discrimination between the groups.

MANOVA analysis found no significant differences between the Auburn samples and the rest of the samples ($p=0.9004$), and between the West Alabama and Macon Mississippi samples ($p=0.1434$). However, catfish from Macon, MS are clustered separately from Stoneville, MS (~169 miles apart from each other) and were statistically different (MANOVA, $p<0.0001$). Yet, Stoneville and West Alabama were not statistically different from each other ($p=0.1036$).

Overall, these results indicate that elemental profiles can be used to distinguish catfish based on country of origin, and may be useful within the same country depending on the geographic proximity, or more accurately the underlying geology and source water (more on this below). Also PCA and DA tend to give the same elements that are deemed to influence the discrimination between groups. The exact order of influence may vary slightly.

3.4.3 Effect of source-water on elemental composition in fish muscle tissue

To evaluate the role of source-water chemistry on the elemental composition in catfish muscle tissue, 15 fish from Macon, MS were compared to 15 fish from Stoneville, MS. The fish from both sites were similar in size and age (estimated to be 2-3 years old, weight from 500 g to 1450 g), and were fed with same diet (28% protein, Delta Western Feed Mill).

Simple Pearson correlation between the fish weight and the elemental concentration in fish tissue showed no significant relationship (r^2<0.36; p>0.08 for all elements). Elemental composition was further evaluated using PCA and Hierarchical Cluster Analysis (HCA) to evaluate grouping tendency of samples. To avoid over-fitting of data, MANOVA and sum contrasts were used instead of DA to test for statistical significance between the two groups. MANOVA and sum contrasts assume independence among response variables and thus are appropriate for examining differences between groups when the number of observations does not greatly exceed the number of response variables. T-tests were also used to determine which elements were influential in distinguishing the groups.

PCA showed the two groups clustered separately from each other with minimal overlap (Figure 3.11). Catfish from Stoneville, MS were clustered on the upper and lower left side of the quadrant while catfish from Macon, MS occupy the other half of the plot. The loading plot (Figure 3.11) showed that catfish from Macon, MS have higher concentration of Cs, Rb, and Sr. While the rest of the elements are higher in Stoneville, MS. The elements responsible for the variance explained in Principal Component 1 are Co, Cu, V, and Zn. These elements played an important role in distinguishing catfish form Macon and Stoneville, MS since the two groups are clustered separately along Component 1.

Results from PCA were further confirmed and strengthened by HCA, where samples grouped based on geographic source (Figure 3.12). MANOVA of the sum of responses showed that the two groups are statistically different from each other (p = 0.0002). Overall least squares means from MANOVA revealed that the elements, V, Co, Mn, and Cu have a big influence on the discrimination between these two groups. In addition, assessment of t-ratio also revealed that these same elements are important in distinguishing the groups.

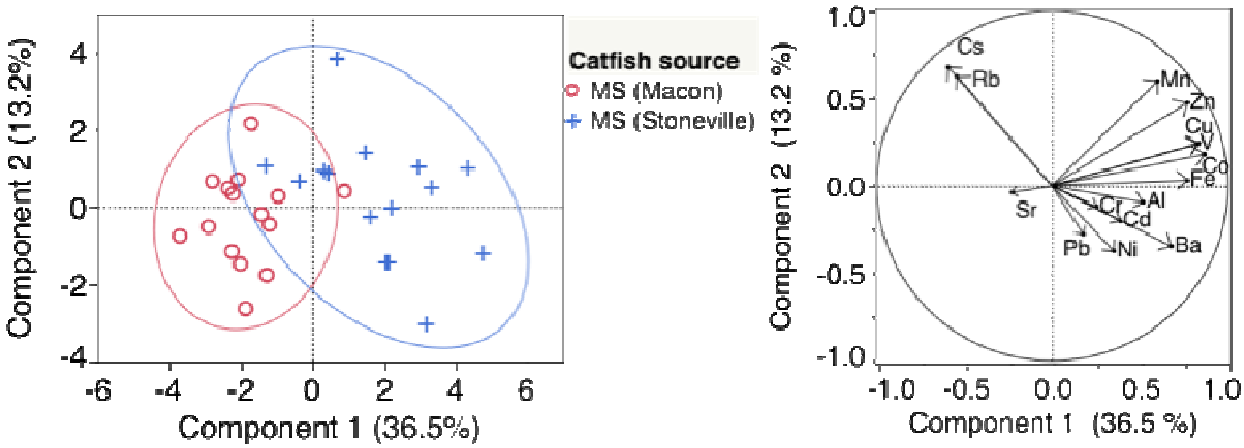

Figure 3.11. PCA score showing 95% density ellipse (left) and loading plot (right) of elemental profiles in catfish from Macon and Stoneville, MS

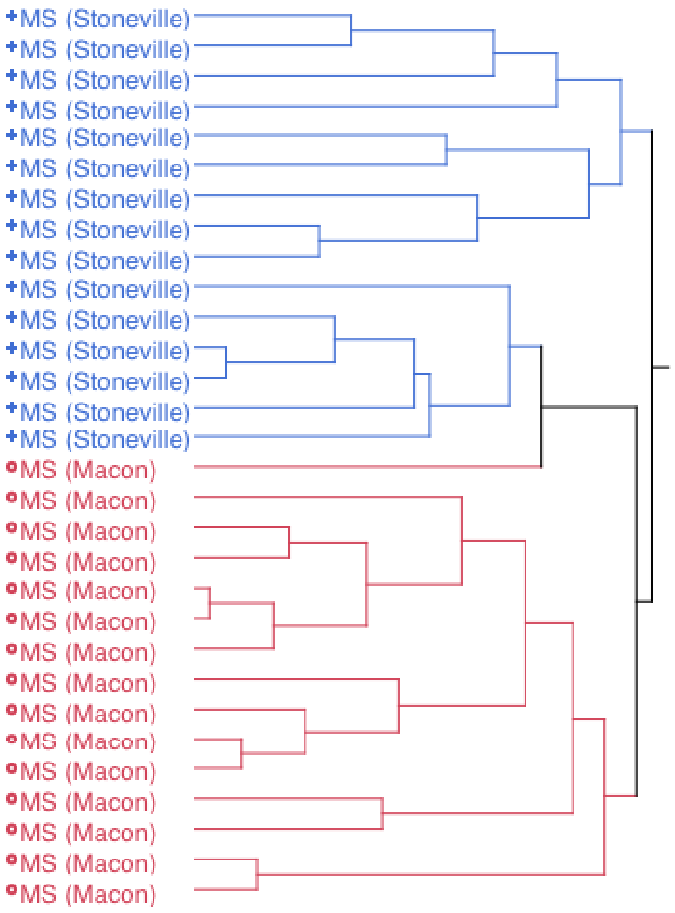

Figure 3.12. Dendogram obtained from HCA for elemental fingerprints catfish muscle tissue from Stoneville and Macon, MS

PCA, MANOVA, and T-tests all show that V, Co, and Cu have a big impact in discrimination between the two groups. In addition, PCA and MANOVA also highlight Zn and Mn (respectively) as elements that contribute toward the discrimination between groups. A box-whisker plot shows that these elements are higher in the Stoneville samples (Figure 3.13). Together this suggests that water chemistry plays an important role in the accumulation of metals in fish muscle tissue.

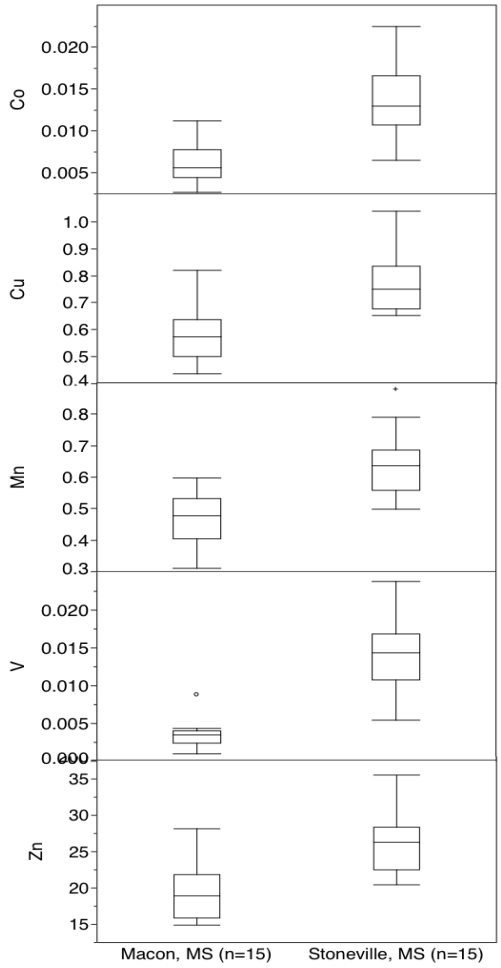

Figure 3.13. Box-Whisker plot showing the concentration (µg/g) of Co, Cu, Mn, V, and Zn in fish muscle tissue in catfish from Macon and Stoneville, MS. These elements were shown to have an important role in the discrimination between catfish from these two sites. The center horizontal line represents the median, the outer lines of the box give the 25 and 75 percentiles, the extreme outer horizontal lines provide the range, and symbols are individual outliers.

The elemental composition of the water from the two sites is summarized in Table 3.4. Data was compared and evaluated using simple Pearson correlations. It should be noted that DA not suitable due to the limited number of samples obtained. Figure 3.14 compares the concentrations of 11 elements in both the water and fish-tissue for both Stoneville and Macon sites. There was a statistical difference in the concentration of Ba and Rb between the two pond waters, and the differences are also reflected in the tissue concentrations. Cobalt and Mn also show the trend but it was not significant. Simple Pearson Correlations of elemental concentration in fish tissue versus elemental concentration in pond water shows that Ba, Co, Fe, Mn, and Rb have positive correlations, while Al, Co, Cr, Cu, Ni, Sr and V have negative correlations. Copper showed a significant relationship in the concentration between fish muscle tissue and pond water ($r=-0.87$; $p=0.03$) (Table 3.5). As previously discussed PCA, t-ratio MANOVA of the elemental profiles of muscle tissue showed that Cu, in addition to V and Co, play an important role in discriminating fish muscle tissue based on the geographic source

These results suggest that the source water, and thus the geographic origin of the fish, influence the elemental composition in catfish muscle tissue. In an aquatic environment, it is the combination of several variables (e.g. water, diet, environment condition) that affect the elemental concentration in catfish tissue. Some of these effects are directly or indirectly related to concentration; both dietary and waterborne sources affect the amount of metals accumulated in fish tissues [44-46]. A study by Cretì et al. (2010) showed that dietary food is the main source of Cd in tissue, while Pb in tissue is not of food origin [45].

Since the aquatic environment is not a closed system and is a complex aggregation of organism and microorganisms, it is hard to pinpoint which factors have the biggest influence on the accumulation of metals in catfish tissue. To fully understand the effect of source-water

chemistry on the elemental composition in fish muscle tissue, beyond the scope of this work, requires further research using a controlled environment where several variables are monitored and controlled.

Table 3.4. Elements in water from catfish ponds (ng/g); Del=Stoneville; EMP=Macon

Elements	Del 125	Del 126	Del 129	EMP1	EMP2	EMP3
Ba	88.5	91.4	93.263	51.728	37.985	62.224
Rb	1.26	1.32	1.195	5.056	7.638	7.658
Al	29.3	93.3	40.634	1283.797	43.691	524.279
Cr	0.142	0.128	0.14	2.085	0.097	1.072
Mn	1.95	3.26	80.122	1.431	5.793	2.974
Fe	47.4	90.0	79.978	464.463	123.742	361.401
Co	0.304	0.510	0.791	0.181	0.178	0.36
Ni	2.27	2.28	2.805	0.956	1.385	1.719
Cu	0.470	0.478	0.403	0.942	1.61	1.557
Sr	69.4	73.0	70.129	73.494	86.667	65.822
V	1.22	2.16	1.60	2.72	1.17	1.87
Zn	1.25	0.692	0.846	1.336	0.84	0.866

Table 3.5. Simple Pearson correlations of the elemental concentration in fish-muscle versus elemental concentration in pond water

Element	r	p value
Ba	0.409	0.421
Rb	0.694	0.126
Al	-0.417	0.411
Cr	-0.268	0.608
Mn	0.366	0.76
Fe	-0.609	0.200
Co	0.687	0.131
Ni	0.362	0.481
Cu	-0.872	0.024
Sr	0.031	0.953
V	-0.292	0.575
Zn	-0.268	0.608

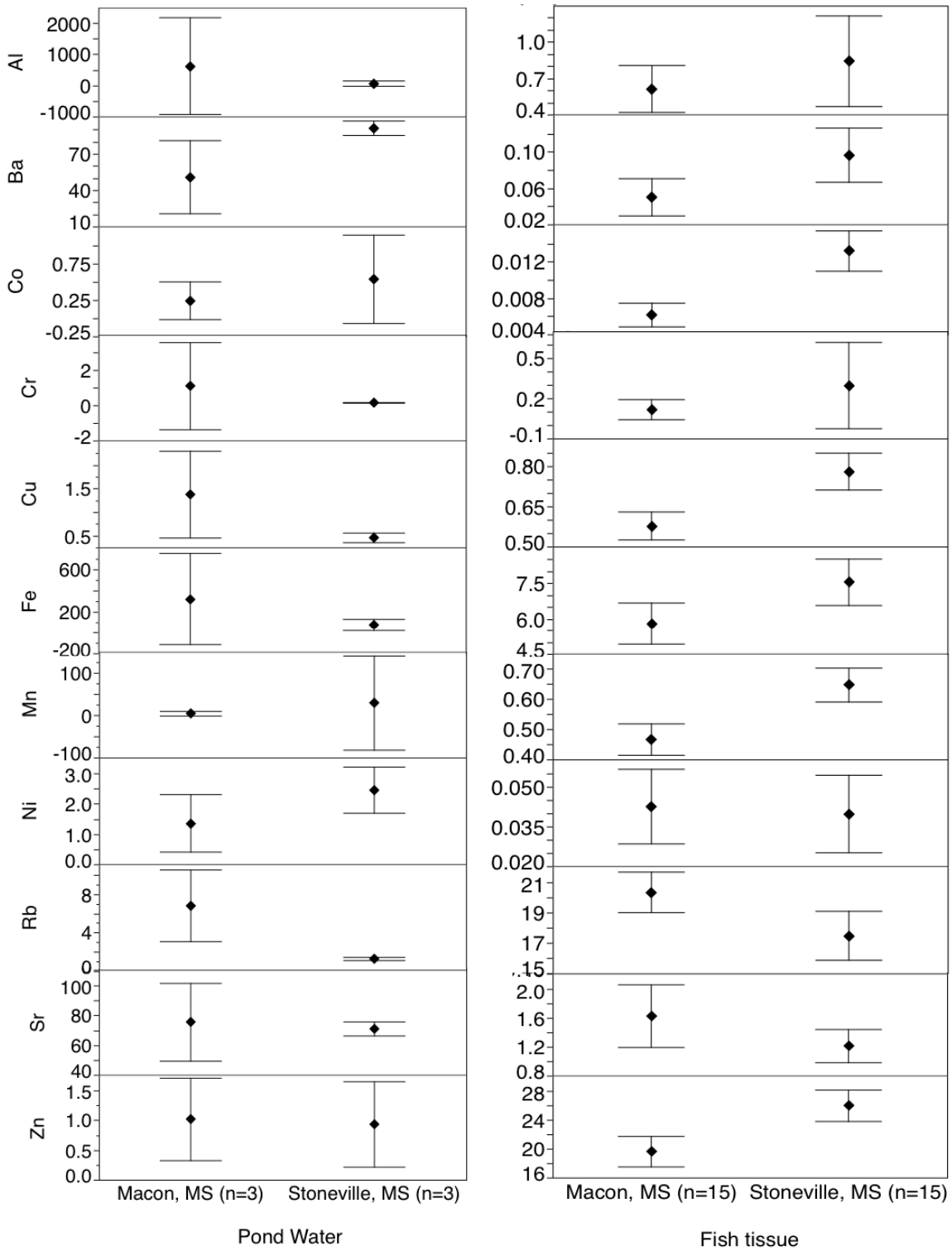

Figure 3.14. Plot showing the mean (diamond) and Confidence Interval (95%) of the concentration for each elements obtained in catfish muscle tissue (µg/g) and pond water (ng/g).

3.4.4 Strontium Isotope Ratio of Bone Samples

Sr isotope ratios in fish bones have been used to study the origins and movements of fish because ratios vary among water bodies due to underlying geology and weathering of rocks (Kennedy 2000). The Sr ratios in fish bones are strongly correlated with the corresponding ratio in ambient water, with water not food being the primary factor (Walther Thorrold 2006).

The $^{87}Sr/^{86}Sr$ ratios determined in bones of catfish from Stoneville and Macon, MS are shown in Figure 3.15. The $^{87}Sr/^{86}Sr$ ratios from Stoneville are higher than Macon, 0.70842 – 0.71027 (mean = 0.70945) and 0.70817 – 0.70939 (mean = 0.70859), respectively. Despite the presence of a few outliers, a two-tailed T-test gave a p-value < 0.0001, suggesting that the $^{87}Sr/^{86}Sr$ ratios in the bones of catfish from Stoneville and Macon are statistically different. Whereas the analysis of Sr isotope ratios is not a technique that would be commonly used for the purposes of provenancing catfish, it does highlight that the two source waters are different and thus can impart a different signal in the fish that can be used for fingerprinting purposes.

There was no significant relationship between the $^{87}Sr/^{86}Sr$ in the bones and weight of the fish (r^2 = -0.286; p = 0.125), nor between the $^{87}Sr/^{86}Sr$ ratios and muscle tissue (p-value = 0.793). Thus the ratios are not a function of fish size (age) but rather the sources of Sr to the fish, including water and diet.

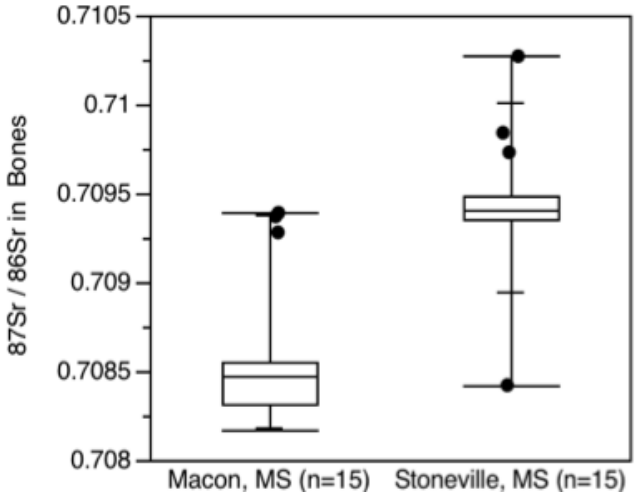

Figure 3.15. A Box-Whisker plot of $^{87}Sr/^{86}Sr$ ratios in catfish bones from two farms in Mississippi

3.5 ACKNOWLEDGMENTS

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3.6 LITERATURE CITED

[1] Hanson, T. R. Catfish Farming in Mississippi. Mississippi History Now. **2006** http://mshistory.k12.ms.us/articles/217/catfish-farming-in-mississippi

[2] Catfish Production Report. National Agriculture Statistics Service (NASS), Agriculture Statistics Board, U.S. Department of Agriculture. Washington D.C. **2010**

[3] Brunson, M. W., and Mott, D. F. A Historical Perspective of Catfish Production in the Southeast in Relation to Avian Predation. *Eastern Wildlife Damage Control Conferences*. **1995**

[4] Price, I. M. and Nickum, J.G. Aquaculture and birds: the context for controversy. In J. V. Hurter, ed. Management of fish eating birds on fish farms: A symposium. National Aquaculture Association/National Audubon Society. **1993**, pp 6-18

[5] Harvey, D. Aquaculture Outlook. Economic Research Service, U.S. Department of Agriculture, Washington, D.C. 20036-5381. **1998**

[6] Catfish Processing Report. National Agricultural Statitsics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. Washington, D.C. **2001**

[7] Catfish Processing Report. National Agricultural Statitsics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. Washington, D.C. **1998**

[8] Martin, M.F. U.S.-Vitenam Economic and Trade Relations: Issues for 111th Congress. Congressional Research Service Report for Congress. 2009

[9] Link, J. E. Mandatory Country of Origin Labeling of Beef, Pork, Lamb, Chicken, Goat Meat, Wild and Farm-raisedFish and Shellfish, Perishable Agricultural Commodities, Peanuts, Pecans, Ginseng, And Macadamia Nuts. *Federal Register*. **2009**, Vol. 74 No. 10

[10] Anderson, K.A., and Smith, B.W. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. *J. Agric. Food Chem*. **2005**, 53, 410–418

[11] Anderson, K.A. et al. Determining the geographic origin of potatoes with trace metal analysis using statistical and neural network classifiers. *J. Agric. Food Chem*. **1999**, 47, 1568-1575

[12] Smith, R.G., Watts, C.A. Determination of the Country of Origin of Farm-raised Shrimp (Family Penaeide) using trace metal profiling and multivariate statistics. *J. Agric. Food Chem*. **2009**, 57, 8244-8249

[13] Keppler, F.; and Hamilton. T.G. Tracing the geographical origin of early potato tubers using stable hydrogen isotope ratios of methoxyl groups. *Isotopes in Environmental and Health Studies* **2008**, 44(4), 337-347

[14] Busetto, M.L.; Moretti, V.M.; Moreno-Rojas, J.M.; Caprino, F.; Giani, I.; Malandra, R.; Bellgamba, F.; and Guillou, C. Authentication of Farmed and Wild Turbot (*Psetta maxima*) by Fatty Acid and Isotopic Analysis Combined with Chemometrics. *J. Agric. Food Chem*. **2008**, 56, 2742 – 2750

[15] Camin, F.; Bontempo, L.; Heinrich, K.; Horacek, M.; Kelly, S.D.; Schlicht, C.; Thomas, F.; Monahan, F.J.; Hoogerwerff, J.; and Rossmann, A. Multi-elements (H, C, N, S) stable isotope characteristics of lamb from different European regions. *Anal. Bioanal. Chem.* **2007**, 389 pp

[16] Ehlringer, J.R., Casale, J.F., Lott, M.J., Ford, V.L. Tracing the Geographical Origin of Cocaine. *Nature*. **2000**, 408

[17] Besacier, F. et al. Isotopic Analysis of 13C as a Tool for Comparison and Origin Assignment of seized heroin samples. *J. Forensic Science*. **1997**, 42 (3), 429 – 423

[18] Bommer, P. et al Determination of the Origin of Drugs by Measuring Natural Iosotope Contents: D/H and 13C/12C Ratios of Some Diazepan Samples. *Z. Natuforsch* **1976**, 31c.

pp. 111 – 114

[19] Renou, J.P., et al. Characterization of Animal Products According to Geographic Origin and Feeding Diet Using Nuclear Magnetic Resonance and Isotope Ratio Mass Spectrometry. Part II. Beef Meat. *Food Chem*. **2004**, 86 pp 251 – 256

[20] Palhol, F., et al. 15N/14N Isotopic Retio and statistical analysis: an efficient way of linking seized Ecstacy tablets. *Anal. Chim. Acta* **2004**, 510 pp 1 - 8

[21] Oda, H., Kawasaki, A., Hirata, T. Determining the Geographic origin of brown-rice with isotope ratios of 11B/10B and 87Sr/86Sr. *Anal. Sci*. **2001**, 17 pp. 1627 - 1630

[22] Crittenden, R.G.; Andrew, A.S.; LeFournour, M.; Young, M.D.; Middleton, H.; and Stockmann, R. Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *International Dairy Journal* **2007** 17, 421–428

[23] Rodrigues, C.; Máguas, C.; an Prohaska, T. Strontium and oxygen isotope fingerprinting of coffee beans and its potential to proof authenticity of coffee. *Eur. Food Res. Technol*. **2011**, 232, 361 – 373

[24] Asfaha, D.G.; Quétel, C.R.; Thomas, F.; Horacek, M.; Wimmer, B.; Heiss, G.; Dekant, C.; Deters-Itzelsberger, P.; Hoelzl, S.; Rummel, S.; Brach-Papa, C.; Bocxstaele, M.; Jamin, E.; Baxter, M.; Heinrich, K.; Kelly, S.; Bertoldi, D.; Bontempo, L.; Camin, F.; Larcher, R.; Perini, M.; Rossmann, A.; Schellenberg, A.; Schlicht, C.; Froeschl, H.; Hoogewerff, J.; Ueckermann, H. Combining isotopic signatures of n(87Sr)/n(86Sr) and light stable elements (C, N, O, S) with multi-elemental profiling for the authentication of provenance of European cereal samples. *Journal of Cereal Sciences* **2011**, 53, 170 – 177

[25] Zampella, M.; Quétel, C.R.; Paredes, E.; Asfaha, D.G.; Vingiani, S.; and Andamo, P. Soil properties, strontium isotopic signatures and multi-element profiles to authenticate the

origin of vegetables from small-scale regions: illustrations with early potatoes form southern Italy. *Rapid Commun. Mass Spectrom.* **2011**, 27, 2721 – 2731

[26] Montgomery, J.; Evans, J.A.; and Wildman, G. 87Sr/86Sr isotope composition of bottled British mineral waters for environmental and forensic purposes. *Applied Geochemistry* **2006**, 21, 1626–1634

[27] Wong, E.H.-K.; Hanner, R.H. DNA barcoding detercts market substitution in North American Seafood. *Food Amercian Research International* **2008**, 41, 828-837

[28] Shackell, G.H.; Mathias, H.C.; Cave, V.M.; and Dodds, K.G. Evaluation of microsatellites as a potential tool for product tracing of ground beef mixtures. *Meat Science* **2005**, 70, 337–345

[29] Alves, E.; Fernández, A.I.; Fernández-Rodríguez, A.; Pérez-Montarelo, D.; Benitez, R.; Óvilo, C.; Rodríguez, C.; and Silió, L. Identification of mitochondrial markers for generic traceability of European wild boars and Iberian and Duroc pigs. *Animal* **2009**, 3, 1216-1223

[30] Herrero, B.; Vieites, J.M.; Espiñeira, M. Authentication of Atlantic salmon (Salmo salar) using real-time PCR. *Food Chemistry* **2001**, 127, 1268–1272

[31] Franke, B.M.; Haldimann, M.; Reimann, J.; Baumer, B.; Gremaud, G.; Hadorn, R.; Bosset, J.; Kreuzer, M. Indications for the applicability of element signature analysis for the determination of the geographic origin of dried beef and poultry meat. *Eur. Food Res. Technol.* **2007**, 225, 501–509

[32] Marcos, A., Fisher, A., Rea, G., Hill, J. Prelimniary Study Using Trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J. of Anal. Atomic Spectrometry.* **1998**, 13 pp. 521 – 525

[33] Anderson, K.A., et al. Determining geographic origin of potatoes with trace metal analysis

using statistical and neural network classifiers. *J. Agric. Food Chem*. **1999**, 47 pp. 1568-1575

[34] Smith, R.G. Determination of the Country of Origin of Garlic (Allum sativum) using trace metal profiling. *J. Agric. Food Chem*. **2005**, Vol 53 pp. 4041-4045

[35] T.L Wellborn and C.S. Tucker. An Overview of Catfish Culture. In: Channel Catfish Culture: Developments in Aquaculture and Fisheries Science, Ed: C.S Tucker. Elsevier Science Publishers Publishing Company, Inc. New York, NY USA, **1985**

[36] F. A. Chapman. Farm-raised Channel catfish. Circular 1052, Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, **2009**

[37] M.H. Beleau. High Density Culture System. In: Channel Catfish Culture: Developments in Aquaculture and Fisheries Science, Ed: C.S Tucker. Elsevier Science Publishers Publishing Company, Inc. New York, NY USA, **1985**

[38] C.E. Boyd. Hydrology and Pond Construction. In: Channel Catfish Culture: Developments in Aquaculture and Fisheries Science, Ed: C.S Tucker. Elsevier Science Publishers Publishing Company, Inc. New York, NY USA **1985**

[39] J.W. Jensen. Watershed Fish Production Ponds: Site Selection and Construction. L-2405 Southern Regional Aquaculture Center Publication No. 102, **1989**

[40] C.S. Tucker and C.E. Boyd. Water Quality. In: Channel Catfish Culture: Developments in Aquaculture and Fisheries Science, Ed: C.S Tucker. Elsevier Science Publishers Publishing Company, Inc. New York, NY USA, **1985**

[41] Anderson, K.A.; Hobbie, K.A.; Smith, B.W. Chemical Profiling with Modeling

Differentiates Wild and Farm-Raised Salmon. *J. Agric. Food Chem*. **2010**, Vol 58 pp. 11768 – 11774

[42] Arribére, M.A.; Ribeiro Guevara, S.; Bubach, D.F.; and Vigliano, P.H. Trace Elements as Fingerprint of Lake of Provenace and of Species of Some Native and Exotic Fish of Northern Patagonian Lakes. *Biological Trace Element Research* **2006**, 111, 71 – 95

[43] Favretto, L.; Favretto, L.G.; Marletta, G.P.; and Saitta, M. Principal Component Analysis: A Chemometric aid for classification of polluted and unpolluted mussels. *Anal. Chim. Acta* **1989**, 220, 135-144

[44] Jezierska, B. and M. Witeska. The Metal Uptake and Accumulation in Fish Living in Polluted Waters. Soil and Water Pollution Monitoring, Protection and Remediation, **2006**, 3,107-114

[45] Cretì, P., F. Trinchella and R. Scudiero. Heavy metal Heavy metal bioaccumulation and metallothionein content in tissues of the sea bream Sparus aurata from three different fish farming systems, Environ Monit Assess, **2010**, 165, 321–329

[46] Miller, P.A., R.P. Lanno, M.C. McMaster, D.G. Dixon. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne copper tolerance in rainbow trout. Can J Fish Aquat Sci 50, **1993**, 1683-1689

[47] Spry, D.J, P.V. Hodson, C.M. Wood. Relative contributions of dietary and waterborne zinc in the rainbow trout. Can J Fish Aquat Sci., **1988**, 45-32

[48] Elnabris, K.J., S.K. Muzyed, N.M. El-Ashgar. Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). Journal of the Association of Arab Universities for Basic and Applied Sciences, **2013**, 13, 44–51

[49] Campbell, K. R. Concentrations of heavy metals associated with urban runoff in fish living in storm water treatment ponds, Arch. Environ. Contam. Toxicol. **1994**, 27, 352–356

[50] Kidwell, J. M., Phillips, L. J., and Birchard, G. F. Comparative analyses of contaminant levels in bottom feeding and predatory fish using the national contaminant biomonitoring program data, Bull. Environ. Contam. Toxicol. **1995**, 54, 919–923.

[51] Ouellet, J.D., M.G. Dubé, S. Niyogi. Influence of elevated alkalinity and natural organicmatter(NOM) on tissue-specific metal accumulation and reproductive performance in fathead minnows during chronic, multi-trophic exposures to a metal mine effluent. Ecotoxicology and Environmental Safety, **2013**, 95, 104–112

[52] Zhou, T., P. Weis, and J.S. Weis. Mercury burden in two populations of Fundulus heteroclitus after sublethal methylmercury exposure, Aquat. Toxicol. **1998**, 42, 37–47.

[53] Linde, A. R., P. Arribas, S. Sanchez-Galan, E. and Garcia-Vazquez. Eel (Anguilla anguilla) and brown trout (Salmo trutta) target species to assess the biological impact of trace metal pollution in freshwater ecosystems, Arch. Environ. Contam. Toxicol. **1996**, 31, 297–302.

[54] Zhou, J. L., S. M. Salvador, Y. P. Liu, and M. Sequeria. Heavy metals in the tissues of dolphins (Delphinus delphis) stranded on the Portuguese coast. Sci. Total Environment. **2001**, 273, 61-76.

[55] Kock, G., M. Triendl, and R. Hofer. Seasonal patterns of metal accumulation in Arctic char (Salvelinus alpinus) from an oligotrophic Alpine lake related to temperature, Can. J. Fish. Aquat. Sci., **1996**, 53,780–786.

[56] Yang, H. N., and H.C. Chen. Uptake and elimination of cadmium by Japanese eel, Anguilla japonica, at various temperatures, Bull. Environ. Contam. Toxicol. **1996**, 56, 670–676.

[57] Faure, G.; and J.L Powell. The Geochemistry of Rubidium and Strontium. In *Strontium*

Isotope Geology; Springer-Verlag: Berlin, Germany, **1972**; pp. 1-8

[58] Stewart, B.W.; R.C. Capo; O.A Chadwick. Quantitative strontium isotope models for weathering, pedogenesis and biogeochemical cycling. *Geoderma* **1998**, 82, 173-195

[59] Knudson, K.J.; T.D. Price; J.E. Buikstra; and D.E. Blom. The Use of Strontium Isotope Analysis to Investigate Tiwanaku Migration and Mortuary Ritual In Bolivia and Peru. *Archaeometry* **2004**, 46, 5-18

[60] Gilli, A.; D.A. Hodell; G.D. Kamenov; and M. Brenner. Geological and archaeological implications of strontium isotope analysis of exposed bedrock in the Chicxulub crater basin, northwestern Yucatán, Mexico. *Geology* **2009**, 37, 723-726

[61] Martin, J.B.; P.J. Moore. Sr concentrations and isotoperatios as tracers of ground-water circulation in carbonate platforms: Examples from San Salvador Island and Long Island, Bahamas. *Chemical Geology* **2008**, 249, 52-65

[62] Almeida, C.M.R.; and M.T.S.D. Vasconcelos. Multi-Element Composition And 87Sr/86Sr Of Wines And Their Potentialities As Fingerprints Of Wine Provenance. *Ciência Téc. Vitiv*. **2003**, 18, 15-27

[63] Kawasaki, A.; H. Oda; and T. Hirata. Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Sci. Plant Nutri*. **2002**, 48, 635-640

[64] Rodrigues, C.; and C. Máguas. Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authenticity of coffee. *Eur. Food Res. Technol*. **2011**, 232, 361-373

[65] Wolff, B.A.; B.M. Johnson; A.R. Breton; P.J.Martinez; D.L. Winkelman. Origins of invasive piscivores determined from the strontium isotop ratio (87Sr/86Sr) of otoliths. *Can. J. Fish. Aquat. Sci*. **2012**, 69, 724-739

[66] Kennedy, B.P.; C.P. Chamberlain; J.D. Blum; K.H. Nislow; and C.L Folt. Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **2005**, 62, 48-57

[67] Capo, R.C.; B.W. Stewart; and O.A. Chadwick. Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* **1998**, 82, 197-225

[68] Price, T.D.; L. Manzanilla; and W.D. Middleton. Immigration and the Ancient City of Teotihuacan in Mexico: a Study Using Strontium Isotope Ratios in Human Bone and Teeth. *Journal of Archaeological Science* **2000**, 27, 903–913

[69] Munro, A.R.; T.E. McMahon; J.R. Ruzycki. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yelloswtone Lake. *Can. J. Fish. Aquat. Sci*. **2005**, 62, 79-87

[70] Schweissing, M.M.; G. Grupe. Stable strontium isotopes in human teeth and bone: a key to migration events of the late Roman period in Bavaria. *Journal of Archaeological Science* **2003**, 30, 1373–1383

[71] Macdonald, N.S.; R.E. Nusbaum; R. Stearns, F. Ezmirlian, C. McArthur; and P. Spain. The skeletal Deposition of non-radioactive strontium. *The Journal of biological chemistry* **1951**, 188, 137-43

[72] Schweissing, M.M.; G. Grupe. Stable strontium isotopes in human teeth and bone: a key to migration events of the late Roman period in Bavaria. *Journal of Archaeological Science* **2003**, 30, 1373-1383

[73] Price, T.D. Immigration and the Ancient City of Teotihuacan in Mexico: a Study Using Strontium Isotope Ratios in Human Bone and Teeth. *Journal of Archaeological Science*

2000, 27, 903–913

[74] SAS Institute Inc. JMP® 10 Basic Analysis and Graphing, Second Edition. Cary, NC: SAS Institute Inc., **2012**

[75] McCune, B. and Grace, J.B. Analysis of Ecological Communities. MGM Software Design. U.S.A. **2002**, pp 205 – 210

CHAPTER FOUR

ELEMENTAL FINGERPRINTING OF SOILS USING ICP-MS AND MULTIVARIATE STATISTICS

(Reidy, L.; Bu, K.; Godfrey, M.; Cizdziel, J.V. Forensic Science International 233 (2013) 37–44)

4.1 ABSTRACT

Students in an instrumental analysis course with a forensic emphasis were presented with a mock scenario in which soil was collected from a murder suspect's car mat, from the crime scene, from adjacent areas, and from more distant locations. Students were then asked to conduct a comparative analysis using the soil's elemental distribution fingerprints. The soil was collected from Lafayette County, Mississippi, USA and categorized as sandy loam. Eight student groups determined twenty-two elements (Li, Be, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cs, Ba, Pb, U) in seven samples of soil and one sample of sediment by microwave-assisted acid digestion and inductively coupled plasmamass spectrometry (ICP-MS). Data were combined and evaluated using multivariate statistical analyses. All eight-student groups correctly classified their unknown among the different locations. Students learn, however, that whereas their results suggest that the elemental fingerprinting approach can be used to distinguish soils from different land-use areas and geographic locations, applying the methodology in forensic investigations is more complicated and has potential pitfalls. Overall, the inquiry-based pedagogy enthused the students and provided learning opportunities in analytical chemistry, including sample preparation, ICP-MS, figures-of-merit, and multivariate statistics.

4.2. INTRODUCTION

Recently it was reported that the application of elemental fingerprinting of soil provided critical supporting evidence for the judicial trial in a murder case that occurred in Italy [1]. The current study, designed as an experiment for an instrumental analysis course with a mixture of undergraduate and graduate students, many of whom are forensic chemistry BS majors, was adapted from that report. The experiment and mock forensic scenario, detailed below, was structured to be hands-on and capture student interest. Students acquired geochemical data (soil metal concentrations) and applied chemometrics (multivariate statistical analyses) to evaluate, for themselves, the potential of soil elemental signatures to assist forensic investigations. The objectives were to: (1) allow students to apply geoscience principles to the forensic arena, (2) reduce student's ''black box'' mentality toward analytical instrumentation, (3) provide students with experience in sample preparation, inductively coupled plasma mass spectrometry (ICP-MS), and multivariate statistical data analysis, including principal component analysis (PCA) and linear discriminant analysis (DA), and (4) use an inquiry-based lab exercise to give students learning opportunities in soil comparative analysis, including philosophical approaches and the potential pitfalls of using geoforensic evidence. Results herein are from the data that the students themselves generated.

4.2.1 Basis for soil elemental fingerprints and potential use in forensic studies

The underlying premise for using trace physical evidence in forensic science is based on (1) the ‘‘locard exchange principle’’, that says there will be cross-transfer of material as a result of physical contact, and (2) the examination and characterization of physical evidence to associate a suspect to the victim, the crime scene, or other material evidence [2–4]. Soil and sediment (when collected and analyzed following a strict protocol) have the potential to provide such trace evidence in forensic investigations because its composition is largely site-specific [1,3–6]. Soil has thousands of different varieties worldwide and is highly complex. Its mineralogy and elemental distribution varies depending on the underlying parent source material (rock), age and weathering of deposits (climate), topography, land use, local pollution, and other factors [7–9]. Other properties of soil that make it suitable as trace physical evidence include high retention and transferability on materials, and ease of collection, separation, and characterization [3]. The basic idea is that because soil particles are readily transferred (often as mud) from the ground to shoes, tires, mats, clothing, and other objects, it is plausible that the material can be used as a source of comparative samples in a forensic investigation. Geoforensic studies dating back to 1904 [10] have taken advantage of the different physical [11–13], chemical [14,15], and biological [16–18] characteristics of soil.

However, it is important to note (especially to forensic students conducting the lab exercise), that there are potential pitfalls in using soil elemental fingerprinting in forensic studies, including mixing of material not related to the forensic event and that the transfer of material varies with physical characteristics, such as grain type and size and organic content [4,6,19–21]. For footwear, mixing of soil from different sources occurs across the shoe sole and through time, and sequential layering of soil in and across a shoe sole may not be preserved [4]. Moreover,

retention and deposition of soil in footwear varies depending on its grain size and type and on organic content [19–21]. This adds complexity because elemental geochemical signatures can vary with particle size; compared to the bulk material, the small fraction (<150 mm) provides less variation and retains most of the inherent elemental information, thus it is recommended for elemental fingerprinting purposes [8].

Thus, when using soil as trace physical evidence in a comparative analysis, several factors have to be considered, including the representativeness of the sample, the analytical technique used, and the philosophical approach used for data interpretation [6]. It is essential that the forensic analyst understands the use and limitation of analytical technique, and whether the methodology has taken into account the homogeneity, representativeness, and spatial variation of the geoforensic evidence [4].

Regarding the philosophical approach, it is imperative that it is clarified when presenting and interpreting results [22]. Forensic geoscience and its sister disciplines have different ways to interpret data. Forensic geoscientists believe that similarity of samples does not mean that the samples came from the same source. Their approach requires exclusion rather than matching samples or scenes. Forensic geoscientists have to prove that a sample is excluded from having been derived from the same source [22]. On the other hand, other geological disciplines usually try to match and classify samples based on their similarity [22].

It is also worth mentioning that elemental fingerprints are routinely used for provenance of plants and animals. The trace element composition of plant materials and products from animals that graze or consume plants as a nutrition source, is known to be affected by the substrate (soil) in which the plant is grown. Watling and others have taken advantage of this in various forensic (provenance) cases, including foodstuffs [23].

4.2.2 Inquiry- and project-based pedagogy

Forensic science investigators require a variety of skills, including gathering reliable data and properly interpreting the results. Students preparing for a career in forensic science need to acquire technical and non-technical skills that are necessary for good scientific practice, such as problem solving, critical thinking, experimental design, data analysis, collaboration, and effective written and oral communication [24, 25]. For analytical chemistry and instrumental analysis courses, laboratory exercises can be an effective medium in acquiring these skills. One successful approach is the inquiry-based learning method which teaches science as practiced by scientists and which makes students take an active role in going through complex reasoning processes to solve a problem [26, 27].

Inquiry-based laboratories can be effective in helping students gain understanding on how science is done in the ‘‘real-world’’ and can either be open-inquiry or guided laboratories [26–28]. In guided-based inquiry, the teacher decides on the scientific problem and help students decide on the experimental method to solve a problem [27, 28]. In open-inquiry laboratories, student designs the experiment from finding a scientific problem to data gathering and analysis with little help from the teacher [27]. It teaches students that there are a variety of ways to solve a problem and that there may not be a single ‘‘correct’’ approach [29]. However, for this method to be effective, students need to have sufficient knowledge before tackling an inquiry problem. Lack of experience and knowledge can lead to poor results [29]. In guidedbased inquiry, students who lack experience are provided with more directions and help. In both cases the laboratories encourage critical thinking and self-reliance.

4.2.3 About the course: some logistics and information for instructors

The course, Advanced Instrumental Analysis (CHEM 512) at the University of Mississippi, typically has 25 students, with a mixture of undergraduate and graduate students. The majority of the students are BS forensic chemistry majors. Briefly, the course was designed to teach students about modern chemical analysis using advanced instrumental techniques, and to provide students opportunities to engage in experiments using select instruments. The goal is to provide students a thorough understanding of not only the theory and principles of analytical instruments but also of their capabilities, limitations, applications, and some practical aspects of sample analyses. After completing the course, students are expected to understand the analytical chemist's approach to problem solving, which includes defining the problem from a scientific point of view, choosing the analytical method, proper sampling and sample preparation, performing the measurement, conducting quality assurance and obtaining figures-of-merit, analyzing and interpreting the data, writing reports, and presenting results.

The course has both lecture and laboratory components. Students meet three hours a week for the lecture; most of the discussions on theoretical underpinnings of the ICP-MS, microwave digestion methods, and chemometrics occurred during this time. The students also meet in small groups for laboratory exercises. Lab schedules are flexible but each lab is to be finished within a specific time frame. Under the guidance of a teaching assistant each student group spent one lab session (3–4 h) preparing their soil samples (see Section 2). Another lab session was used to show the ICP-MS components and demonstrate its operation. Although students were allowed to operate the instrument under supervision during the demonstration, the actual analysis of their digests were done in a single run by a trained technician after combining all of the group's solutions.

4.2.4 About the laboratory exercise: introducing students to ICP-MS and chemometrics in a forensic- and inquiry-based experiment

Following an inquiry-based pedagogy, students are presented the following mock scenario. A murder took place and the victim was found near a muddy cotton field. The suspect's car mat was found to contain large clumps of dried mud. Subsequently, soil was collected from the crime scene, as well as adjacent areas, more distant agricultural fields, and control sites (outside the state) to compare with the dried mud (soil) from the car. Students try to answer the following question: Based on soil geochemical (elemental) signatures, can the soil from the car be excluded with that of the crime scene (body disposal site)? Their primary tools are ICP-MS, to measure multiple elements in the soil, and multivariate statistics, to find patterns within the data. The subsections below briefly introduce the major analytical, instrumental, and statistical analysis concepts that the students are expected to learn, including figures-of-merit, sample preparation, ICP-MS, and multivariate statistics. Details of the methodology are presented in Section 4.3.

Students worked in eight groups of three to carry out the experiment and generate the data, but were required to submit their own individual reports. We found that partnering undergraduate students with graduate students provided an interesting dynamic that may encourage some undergraduates to consider graduate school in sciences. Moreover, partnering students saves supplies and teaches teamwork. Students are required to determine several important figures-of-merit, performance characteristics of an analytical method, including accuracy (using a certified reference material), precision (replicate analysis), and method detection limits (by analyzing a series of blanks).

4.2.5 Sample preparation

Too often students have to rely on non-tactile ways (e.g., textbooks) to become familiar with sample preparation techniques, which are often the most-time consuming and error-prone part of analytical measurements. For ICP-MS, samples are analyzed typically as liquids. For solids such as soils, a total-dissolution or a strong-acid leach followed by filtering and dilution of the digest with deionized water is common. Compared to open-beaker digestions, microwave assisted acid digestion can minimize loss of volatile elements (e.g., Hg, Se, As, Cd) and decrease the likelihood of contamination because the sample is sealed in a Teflon vessel. Moreover, higher temperatures and pressures can be attained speeding digestion of refractory materials. In this study, students gain hands-on experience using microwave-assisted acid digestion. Because students prepare the samples themselves as a team, they become ‘‘invested’’ in the outcome. An alternative to dissolution methods is direct analysis by laser ablation (LA) ICPMS. This approach is advantageous because it eliminates the use of acids, generates no waste, and provides spatially resolved information; however, quantitation can be a challenge due to the lack of standards, and precision is generally worse than solution-based methods.

4.2.6 ICP-MS

ICP-MS is a powerful multi-element atomic spectrometric technique used to determine trace elements and isotope ratios in a wide-variety of sample matrices. The students were instructed to review the basic theory of ICP-MS in their instrumental analysis textbook; there are also several good introductory books on the subject [30]. Briefly, liquid samples are typically introduced into the ICP-MS using a nebulizer which creates an aerosol that is swept through a spray chamber (to remove large droplets) into an argonbased plasma. There some of the sample’s

atoms are converted to ions which enter the mass spectrometer through two small orifices. The positive ions are focused into the mass analyzer where they are separated by their mass-to-charge ratio, and are subsequently counted using an electron multiplier or faraday detectors. The resulting mass spectra can be used for both qualitative and quantitative analyses. Because of the large class size (24) and high number of samples to be analyzed, samples were collected from all groups and analyzed together. Raw ICP-MS intensity data (counts per second) and concentration data was provided to the students in spreadsheet format.

4.2.7 Multivariate statistics

Finding patterns in data and the interpretation of differences therein is a frequent task for the forensic chemist. ICP-MS analyses and elemental fingerprinting provides an excellent way to introduce students to the basics of multivariate statistics because the method can generate a tremendous amount of elemental and isotopic information. For instance, a single sample may be analyzed for more than 20 elements with a wide-range of concentrations and different sources for the elements. With so much data it is often difficult to observe patterns using standard statistical techniques that students are familiar with (e.g., Pearson correlations, t-tests). Multivariate statistical techniques, such as principal component analysis (PCA) and discriminant analysis (DA), are robust methods to assess the similarities and dissimilarities between samples and sample groups.

PCA is commonly used to evaluate complex dataset for patterns that can be seen visually as clustering of groups in a score plot, a projection of the data onto the axes of the two principal components. The technique uses a linear combination of the variables to reduce a data set with multiple variables to a smaller number of composite variables that represent most of the

information in the original data set. This is accomplished by determining the strongest linear correlation structure among variables [31].

DA is routinely used to summarize differences between groups, predict membership of sample points in a group, and to determine which elements are most responsible for the differences between groups. DA is an eigen-analysis technique that determines canonical axes that best discriminate between groups. It maximizes the among group variation with respect to the within group differences by determining the optimum linear combination of variables [31].

Importantly, these methods can provide statistical significance of differences in patterns, have a documented history, and are accepted by the scientific community provided the assumptions of the technique are met (in particular, multivariate normality and a sufficient number of observations). There are many forensic examples of classification of materials and evidence using multivariate statistics [32], including accelerants [33], currency [34], documents [35], drugs [36], fibers [37], and glass [38]. Whereas the ability to understand and interpret standard statistical tests has always been important to the forensic scientist, increasingly the same can be said for multivariate statistics. Students were provided access to a statistical software package and given three weeks to analyze the data and prepare their reports, which were to be in typical journal format and include assessment of accuracy, precision, and method detection limits.

4.3 METHODS

4.3.1 Soil sampling

Bulk samples of surface soil were collected from the top 5 cm of soil from a ~10 cm x~10 cm area using a plastic scoop and placed into Ziplock bags. Six different locations in Lafayette County, Mississippi were targeted for soil collection (Table 4.1 and Figure 4.1). The soil from each of the sites can be generally categorized as Chenneby silt loam from silty alluvium deposits [39]. Soil near Yocona River is occasionally flooded. From a forensic investigation standpoint, these multiple sites were selected to determine the local scale variability of the soil elemental fingerprint. To that end, three samples were collected from the same agricultural (cotton) field, designated the crime scene where the corpse was found, one in an adjacent forested area, at two others in cotton fields at more distant locations. Sample A was collected directly next to footprints at the crime scene. Samples B and C were collected nearby (~10 and ~50 m away) in the same agricultural field. A fourth sample was collected from a forested area bordering the cotton field where the victim's body was found to determine the effect of land use on the elemental composition of the soil. The remaining two samples (Ag1 and Ag2) were collected from cotton fields at more distant locations. Ag1 is located about 3 km to the east of the crime scene, and like samples A, B and C is within the Yocona River floodplain. Ag2 is located 30 km to the northwest away from the river. Other samples included NIST reference materials, SRM 8704 (Buffalo River sediment) and SRM 2709 (San Joaquin soil),

which, being from outside the state, served as ‘‘control’’ samples. The former was also used to determine recoveries. In addition, the students were provided a separate sample labeled ‘‘Car’’ and were told that it was taken from the mat of the suspect’s impounded car. For ‘‘Car’’ sample we used sample A for half of the students (groups 1–4) and sample Ag2 for the other half (groups 5–8).

Table 4.1. Sample information

Sample ID	Soil Type/Use	Description	Coordinates N	Coordinates W
A	Agriculture	“Crime scene” margin of cotton field	34.162180	89.312006
B	Agriculture	10m from A (inside cotton field)	34.161892	89.311958
C	Agriculture	50m from A (inside cotton field)	34.161583	89.312408
F1	Forest	Forest bordering cotton field	34.162180	89.311951
Ag1	Agriculture	Different cotton field ~3Km from A	34.170603	89.301271
Ag2	Agriculture	Different cotton field ~30Km from A	34.210717	89.385684
NIST	Sediment	SRM 8704,; Buffalo River, NY, USA	42.514287	78.520298
CaS	Agriculture	San Joaquin, CA, USA	120.15	36°30’
Car	Agriculture	“Suspect” sample (A or Ag2)*		

*Student “unknown” varied

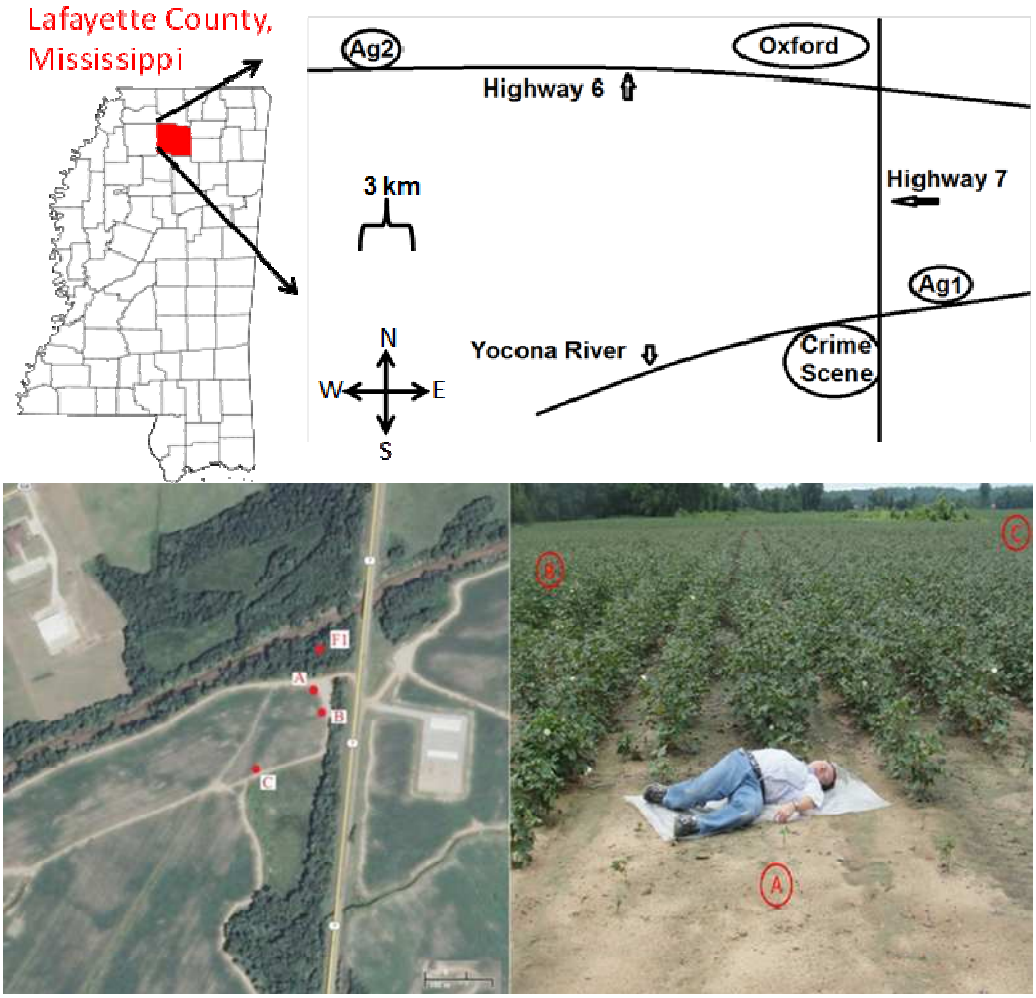


Figure 4.1. Map of study area (top) showing location of Lafayette County in Mississippi and proximity of sampling sites to Oxford, MS and the Yocona River. Arial view (bottom left) and photo (bottom right) of the mock crime scene showing sampling locations A, B, C and F and one of the authors (J.V.C.).

4.3.2 Sample preparation and microwave-assisted acid digestion

Samples were air-dried in a clean laminar flow hood, passed through a 1 mm sieve to remove stones, twigs and other debris, and transferred into labeled plastic bags. Students were instructed on laboratory safety protocols and the proper use of the microwave digestion system. About 0.25 g of sample was weighed to 0.1 mg and transferred into a digestion vessel. Digestion was carried out using an Ethos microwave digestion system (Milestone Inc. Shelton, CT, USA)

equipped with a 41-vessel (PFA) multi-prep rotor. The suspect (car) sample was determined in triplicate, other samples were single analyses. Three blanks were included in each analytical batch. In a fume hood, 4.5 mL HCl, 1.5 mL HNO3 and 1.5 mL H2O2 was added to each vessel (trace metal grade; Fisher Scientific). Students were instructed to allow reactions to subside before closing the vessels; HCl will react with carbonates in soil. The vessels were loaded into the microwave system and the samples brought to 180 8C in 20 min and held at that temperature for 40 min. Samples were allowed to cool to <50 ^{0}C before removal and opening in a hood. The solutions were passed through a 0.25 mm Teflon filter into a labeled 50 mL centrifuge tube and diluted to the 50 mL mark with de-ionized (>18.2 MΩ) water. The tubes were covered and gently mixed. 2.5 mL of each solution was pipetted into a labeled 15 mL centrifuge tube and diluted to 15 mL mark with DI water. The digests were stored at ~4 ^{0}C until analysis.

4.3.4 ICP-MS analyses

Quadrupole-based mass analyzers are the most common type ICP-MS instruments because they are robust, fast scanning, and relatively inexpensive. Sector field ('high resolution') ICP-MS, on the other hand, offers a much higher sensitivity and mass resolution, and allows many common polyatomic and isobaric interferences to be resolved (e.g., $^{40}Ar^{160+}$ on $^{56}Fe^{+}$, and $^{48}Ca^{++}$ and $^{12}C^{12}C^{+}$ and $^{48}Ti^{++}$ on $^{24}Mg^{+}$). Whereas both types are perfectly suited for elemental fingerprinting purposes, we used a sector field ICP-MS (Element-XR; Thermo-Fisher Scientific, Waltham, MA, USA). It employs a double-focusing magnetic sector design and is equipped with slit mechanism that allows three resolution settings: ''low'' ($m/\Delta m \approx 400$), ''medium'' ($m/\Delta m \approx 4000$) and ''high'' ($m/\Delta m \approx 10\,000$).

A glass concentric nebulizer with a cyclonic spray chamber was used for sample

introduction. The ICP-MS operational settings are summarized in Table 4.2. Before the samples were analyzed, the system was optimized for sensitivity, stability, and oxide levels. The following was achieved for 1 mg/kg 115In in low resolution mode: ~10 million counts per second; <3% RSD (short-term); and <5% oxides. For calibration, a series of standards ranging from 0.1 mg/kg to 40 mg/kg in 2% nitric were prepared from a multi-element standard (Spex Certiprep; Metuchen, NJ, USA). All the sample, blank, and standard solutions were spiked with 103Rh internal standard (2 mg/kg) using on-line addition.

Table 4.2. ICP-MS instrument settings

Plasma	
Cool gas flow	16 L min^{-1}
Auxiliary gas flow	0.9 L min^{-1}
Sample gas flow	1.19 L min^{-1}
RF power	1300 W
Data Acquisition	
Isotopes Monitored	LR: ^{7}Li, ^{9}Be, ^{85}Rb, ^{88}Sr, ^{133}Cs, ^{138}Ba, ^{208}Pb, ^{238}U MR: ^{24}Mg, ^{27}Al, ^{44}Ca, ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{62}Ni, ^{65}Cu, ^{66}Zn HR: ^{39}K
Mass window	20% for LR 150% for MR and HR
Integration (ms)	20 LR, 50 MR and HR
Runs/passes	3/2
Scan type	E-scan

4.3.5 Data analysis

Data was analyzed using JMP software1 10.0 (SAS; Cary, NC, USA). ICP-MS data were normalized using z-scores prior to statistical analysis to compensate for the varying ranges of elemental concentration. Results were emailed to the students as an Excel spreadsheet. All the data were used for the analysis, except that from group 1 (see below). Students then cut and paste

the data into JMP for PCA and DA analysis. Additionally, we used the PCA loading plot to select elements (orthogonal to each other) for a bivariate plot.

4.4 RESULTS AND DISCUSSION

4.4.1 Concentrations and figures-of-merit

Soil elemental concentrations are presented in Table 4.43. These results, obtained by one group, are typical of what was found by all groups, except one. One group had difficulty carrying out the experiment and had substantially higher concentrations, possibly a result of contamination or improper weighing. DA clearly showed that this group was an outlier among the eight groups (data not shown). Given that the student group was consistently the poorest performing group in the class and that their data showed high blank levels, the problem more likely stems from poor laboratory practice and not some fundamental limitation of the approach or technology. Because the data from this group were suspect, we did not include it in subsequent data analysis.

Students used a reference material (NIST SRM 8704; Buffalo River sediment) to gauge the accuracy of their elemental concentration data. For those elements with reference values, there were two categories: recoveries that were good (the majority of the elements ranged from 87% to 102%, for Fe, Mg, Cs, Cr, Co, Pb, Mn, Ni, and V) and recoveries that were moderate to poor (Al 72%, Ca 62%, K 67%, Ba 63%, Zn 134%, and U 46%). Certified values for this material are based on total-dissolution, not acid leaching so low recoveries are not that surprising. However, for elemental fingerprinting, it less critical to have accurate data than to have reproducible results obtained from methods that are applied consistently to all samples.

Table 4.3. Concentrations (mg/g, dry weight) of elements in soil determined by ICP-SFMS.

Sample ID	Li	Be	Rb	Sr	Cs	Ba	Pb	U	Mg	Al	Ca
A	5.04	0.292	17.6	10.3	0.722	77.9	12.3	0.69	775	13000	426
B	7.62	0.592	24.7	16.1	1.18	113	12.9	1.07	1170	20100	746
C	4.91	0.292	16.3	12.0	0.709	80.2	10.2	0.61	842	14300	461
Ag1	5.16	1.04	17.2	13.3	1.62	81.4	9.42	1.52	909	13600	820
Ag2	3.55	0.123	13.8	10.4	0.401	57.2	22.8	1.38	693	13000	754
F1	7.04	0.518	24.5	33.1	1.18	203	20.2	0.52	1700	19700	1830
Cas	66.1	1.31	54.2	124	4.37	500	13.6	1.84	13700	42400	9880
NIST (8704)	51.8	1.79	82.1	83.5	5.11	259	143	1.43	10600	44100	16300
Car	5.15	0.345	17.6	11.5	0.750	85.8	13.1	0.78	875	14700	475
Car duplicate	5.21	0.367	18.9	12.3	0.811	90.3	13.3	0.84	935	15400	512
Car triplicate	5.53	0.405	18.8	12.0	0.853	88.6	14.0	0.88	939	15200	507
Car Average	5.30	0.370	18.5	11.9	0.800	88.2	13.5	0.84	916	15100	498
Car SD	0.200	0.03	0.73	0.40	0.05	2.25	0.46	0.05	35.5	342	20.3
Car RSD (%)	3.85	8.17	3.98	3.37	6.45	2.56	3.40	5.72	3.88	2.27	4.08

Sample ID	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	K	As	Se
A	23.7	9.90	276	7540	3.81	5.44	0.200	30.5	1890	7.31	0.7
B	35.8	18.3	429	10800	5.65	8.89	1.94	43.5	2650	10.5	1.43
C	26.4	11.7	440	7920	5.21	5.54	0.49	29.7	2040	6.34	1.12
Ag1	29.3	11.7	321	8190	3.85	5.61	1.69	20.9	2190	1.31	1.23
Ag2	26.6	18.4	165	8720	2.5	6.13	0.79	36.9	1510	2.70	1.17
F1	35.6	16.5	634	11100	5.18	9.83	1.62	52.1	2450	2.33	1.36
Cas	107	111	523	33500	12.6	83	24.0	130	6650	20.2	2.24
NIST (8704)	88.5	106	553	39900	12.9	38.4	72.5	546	13400	18.5	3.45
Car	26.5	12.3	301	8190	4.13	6.29	0.78	33.9	2030	7.78	0.26
Car duplicate	28.6	15.7	315	8680	4.37	7.65	1.23	36.8	2050	8.10	0.48
Car triplicate	27.7	12.8	325	8670	4.57	6.70	0.97	35.5	2020	8.54	<DL
Car Average	27.6	13.6	313	8510	4.36	6.88	1.00	35.4	2030	8.14	0.37
Car SD	1.03	1.85	12.1	281	0.22	0.70	0.23	1.49	14.7	0.38	0.15
Car RSD (%)	3.73	13.7	3.86	3.30	5.08	10.1	22.6	4.22	0.72	4.65	42

To estimate precision, each group analyzed the soil from the suspect's car three times. Precision for individual elements was generally <10% relative standard deviation (RSD). Higher values were found for elements with concentrations near the detection limit such as Se, Cu, and Cr. Method detection limits (3 sigma criteria) were estimated by replicate analyses of reagent

blanks carried through the entire analytical process. Detection limits ranged from 0.1 mg/kg for U to 20 mg/kg for Fe.

4.4.2 Soil elemental fingerprints, pattern recognition, and forensic implications

Preliminary evaluation of data was conducted by plotting the sum of normalized concentration of all elements using Box–Whisker plot (Figure 4.2). Two groups (CaS and NIST) were clearly different, with much higher sums that do not overlap with the rest of the groups. These two ‘‘outliers’’ are soil from California and sediment from the Buffalo River; the remaining groups are all soils from Lafayette County, Mississippi. Among the Mississippi soils, the forest soil (F) had elevated concentrations compared to the others, which were agricultural soils. Figure 4.2 also shows some differences among the agricultural samples, but this more clearly observed in the results from the multivariate analysis.

Sample grouping tendency was further evaluated using PCA, DA, and a bivariate plot. DA and PCA describe, graphically, the relationship among groups and individual samples using linear combination of variables. DA maximizes the separation of groups while PCA maximizes the variation among the individual points [30]. The use of multivariate statistical analysis techniques for discriminating forensic analytical chemical data has been the subject of a number of reports [31–36].

For the student-derived data in the current study, the PCA score plot showed that the Mississippi soil samples were clustered together, separate from NIST SRM 8704 and SRM 2709 (Figure 4.3). Examining only the Mississippi soil samples (Figure 4.4), the PCA score plot revealed three clusters: (1) Site Ag2 and Cars 5, 6, 7, and 8 (upper left); (2) Site A, B, C, and Cars 2, 3, and 4 (lower center); (3) Site F (center to upper right). Soils in the near range (A, B,

and C) showed similarities and were grouped together. The grouping of samples for Cars 2–4 with Ag2 and Cars 5–8 with site A was as expected because student groups 2–4 and 5–8 were given Ag2 and A, respectively, as their car mat samples.

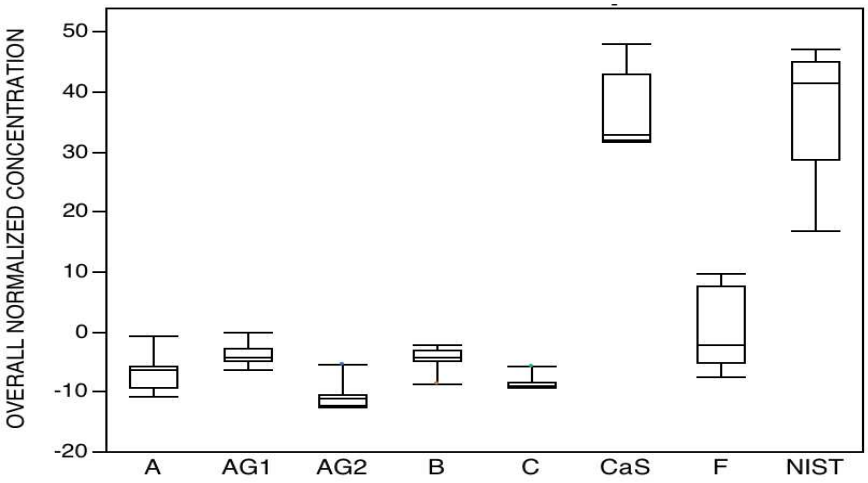


Figure 4.2. Box–Whisker plot of overall normalized elemental concentration in soil samples.

Interestingly, the PCA plot shows Ag1 overlapped with sites A, B and C, despite being ~3 km away. This serves to illustrate that caution should be employed when using elemental fingerprinting in forensic investigations for two reasons. First, it is feasible that relatively wide areas of soil may have comparable elemental profiles if the soil type and source material are similar. In this case, soil from both locations (Ag1 and A, B, and C) are similar because both stem from cultivated fields (cotton crop) that are adjacent to the Yocona River, falling in its flood plain/depositional basin. Second, PCA alone may not be the best tool for data discrimination purposes. Indeed, DA canonical plots showed much better discrimination despite the similarities previously mentioned (Figure 4.5). In the DA plot Ag1 is clearly clustered separate from site A, B, and C. Even site C located about 50 m from the ‘‘crime scene’’ (site A) was separate from site A and B. Site B located a mere 10 m away overlapped with site A. Also,

the forest soil, which was collected mere meters from the adjacent agricultural field, had significantly different elemental patterns, showing that land use is a major factor in the discrimination and data clustering.

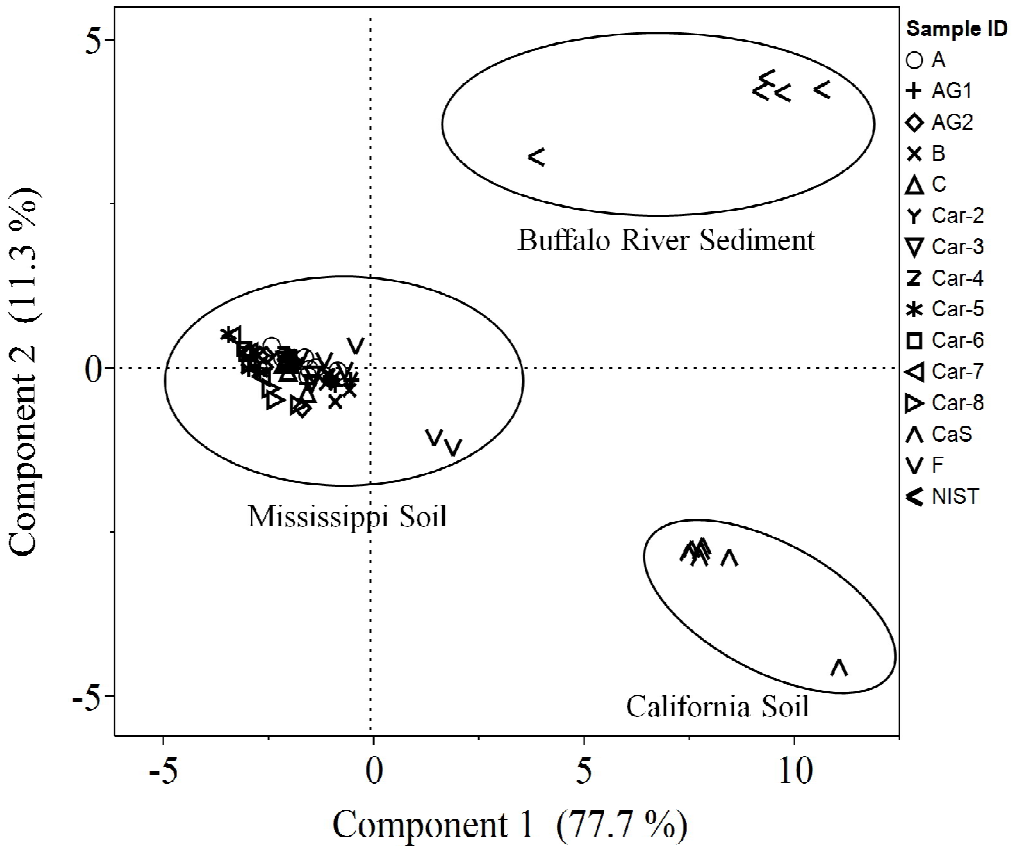


Figure 4.3. Plot for the first and second principal components using data for 22 elements determined by ICPMS for all soil samples.near range (A, B, and C) showed high

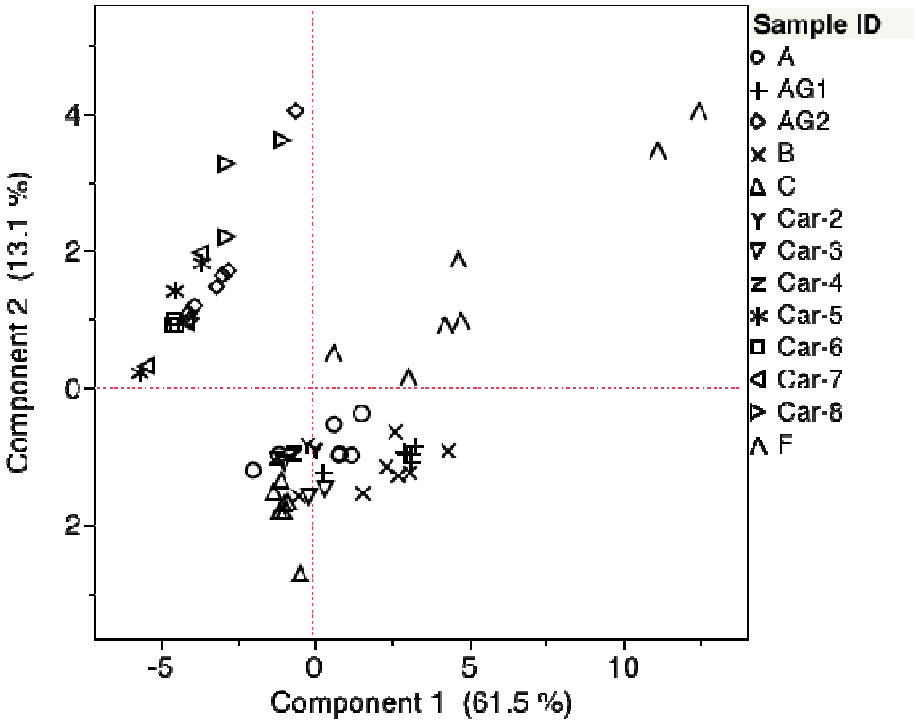
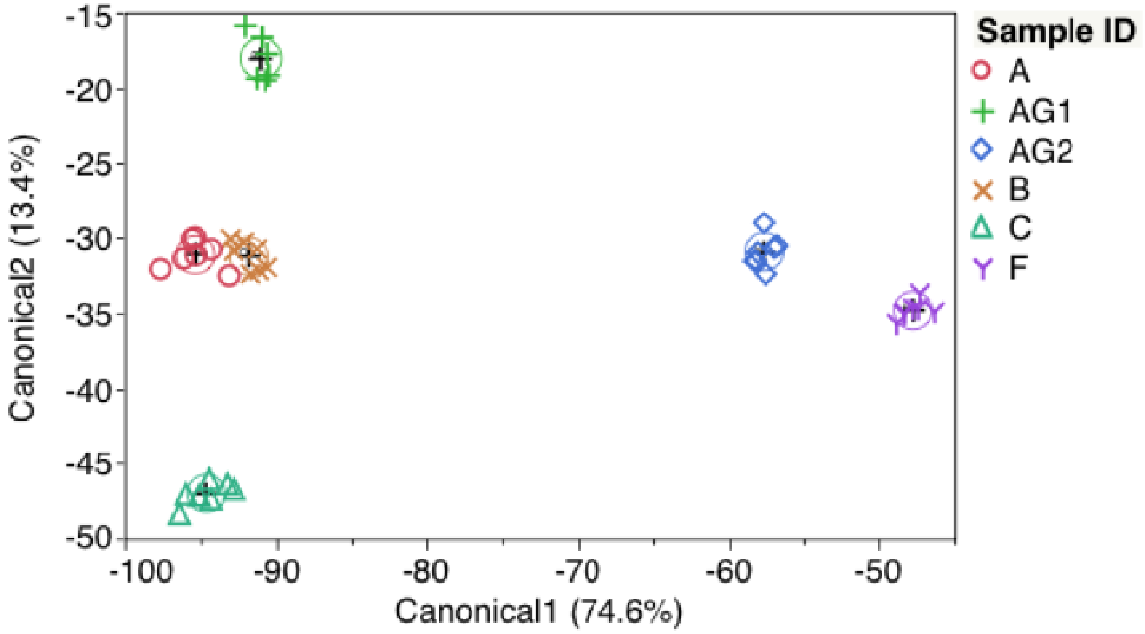


Figure 4.4. Plot for the first and second principal components using data for 22 elements determined by ICPMS for only the soil samples from Mississippi.



Figue 4.5. Discriminant analysis (DA) canonical plot using data for 22 elements determined by ICPMS for the Mississippi soils.

Yet another common approach for elemental fingerprinting involves the use of bivariate plots. The loading plot was used to select elements for the bivariate plot. A PCA loading plot shows the relationship between variables (elements) in the space of the first two components. Elements that are correlated will be grouped together and have arrows pointing in similar directions. The greater the length of the arrow, the stronger the influence the element has on the separation of the groups in the score plot. Here, elements Pb and V were chosen from the PCA loading plot because their arrows (vectors) had relatively high magnitudes and were orthogonal to each other (Figure 4.6). The lack of correlation between the Pb and V may partly reflect different sources for these elements in the samples. For Pb, a likely source is leaded gasoline, phased out in the 1970s. Soil collected near a busy roadway, in our study highways 6 and 7, will certainly contain Pb from vehicle exhaust. The Pb and V bivariate plot for the Mississippi soils showed the same clusters of groups observed in the PCA and DA analysis (Figure 4.7).

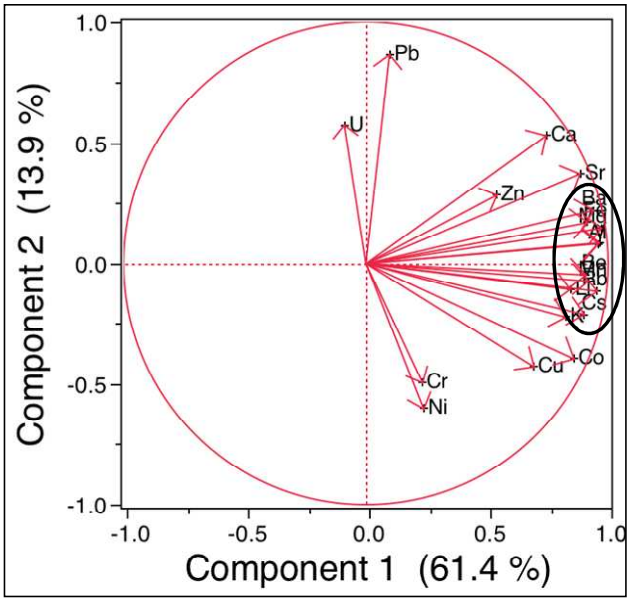


Figure 4.6. Principal component analysis (PCA) loading plot for elemental profiles for the Mississippi soil samples. Elements clustered in the circle along component 1 axis include V, Ba, Fe, Mg, Al, Be, Mn, Rb, Li, Cs, and K.

We wish to emphasize that the philosophy of forensic soil analysis is fundamentally different to that of conventional geological and soil analyses, for a several reasons, some of which were discussed in the introduction of this paper. Thus, applying soil elemental fingerprinting for forensic studies requires techniques of exclusion rather than inclusion [e.g., 24, and references therein]. Furthermore, databases for evaluation of soil comparative results, in many cases, do not exist, or are based on bulk samples, which may have different homogeneity and particle size distributions (and thus elemental patterns) compared to samples picked up by footwear [6,22]. In the current study, we essentially developed our own database, analyzing both near field and more distant samples from agriculturally similar fields. Whereas the results show that bulk soil from Lafayette County, Mississippi, can be effectively discriminated based on land-use and geographic location, attempting to link a suspect to a location based on soil elemental fingerprints would require additional scrutiny and lines of evidence, beyond the scope of this lab exercise, which in turn makes for good class discussion.

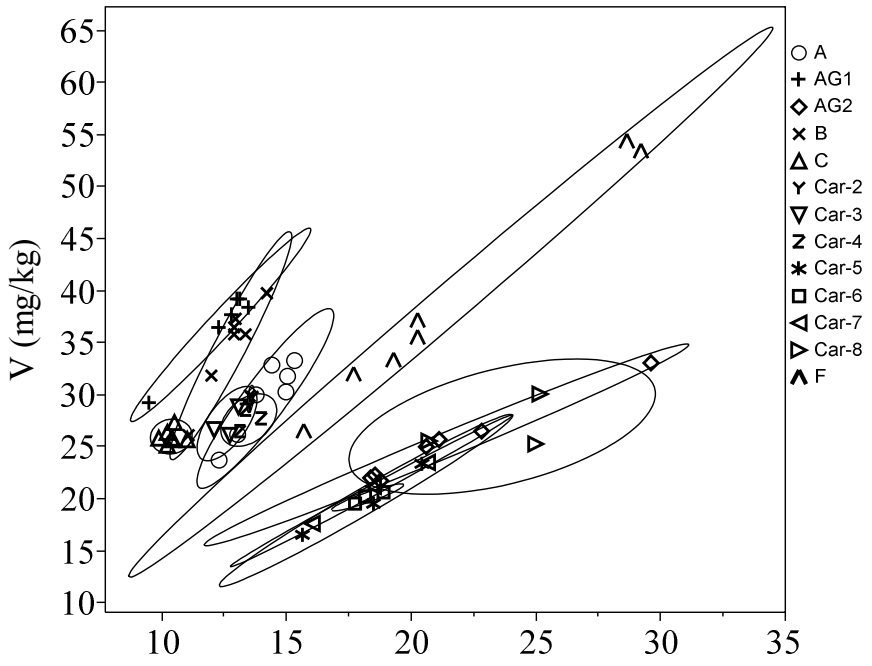


Figure 4.7. Bivariate plot of V vs. Pb for all soils. Ellipsoids represent 95% confidence intervals.

4.4.3 Using ICP-MS and multivariate statistics in an inquiry-based experiment

Following an inquiry-based pedagogy, students were required to evaluate elemental fingerprinting using ICP-MS and chemometrics as a forensic tool. The laboratory exercise is applicable to instrumental analysis courses (even without forensic majors) because it provides an opportunity to teach the principles and application of analytical methodology in a relevant and hands-on manner. The experiment can be tailored by the instructor introduce various analytical and forensic concepts to reduce the ‘‘black-box’’ mentality toward instrumentation. In the current study, we used it to address sample preparation strategies, figuresof-merit, and multivariate statistics, among other subjects.

Each of the student groups correctly classified their unknown ‘‘suspect sample’’ among the soils. This was accomplished despite the student groups working independently to generate their data, and then grouping data to be used in the statistical analysis. The low recoveries for some elements in the reference material served as a good lesson for students to read the certificate associated with reference materials for proper use. Students were asked to speculate possible reasons for the low recoveries and several answered that leaching is not equivalent to total-dissolution, which is what the reference data is based on.

From a pedagogical standpoint, the hands-on lab exercise, which included sample preparation using microwave digestion, analysis by ICP-MS, and analysis of raw data using multivariate statistics, gave students a greater understanding of a ‘‘holistic’’ analytical approach and reduced their ‘‘black box’’ mentality toward instrumentation and analysis. The lab also gave student opportunities to work in collaborative manner, compare their results to that of their peers, and enhance their communication skills through class discussions and written reports. In addition, by determining accuracy, precision, and detection limits, students gain a better

appreciation for key figures of merit.

Limitations to this approach may involve resources and time. Whereas ICP-MS is becoming more common in academic departments with forensic programs, not every department has access an instrument and microwave digestion unit. However, often an ICP-MS is available in geology departments or at neighboring institutions and open-beaker digestions are possible though not recommended because of safety and contamination concerns. The experiment also takes two lab sessions, one for sample preparation and the other for ICP-MS demo/analysis.

A formal class evaluation on this specific lab experiment was not conducted but will be done in the future. However, overall, students were enthused about the experience and had favorable comments, including: ‘‘This was the first time that I used multivariate statistics. Studying data that I helped generate allowed me to better understand how statistics is applied in real forensic scenarios’’ and ‘‘although the sample preparation was tedious it helped me appreciate the importance of contamination control and blank measurements’’.

4.5 CONCLUSIONS

This laboratory experiment introduces students, in an interesting forensic-based application, to: (1) sample preparation and the considerations therein, (2) analytical chemistry figures-of-merit, (3) the theory and application of ICP-MS, multivariate statistical methods, and elemental fingerprinting, and (4) the philosophy and practice of forensic soil analysis. The students determined the proportional distribution of elements in eight different soils using ICP-MS and examined the data using multivariate statistics. Each of the student groups correctly classified their unknown ‘‘suspect sample’’ among the soils. Results suggest that elemental fingerprinting of soil can be a reliable tool to distinguish soils from different land-use areas and geographic locations; however, application to forensic cases requires additional considerations highlighted herein. Students were enthusiastic about the experiment, which provided collaborative learning opportunities in analytical chemistry, including sample preparation using microwave digestion, analysis by ICPMS, figures-of-merit, and multivariate statistics.

4.6 ACKNOWLEDGMENTS

We thank the students in the University of Mississippi's Advanced Instrumental Analysis class (CHEM 512) for their insightful comments and suggestions on the experiment. The ICPMS used in this study was obtained through a NSF grant (Award #0923080).

4.7 LITERATURE CITED

[1] G. Concheri, D. Bertoldi, E. Polone, S. Otto, R. Larcher, A. Squartini, Chemical elemental distribution and soil DNA fingerprints provide the critical evidence in murder case investigation, PLoS ONE 6 (2011) 1–5.

[2] W.J. Chisum, B.E. Turvey, Evidence dynamics: locard's exchange principle and crime reconstruction, Journal of Behavioral Profiling 1 (2000) (accessed 20.06.13) http://www.profiling.org/journal/vol1_no1/jbp_ed_january2000_1-1.html.

[3] R.W. Fitzpatrick, M.D. Raven, S.T. Forrester, A systematic approach to soil forensics: criminal case studies involving transference from crime scene to forensic evidence, in: K. Ritz, D. Miller, L. Dawson (Eds.), Criminal and Environmental Soil Forensics, Springer Science + Business Media B.V., 2009 , pp. 105– 127.

[4] R.M. Morgan, J. Freudiger-Bonzon, K.H. Nichols, T. Jellis, S. Dunkerley, P. Zelazowski, P.A. Bull, The forensic analysis of sediments recovered from footwear, in: K. Ritz, D. Miller, L. Dawson (Eds.), Criminal and Environmental Soil Forensics, Springer Science + Business Media B.V., 2009, pp. 251–269.

[5] A. Ruffell, Forensic pedology, forensic geology, forensic geosciences, geoforensics and soil forensics, Forensic Sci. Int. 202 (2010) 9–12.

[6] B.G. Rawlins, M. Cave, In vestigating multi-element soil geochemical signatures and their potential for use in forensic studies, Geol. Soc. London Spec. Publ. 232 (2004) 197–206.

[7] J.D. Ingram, J.C. Lin, Geochemcial tracers of sediment sources to San Francisco Bay, Geology 30 (2002) 575–578.

[8] K. Pye, S.J. Blott, D.J. Croft, S.J. Witton, Discrimination between sediment and soil samples for forensic purposes using elemental data: an investigation of particle size effects, Forensic Sci. Int. 167 (2007) 30–42.

[9] K. Pye, D. Croft (Eds.),Forensic Geoscience: Principles, Techniques, and Applications Geol. Soc. London Spec. Publ. 232 (2004) 318.

[10] R.C. Murray, J.F. Tedrow, Forensic Geology: Earth Science and Criminal Investigation, Quinn and Boden Company, Inc., New Jersey, 1975, pp. 6–8.

[11] E.P. Junger, Assessing the unique characteristics of close-proximity soil samples: just how useful is soil evidence? J. Forensic Sci. 41 (1996) 27–34.

[12] N. Petraco, T. Kubic, A density gradient technique for use in forensic soil analysis, J. Forensic Sci. 45 (2000) 872–873.

[13] D.W. Janssen, W.A. Ruhf, W.W. Prichard, Use of clay for soil colour comparisons, J. Forensic Sci. 28 (1983) 773–776.

[14] J. Linderholm, E. Lundberg, Chemical characterization of various archaeological soil samples using main and trace elements determined by inductively coupled plasma atomic emission spectrometry, J. Archaeol. Sci. 21 (1994) 303–314.

[15] R.J. Cox, H.L. Peterson, J. Young, C. Cusik, E.O. Espinoza, Forensic analysis of soil organic by FTIR, Forensic Sci. Int. 108 (2000) 107–116.

[16] R. Bruce, M. Dettmann, Palynological analyses of Australian surface soils and their potential use on forensic science, Forensic Sci. Int. 81 (1996) 77–94.

[17] M. Horrocks, K.A.J. Walsh, Fine resolution of pollen patterns in limited space: differentiating a crime scene from an alibi scene seven metres apart, J. Forensic Sci. 44 (1999) 417–420.

[18] D.C. Mildenhall, Deer velvet and palynology: an example of the use of forensic palynology in New Zealand, Tuatara 30 (1988) 1–11.

[19] V. Chazottes, C. Brocard, B. Peyrot, Particle size analysis, particle size analysis of soils

under simulated scene of crime conditions: the interest of multivariate analyses, Forensic Sci. Int. 140 (2004) 159–166.

[20] V. Virtanen, H. Korpelainen, K. Kostamo, Forensic botany: usability of bryophyte material in forensic studies, Forensic Sci. Int. 172 (2007) 161–163.

[21] J. Horswell, S.J. Cordiner, E.W. Maas, T.M. Martin, K.B. Sutherland, T.W. Speir, B. Nogales, A.M. Osborn, Forensic comparison of soils by bacterial community DNA profiling, J. Forensic Sci. 47 (2002) 350–353.

[22] R.M. Morgan, P.A. Bull, The philosophy, nature, and practice of forensic sediment analysis, Prog. Phys. Geogr. 31 (2007) 43–58.

[23] R.J. Watling, G.S. Lee, C.J. Scadding, T.S. Pilgrim, R.L. Green, A.E. Martin, C.D. May, J.L. Valentin, The application of solution and laser ablation based ICP-MS and solution based AES for the provenance determination of selected food and drink produce, Open Chem. Biomed. Methods J. 3 (2010) 179–196.

[24] G. Adami, A new project-based lab for undergraduate environmental and analytical chemistry, J. Chem. Educ. 83 (2006) 253–256.

[25] W.W. Hope, L.P. Johnson, Urban air: real samples for undergraduate analytical chemistry, Anal. Chem. 72 (2000) 460A–467A.

[26] J. Handelsman, D. Ebert-May, R. Beichner, P. Bruns, A. Chang, R. DeHaan, J. Gentile, S. Lauffer, J. Stewart, S.M. Tilghman, W.B. Wood, Scientific teaching, Science 304 (2004) 521–522.

[27] M. Windschitl, Inquiry projects in science teacher education: what can investigative experiences reveal about teacher thinking and eventual classroom practice? Sci. Educ. 87 (2003) 112–143.

[28] C.M. Gliddon, R.J. Rosengren, A laboratory course for teaching laboratory techniques, experimental design, statistical analysis, and peer review process to undergraduate science students, Biochem. Mol. Biol. Educ. 40 (2012) 364–371.

[29] D.R. Marshall, N.L. Owen, A.E. Underhill, Analysis of ‘‘industrial waste’’: a quantitative laboratory project, J. Chem. Educ. 54 (1977) 584.

[30] R. Thomas, Practical Guide to ICP-MS: A Tutorial for Beginners, second ed., CRC Press, Boca Raton, 2008.

[31] B. McCune, J.B. Grace, Analysis of Ecological Communities, MGM Software Design, USA, 2002, pp. 205–210.

[32] S.L. Morgan, E.G. Bartick, Discrimination of forensic chemical data using multivariate statistics, in: R.D. Blackledge (Ed.), Forensic Analysis on the Cutting Edge: New Methods for Trace Evidence Analysis, John Wiley & Sons, New York, 2007, pp. 331–372.

[33] B. Tan, J.K. Hardy, R.E. Snavely, Accelerant classification by gas chromatography/ mass spectrometry and multivariate pattern recognition, Anal. Chem. Acta 422 (2000) 37–46

[34] M. Hida, H. Sato, H. Sugawara, T. Mitsui, Classification of counterfeit coins using multivariate analysis with X-ray diffraction and X-ray fluorescence methods, Forensic Sci. Int. 115 (2001) 129–134.

[35] A. Kher, M. Mulhooland, P. Maynard, Classification of document papers by infrared spectroscopy and multivariate statistical techniques, Appl. Spectrosc. 55 (2001) 1192–1198.

[36] C.S. Jonson, Amphetamine profiling-improvements of data processing, Forensic Sci. Int. 69 (1994) 45–54.

[37] L. Morgan, S.H. Hall, J.E. Hendrix, E.G. Bartick, Pattern recognition methods for the classification of trace evidence textile fibers from UV/visible and fluorescence spectra, in:

Proceedings of the FBI Trace Evidence Symposium, Clearwater, FL, 15 August 2007, 2007.

[38] R.D. Koons, C. Fiedler, R.C. Rawalt, Classification and discrimination of sheet and container glasses by inductively coupled plasma atomic emission spectrometry and pattern recognition, J. Forensic Sci. 33 (1998) 49–67.

[39] Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed (June 12, 2014).

CHAPTER FIVE

PROVENANCE OF EPHEDRA USING ELEMENTAL FINGERPRINTS AND MULTIVARIATE STATISTICS

5.1 ABSTRACT

Dietary supplements containing ephedra and its active ingredients, ephedrine alkaloids, became widely available in the United States to treat common colds and asthma, aid in weight loss, and enhance athletic performance. Due to stimulant properties of ephedrine alkaloids and its structural similarity to methamphetamine, manufacturer may sometimes add synthetic stimulants or synthetic ephedrine alkaloids to other plant material and ephedra's devoid with these constituents. Mislabeling, adulteration, and contamination of dietary supplements may potentially cause adverse effects to consumers. The ability to determine the authenticity of the dietary supplement and its botanical source is important for public safety. In the present work, we studied the feasibility of using elemental profiles and multivariate statistics to identify the species and determine geographical source of ephedra. A variety of ephedra species from United States and China were examined. Samples were microwave digested and fourteen elements (Ba, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr, U, V, and Zn) were determined using sector-field ICPMS. Principle Component Analysis, Multivariate Analysis of Variance, and Hierarchical Clustering Analysis show that elemental patterns are capable of discriminating ephedra from U.S. and China, and can distinguish *E. przewalskii* from the other ephedra species tested. However, the approach did not have the discriminatory power to separate species other than *E. przewalskii*.

5.2 INTRODUCTION

For thousands of years, plants have been used to treat illnesses and thus continue to play an important role in medical field [1]. Medicinal plants are used as complementary alternative medicine/traditional medicine, as botanical supplements, and as a source of lead compounds for drug development [2-4]. Today, approximately 40% of pharmaceutical products come from plants [5].

The demand for herbal medicine around the world is growing 5 to 15% annually [6]. In 2000, the Secretariat on the Convention on Biological Diversity reported $60 billion-sales worldwide [7]. The increased demands for medicinal plants for pharmaceutical industry, cosmetics, dietary supplements and other botanical products call for quality control measures. The quality of herbal medicine and other botanical products relies on the quality of the starting raw material. Correct identification and authentication of the plant is a critical step to obtain a reproducible quality of the product [8].

5.2.1 Ephedra

Ephedra has been used in China as Traditional Chinese Medicine for treatment of asthma or bronchitis. The main active ingredients in ephedra are ephedrine alkaloids [9]. It wasn't until 1926 that dietary supplements containing ephedrine alkaloids became widely available in the United States after it has been approved by American Medical Association for use in medicinal

purposes [10]. Ephedrine alkaloids can be derived from the raw botanicals and their extracts. The primary source of ephedrine alkaloids is the species *Ephedra sinica*. However, other ephedra species found throughout Eurasia also contain ephedrine alkaloids. These species include *E. equisitina, E. intermedia, E. distachya, E. alata, E.fragilis, E. botschantzevii, E. major, E. minuta, E. pachylada, E. monosperma, E. likiagensis, E. lomatolepis, E. saxatilis, E. lepidosperma, E. przewalskii,* and *E. regeliana*. Ephedrine alkaloids are considered to be absent in the ephedra species from the Americas [11]. Ephedrine alkaloids include ephedrine, pseudoephedrine, norephedrine, methylephedrine, mehtylpseudoephedrine, and norseudoepherine. The concentration of these alkaloids may differ between species but the predominant alkaloid is the ephedrine [12].

Ephedrine alkaloids belong to a large family of pharmacological compound called sympathomimetics, which mimics the effects of norepinephrine and epinephrine. Sympathomimetics can raise blood pressure and increase heart rate [12]. It was found that ephedrine have the same physiological, subjective and behavioral effect as amphetamine [13]. Despite the strong stimulant properties of ephedrine, ephedrine products are available over-the-counter to treat a variety of disorders including common colds and bronchial disorders such as asthma. Dietary supplements containing ephedrine alkaloids are used in the U.S. primarily as stimulants, aids in weight control and enhancers of athletic performance. Throughout the 1980s, ephedrine became a widely abused stimulant. In addition, misuse was prevalent after it gained popularity as a precursor to illicit production of methamphetamine after another precursor, 1-phenyl-2-propanone [14]. In the last decade, adverse events have been reported due to use and misuse of ephedrine and it's herbal source, ephedra [10]. The Food and Drug Administration (FDA) received over 18,000 adverse event reports (AER) in 2004 [12]. Ephedra products are

now tightly controlled and Federal regulation allows ephedrine to be available behind the counter [10]. The history of the use and misuse of ephedra products is summarized in Table 5.1.

5.2.2 Provenance and authentication of ephedra

Consumer misuse, manufacturer abuse and contraindication, hypersensitivity and/or drug interaction can cause adverse effects of ephedra [15]. Manufacturer abuse may include adding synthetic stimulants or synthetic ephedrine alkaloids to other plant material and ephedras devoid with these constituents [16]. Mislabeling, adulteration, and contamination of dietary supplements may potentially cause adverse effects to consumers and can be fatal [17]. To ensure the quality of dietary supplements available in the market, US FDA established Rule 21 CFR 111, which requires products to be accurately labeled and to not contain hazardous s contaminants [18]. This rule regulates current good manufacturing practices during the manufacture, labeling, and storage of herbal and botanical dietary supplements. The ability to determine the authenticity of the dietary supplement and its botanical source is important for public safety. Method to characterize the raw processed, and finished botanical products is necessary to verify authenticity of dietary supplements [17].

Table 5.1. Timeline of ephedrine use [10]

Timeline	Events
Early 1920s	Introduction of ephedrine to the US to treat ailments such as asthma
Late 1930s	Reports of false product claims started and ephedrine was grandfathered into the Food, Drug, and Cosmetics Act as a "safe" drug
1950s	Stimulant misuse became prevalent in the US
1960s	Ephedrine abuse was first documented
1970	Ephedrine wasn't included in the schedules of the newly introduced Controlled Substances Act. The amphetamine "look-alike" phenomenon started.
1980	Ephedrine became the new key ingredient to manufacture illicit methamphetamine
Early 1980s	Federal agencies had a hard time banning ephedrine-containing "look-alikes".
1994	The Dietary Supplement Health and Education Act (DSHEA) of 1994 classified ephedra, the herbal source of ephedrine, a dietary supplement and thus was not regulated
1995	FDA withdrew their proposal to limit ephedrine availability to prescription. Medical benefits were said to outweigh risks.
1998	FDA received 800 Adverse Event Reports (AERs) and proposed a ban on ephedra products, but the rule was not passed due to lack of evidence
2003	Ephedra become demonized by the media after Baltimore Orioles pitcher, Steve Bechler, died due to ingestion of ephedra products
2004	FDA banned ephedra products after they received 18000 AERs. Over-the counter ephedrine products are still available to the public without prescription
Late 2000s	FDA regulations allow ephedrine product to be available "behind the counter".

A number of methods, capillary electrophoresis [19], chiral gas chromatography [20], High Performance Liquid Chromatography (HPLC) [21-22], have been developed to quantitatively measure ephedrine alkaloids. These studies, however, were not geared towards

identifying authenticity of products. A number of methods have been developed and reported by Schaneberg, et al [15] and Joshi and Khan [23] to verify the identity of ephedra species. Schaneberg, et al [15] used chemical fingerprinting to characterize ephedra species using reverse phase high performance liquid chromatography with photodiode array detection. Joshi and also used macroscopic identification and light microscopy to verify authenticity of ephedra and ephedra products. Both methods were successful in detecting the presence of ephedra species in the ground plant material in dietary supplements and ephedra botanical products. Both methods were able to distinguish ephedra species from North America, South America, and Eurasia. Joshi and Khan (2005) also found out that leaf and internode lengths are major identifying characteristics of ephedra species [23].

5.2.3 Elemental Fingerprinting of Plants

Elemental profiling along with multivariate statistics to trace geographical origin of plant samples has been cited in the literature since the 1980s [24]. Nikdel et al used the method to determine the country of origin and detection of adulteration of orange juice [24]. Schwarts and Hecking determined geographic origin of agricultural products [25]. Anderson et al used trace metal profiling along with multivariate statistics and neural network classifier to determine geographic origin of potatoes [26]. Anderson and Smith studied geographic growing origins of coffee [27]. Samsoe-Petersen determined the uptake of trace elements and PAHs by fruits and vegetables from contaminated soils [28].

Many factors can affect the elemental concentration in plants. These factors include soil characteristic and environmental condition. Soil has thousands of different varieties worldwide and is highly complex. Its mineralogy and elemental distribution varies depending on the

underlying parent source material (rock), age and weathering of deposits (climate), topography, land use, local pollution, and other factors [29-31]. The elemental composition in soil will reflect the elemental composition in plants [32-33]. Uptake of elements by plants depends on the soil-plant interaction, which is influences by plant species, environmental condition, and the bioavailability of elements [34-36].

5.2.4 Purpose of the study

In the present study, a different approach was used to identify ephedra species and determine the geographical source of epehdra. Fingerprinting of ephedra was conducted using elemental profiles determined by ICPMS. The purpose was to evaluate the feasibility of and discriminatory power of elemental profiling for: (1) distinguishing ephedra based on geographical source; and (2) distinguishing ephedra based on species.

5.3 EXPERIMENTAL

5.3.1 Samples

Samples of ephedra, primarily consisting of stems or ground stems (Figure 5.1), were obtained from the National Center for Natural Products Research (NCNPR) Repository of Botanicals at the University of Mississippi. The origin of the plant material was the southwestern U.S. (Figure 5.2) and various provinces in China (Figure 5.3). The U.S. samples include the following species: *E. antisyphilitca, E. aspera, E. aspera trifurca, E. californica, E. coryi, e. cutleri, E. fasiculata, E. nevadensis, E. pedunculata, E. torreyana, E. trifurca, and E. viridis*. For China, the species include: *E. equisetina, E. intermedia, E. przewalskii, and E. sinica*. A summary of samples used in this study is given in Table 5.2.

Figure 5.1. Ephedra samples obtained from NCNPR

Figure 5.2. States in the U.S. where ephedra samples originated. Number represents the number of sample originated from that location.

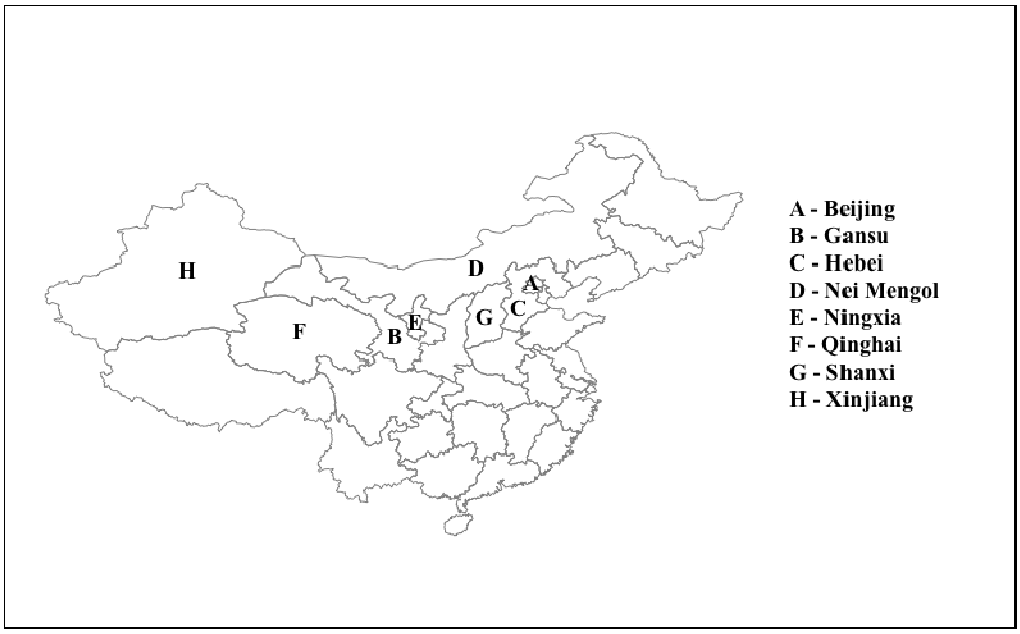


Figure 5.3. Provinces in China where ephedra samples originated.

Table 5.2. Summary of ephedra species collected from provinces in China and United States

Province	Species (number of samples)
Beijing	*E. intermedia* (1)*; E. sinica* (1)
Gansu	*E. intermedia* (7); *E. przewalskii* (1)
Hebei	*E. intermedia* (1); *E. sinica* (3)
Nei Mengol	*E. equisetina* (2); *E. intermedia* (1); *E. przewalskii* (4); *E. sinica* (4)
Ningxia	*E. intermedia* (4); *E. sinica* (4)
Qinghai	*E. intermedia* (2); *E. przewalskii* (1); *E. sinica* (1)
Shanxi	*E. intermedia* (1); *E. sinica* (2)
Xinjiang	*E. equisetina* (1); *E. intermedia* (1); *E. przewalskii* (1)
State	**Species (number of samples)**
Arizona	*E. fasiculata* (3); *E. nevadensis* (2)
California	*E. aspera* (1); *E. californica* (1) *E. nevadensis* (2); *E. viridis* (2)
New Mexico	*E. coryi* (1); *E. cutleri* (1); *E. nevadensis* (1); *E. torreyana* (1); *E. trifurca* (1)
Texas	*E. antisyphilitca* (1); *E. aspera* (1); *E. aspera trifurca* (1); *E. predunculata* (1); *E. trifurca* (3);

5.3.2 Sample Digestion and Preparation

Ephedra samples were ground using PM 400 Planetary Ball Mill (Retsch, Germany) at a speed of 400 rpm for 5 minutes and dried to constant weight in an oven at 70^0C. Moisture content of the samples was determined to be 7.68±0.90%. Concentration of elements is reported in dry-weight basis. Approximately 0.1 g of sample was weighed into an acid-washed Teflon (pfa) vessel and 5 mL of high purity concentrated HNO_3 (Optima, Fisher Scientific), 1 mL of 30% hydrogen peroxide (Optima, Fisher Scientific), and 2 mL DI water were added. The mixture was allowed to cold-digest for an hour. Digestion was completed using a closed-vessel microwave digestion system (Ethos; Milestone Inc., Shelton CT, USA) equipped with a multi-prep rotor (41 pfa vessels). The digestion program consisted of a 25-min ramp to 120^0C, 20-minute ramp to 160^0C, then 35 min ramp to 180^0C, where the temperature was held for 20 minutes. The resulting digest was transferred to an acid-washed polypropylene tube and diluted

to 50-mL with DI water. This solution was further diluted, 5-fold with DI water, to bring the concentration of HNO_3 to 2%.

5.3.3 Elemental Analysis using sector-field ICPMS

Digested sample was introduced into a high-resolution SF-ICPMS (Element-XR; Thermo Scientific) for multi-element analysis using concentric nebulizer with glass cyclonic spray chamber. An internal standard containing 1 ppb ^{103}Rh was added inline using a T-junction. The instrument was optimized prior to analysis for sensitivity and stability. Instrument and data acquisition parameters are given in Table 5.3. Elements, Ba, Cd, Pb, Rb, Sr, and U were analyzed in low resolution. Elements, Co, Cu, Fe, Mg, Mn, Ni, V, and Zn, were analyzed in medium resolution. Internal standard, ^{103}Rh, was used for all elements.

External calibration was used to quantify elements. A series of multi-element standards, ranging from 0.01 ng g^{-1} to 20 ng g^{-1}, were prepared in 2% HNO_3. A stock solution of multi-element standard was purchased from SpexCertiPrep. Results were validated using peach leaves certified reference material, NIST 1547 (Peach leaves). Recoveries of the elements are within ±20%, and are given in Table 5.4.

5.3.4 Statistical Evaluation of Data

Elemental data were evaluated using JMP software ®10.0 (SAS; Cary, NC, USA). Data were standardized using Z-score to compensate for the varying ranges of the elemental concentration. Data that showed poor homogeneous dispersion were standardized using log-transformation. Since, the dataset contain multiple response variables for each sample, multivariate statistics, such as Principal Component Analysis (PCA), Hierarchical Clustering

Analysis (HCA), and Multivariate Analysis of Variance (MANOVA) using sum contrasts. were used to evaluate data. PCA and HCA were used to assess grouping tendency of ephedra. MANOVA was used to test for statistical significance ($\alpha = 0.05$) between the groups. These statistical analyses are described in Chapter 1.

Table 5.3. Data acquisition and instrument parameters for the ICPMS analysis

Plasma	
Auxiliary gas flow	1.15 L min^{-1}
Sample gas flow	1.270 L min^{-1}
Cool gas flow	16.00 L min^{-1}
RF power	1450 W
Data acquisition	
Isotopes monitored in LR	^{37}Ba, ^{111}Cd, ^{208}Pb, ^{85}Rb, ^{88}Sr, ^{238}U
Isotopes monitored in MR	^{59}Co, ^{63}Cu, ^{56}Fe, ^{24}Mg, ^{55}Mn, ^{60}Ni, ^{51}V, ^{66}Zn,
Integration time	10ms for LR 30ms for MR
Sample per peak	50 for LR 20 for MR

LR = low resolution; MR = medium resolution

Table 5.4. Recovery for NIST 1547, Peach leaves reference material (n = 13)

Elements	**Certified (ppm)**	**Found (ppm)**	**Recovery (%)**
Al	249 ± 8	239±17	96.0
Ba	124 ± 4	116±7	105.9
Cd	0.03	0.03±0.00	80.7
Cr	1	0.81±0.10	98.6
Co	0.07	0.07±0.01	91.2
Cu	3.7 ± 0.4	3.4±0.13	93.6
Fe	220	206±9	83.6
Pb	0.87 ± 0.03	0.73±0.08	95.1
Mg	0.432 ± 0.008 (%)	0.395±0.058	97.5
Mn	98 ± 3	93±4	87.3
Ni	0.69 ± 0.09	0.60±0.15	92.2
Rb	19	19±2	99.8
Sr	53 ± 4	55±5	91.5
U	0.015	0.013±0.002	93.4
V	0.37 ± 0.03	0.34±0.02	103.8
Zn	17.9 ± 0.4	17.9±1.2	83.8

5.4 RESULTS AND DISCUSSION

5.4.1 Elemental Composition in Ephedra

Among the fourteen elements measured, Ephedra samples were highest (mean concentration for all samples) in Mg (2420±900 µg/g), Fe (324±338 µg/g), and Sr (34±53 µg/g), and lowest in Pb (0.500±0.423 µg/g), Cd (0.111±0.276 µg/g), and U (0.0204±0.0216 µg/g). A detailed summary of the elemental concentrations is given in Table 5.5 (Appendix A).

5.4.2 Elemental profiling based on species

The first part of the study was to determine if elemental profiles could distinguish ephedra based on species. To do this, ideally the geographic source should be the same for the species involved. Here we chose two data sets, U.S. and China, as a first approach. Elemental profiles were evaluated using PCA and HCA to assess grouping tendency of ephedra sample. Once clustering of groups is observed, MANOVA of sum of responses is performed to test for statistical significance and determine which elements are responsible for the discrimination between groups.

Ephedra species collected from China were *E. equisetina, E. intermedia, E. przewalskii, and E. sinica*. Data were grouped based on species. Z-scores were used for statistical analysis. Log-transformed data showed the same results as Z-scores. PCA score plot showed that *E. przewalskii*, is clustered separately from the other groups (Figure 5.4). Species, *E. equisetina, E.*

intermedia, and E. sinica, are clustered together and overlap each other such that the groups are indistinguishable. The same clustering of groups in PCA score plot was observed in the HCA dendogram (Figure 5.5). In the dendogram, *E. przewalskii* is clustered completely separate from the all the other groups indicating its elemental composition is substantially different from the other groups. MANOVA revealed that the difference is indeed significant (p=0.0002). The least square means obtained from MANOVA showed that *E. przewalskii* and *E. intermedia* are the most different in terms of elemental composition and that Fe, V, U, Pb, and Co are influential in distinguishing the two.

The PCA loading plot showed that *E. przewalskii* has the highest concentration of Fe, V, U, Co and Mn (Figure 5.4). The same elements are highly correlated with Component 1, which explains 38.3% of the total variance. This suggests that these elements are responsible for distinguishing *E. przewalskii* from the other species. Both PCA and MANOVA agreed that Fe, V, U, and Pb are influential in distinguishing species. This suggests that species type does play a role on the elemental composition of ephedra, at least for *E. przewalskii*.

Geographic source did not seem to have a big impact on the discrimination between the *E. przewalskii* from the other species (Figures 5.5 and 5.6). However, MANOVA of sum responses between the *E. intermedia,* and *E. sinica* showed that the two groups are not statistically different (p=0.1116). These results suggest *E. przewalskii* can be discriminated against the other species, but that other species may not be distinguished among themselves (e.g. *E. intermedia* versus *E. sinica*).

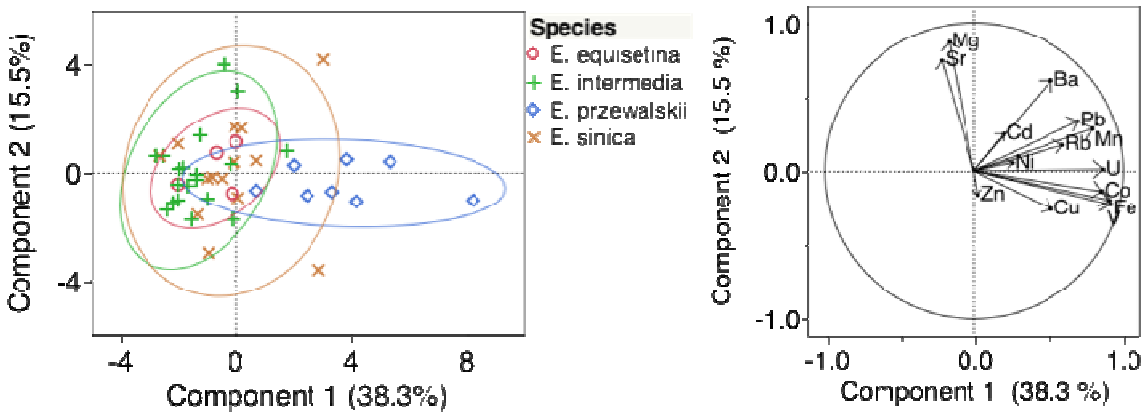


Figure 5.4. PCA loading plot (left) showing 95% density ellipses, and associated score plot (right) of the elemental profile of ephedra species from the China.

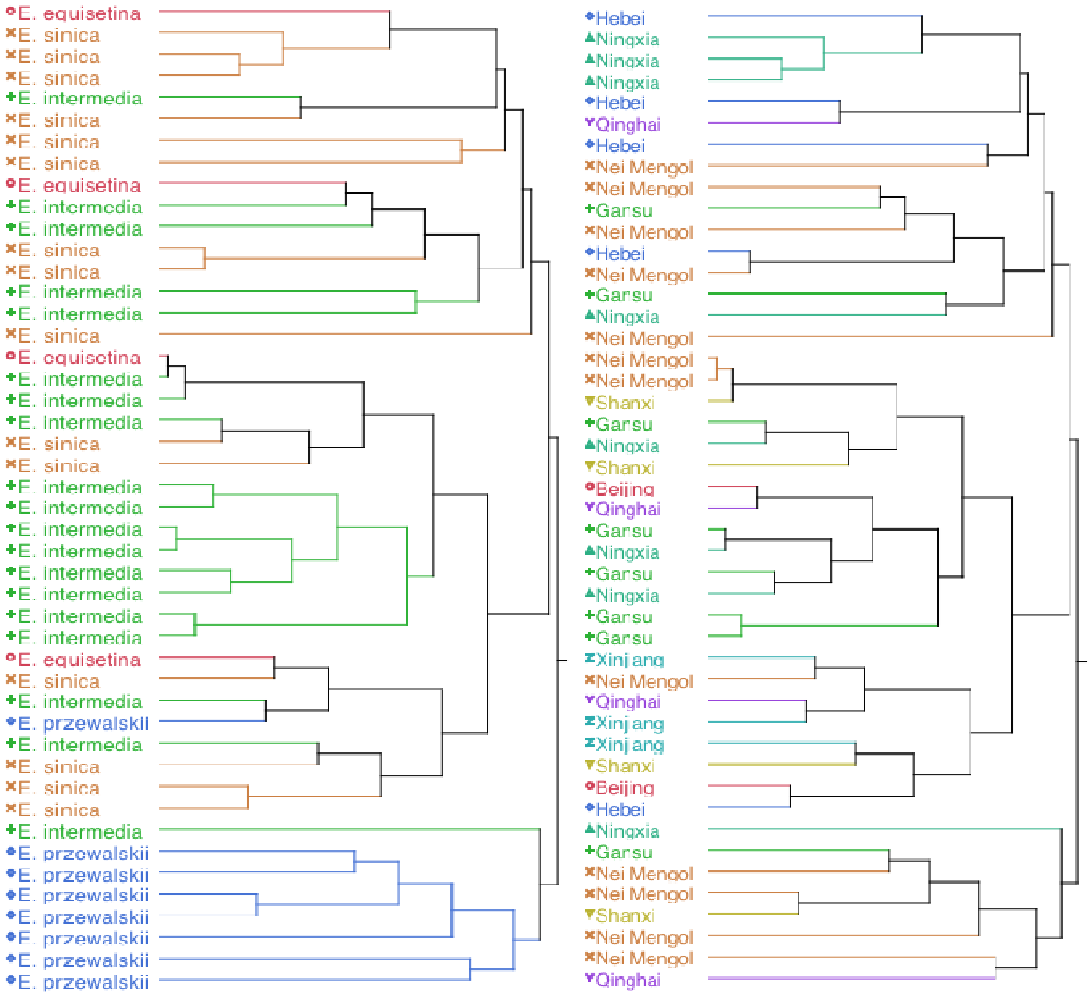


Figure 5.5. Dendogram obtained from HCA for elemental fingerprints of different ephedra species obtained from China. The left dendogram shows samples represented by species, while the right dendogram shows samples represented by its geographic source (provinces in China).

Ephedra species collected from the US were *E. antisyphilitca, E. aspera, E. aspera trifurca, E. californica, E. coryi, e. cutleri, E. fasiculata, E. nevadensis, E. pedunculata, E. torreyana, E. trifurca, and E. viridis*. The PCA score plot and HCA dendogram for this dataset showed no clustering of groups based on species and geographic source (U.S. State) (Figures 5.7 and 5.8). However, the number of sample points is limited and is reducing the discriminatory power of the statistical method.

The top three elements responsible for the variance explained by the first component are V, Fe, and Pb (Figure 5.9). PCA score plot showed that species, *E. antisyphilitca, E. coryi, and E. viridis*, have high concentration of this elements. Box-whisker plot of these elements are shown in Figure 5.10. This may suggest that the variance explained by the first component is related to distinguishing species rather than geographical origin. But then again, statistic's distinguishing power is not verified due to limited number of sample. Also, the variance explained by Component 1 is relatively low, 28.4%.

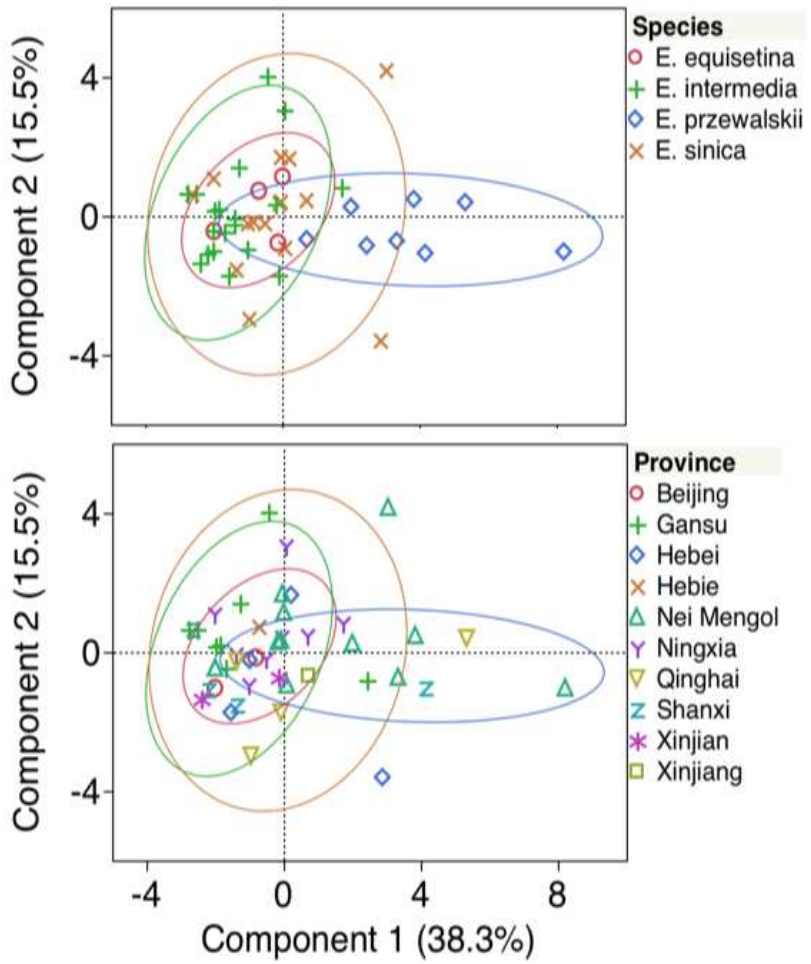


Figure 5.6. PCA score plot of the elemental profile of ephedra species collected from China. Plot above shows samples represented by species while plot below shows samples represented by geographic source (provinces in China).

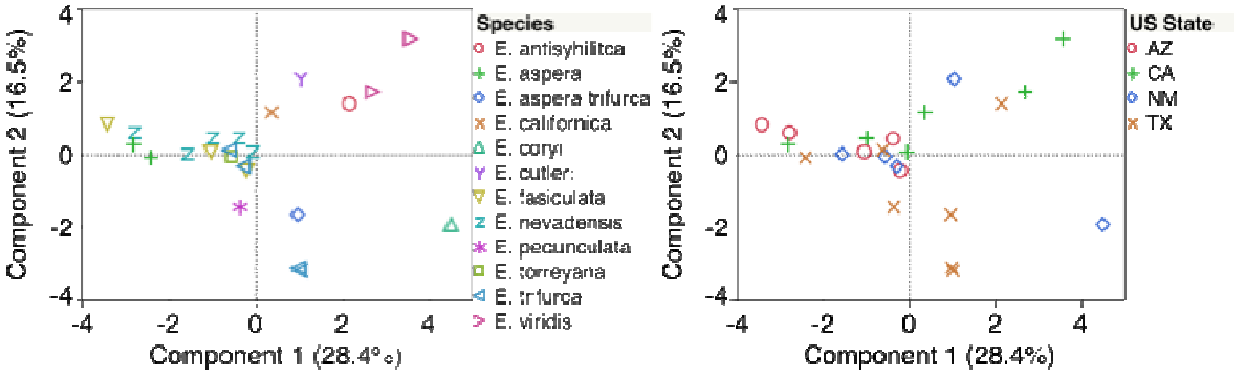


Figure 5.7. PCA score plot of the elemental profile of ephedra species collected from the US. Plot above shows samples represented by species while plot below shows samples represented by geographic source (US States).

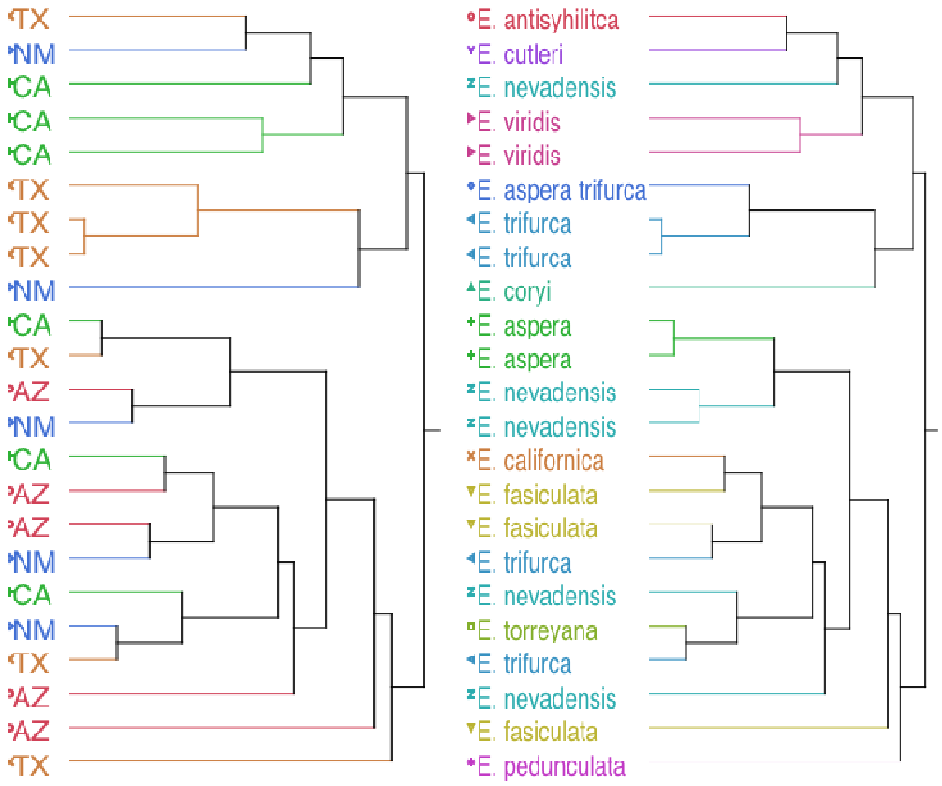


Figure 5.8. Dendogram obtained from HCA for elemental fingerprints of different ephedra species collected from the US. The left dendogram is showing samples represented by its geographic source while the right dendogram showed samples represented by species.

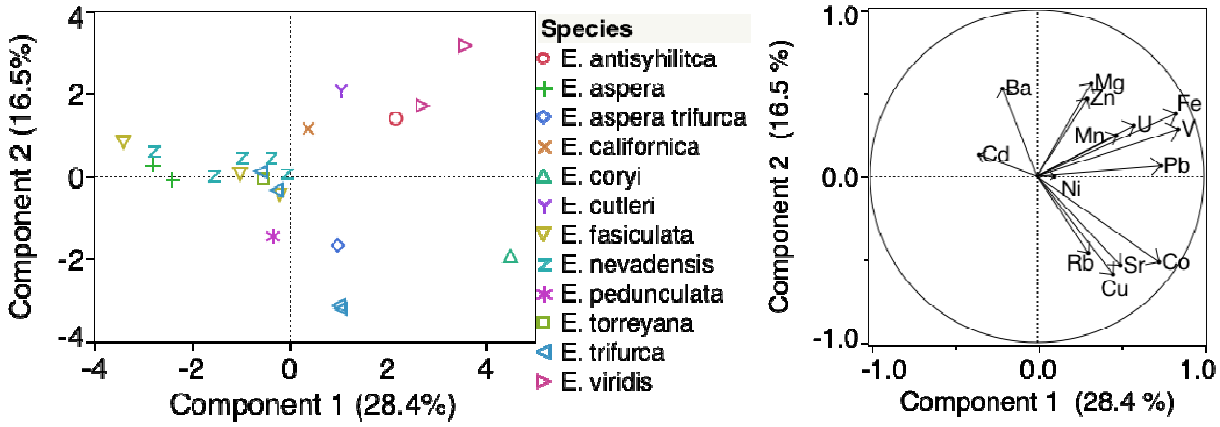


Figure 5.9. PCA score plot (right) and loading plot (left) of elemental profile of ephedra species from the US

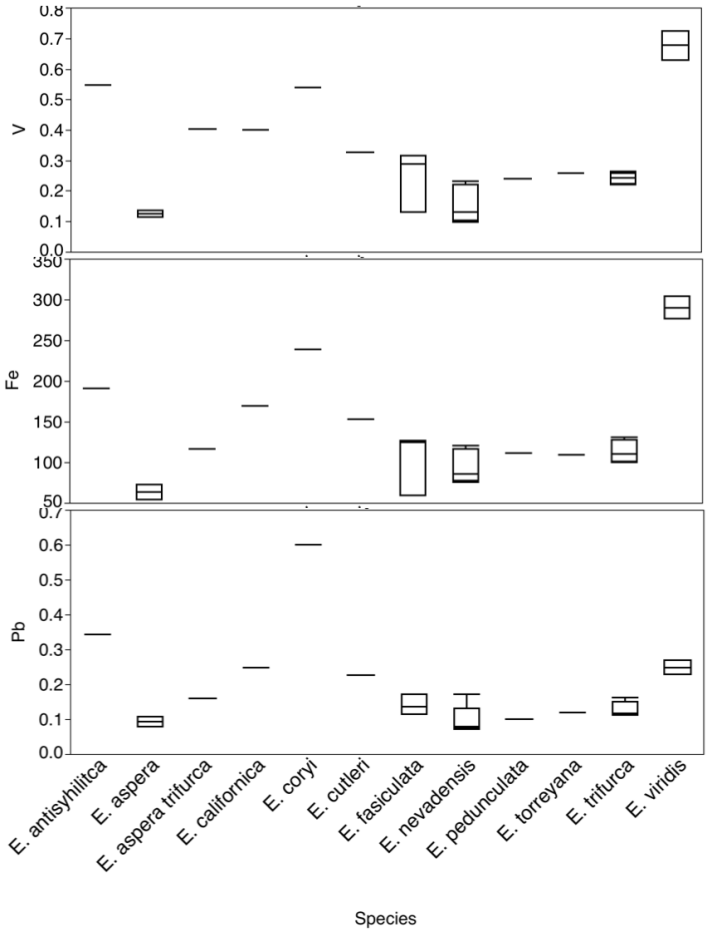


Figure 5.10. Concentration of V, Fe, and Pb in ephedra originated in the US. These elements are the responsible elements for the variance explained by Component 1 in Figure 5.9

In summary, with the possible exception of *E. przewalskii*, species does not affect the discrimination based on elemental profiling and thus the samples can be grouped to study the influence of geographic origin (discussed next).

5.4.3 Elemental profiling based on geographic source

The second part of the study was designed to determine if elemental profiles could distinguish ephedra based on geographic source. The U.S. and China are the two major geographic sources of samples used for this study. To do this, two tests were carried out: (1) test

for difference in the elemental composition of ephedra based on country of origin, China and US regardless of the ephedra species; (2) test for differences in elemental composition of ephedra based on geographic source of the same ephedra species.

5.4.3.1 Elemental profiling based on geographic origin (U.S. versus China)

Clustering of groups was observed in the PCA score plot and HCA dendogram of ephedra samples based on country of origin (Figures 5.11 and 5.12). MANOVA of sum of responses indicates that the two groups are statistically significant ($p=0.0003$). T-test showed that the elements Pb, Fe, Mg, U, and V were influential in these differences. The PCA loading plot showed that elements Fe, V, U, Co, and Pb showed a high correlation with Component 1 indicating that these elements are responsible from the variance explained by that component. PCA, and T-tests gave the different results with respect to the relative importance of elements in discriminating the group. This suggests that other factors aside from geographic source affect the elemental composition in ephedra.

It should be noted that certain species grow predominantly in different regions of the world. The species *E. przewalskii* is most likely be found in Asia (e.g. China) while *E. nevadensis* are predominant in North and South America. Our results showed that other factors are affecting elemental composition in ephedra. Elemental composition may also be species-dependent. This is validated in the HCA dendogram (Figure 5.12) and the PCA score plot showing samples represented as species (Figure 5.13). Both of these figures show that the elemental composition of *E. przewalskii* played a role in distinguishing the group. HCA dendogram showed that *E. przewalskii* is clustered completely separated from the rest of the group. As discussed in the previous sections, *E. przewalskii* has the highest concentration of Fe,

V, U, Co, and Pb. These same elements were responsible for the differences between ephedra from US and China.

HCA dendogram showed that *E. przewalskii* is a separate cluster from the rest of the samples (Figure 5.12, left). The rest of the groups are furthered clustered into two groups: US and China (Figure 5.12, right). MANOVA of sum of responses of elemental composition between U.S. and Chinese ephedra revealed that the two groups are statistically different (p=0.0003) and that the elements, Fe, Pb, V, Mg, and U are influential in distinguishing the two. PCA also agreed that Fe, V, and U are important in discriminating Chinese and U.S. ephedra. This result suggests that country of origin (soil, climate, etc.) plays an important role in the accumulation of metals in ephedra.

To evaluate the discriminatory power of elemental profiles for provenance of ephedra, the same ephedra species collected from different geographic source should be used for evaluation. In that way the species variable is held constant. Unfortunately, the number of samples collected (same species but different sources) was too limited for us to carry out valid statistical tests.

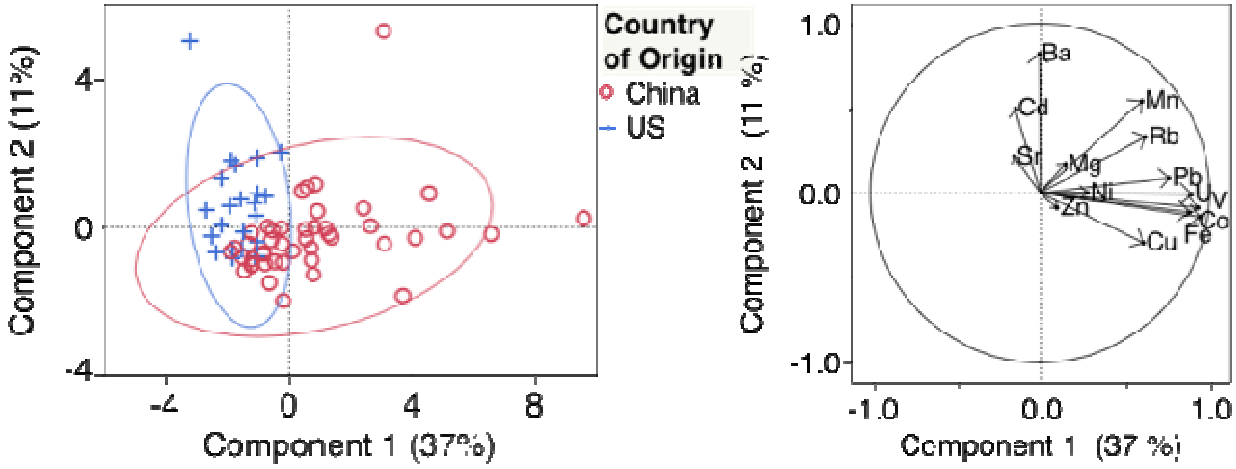


Figure 5.11. PCA score plot (left) and loading plot (right) of the elemental profile of ephedra collected from the US and China. Samples were grouped based on country of origin

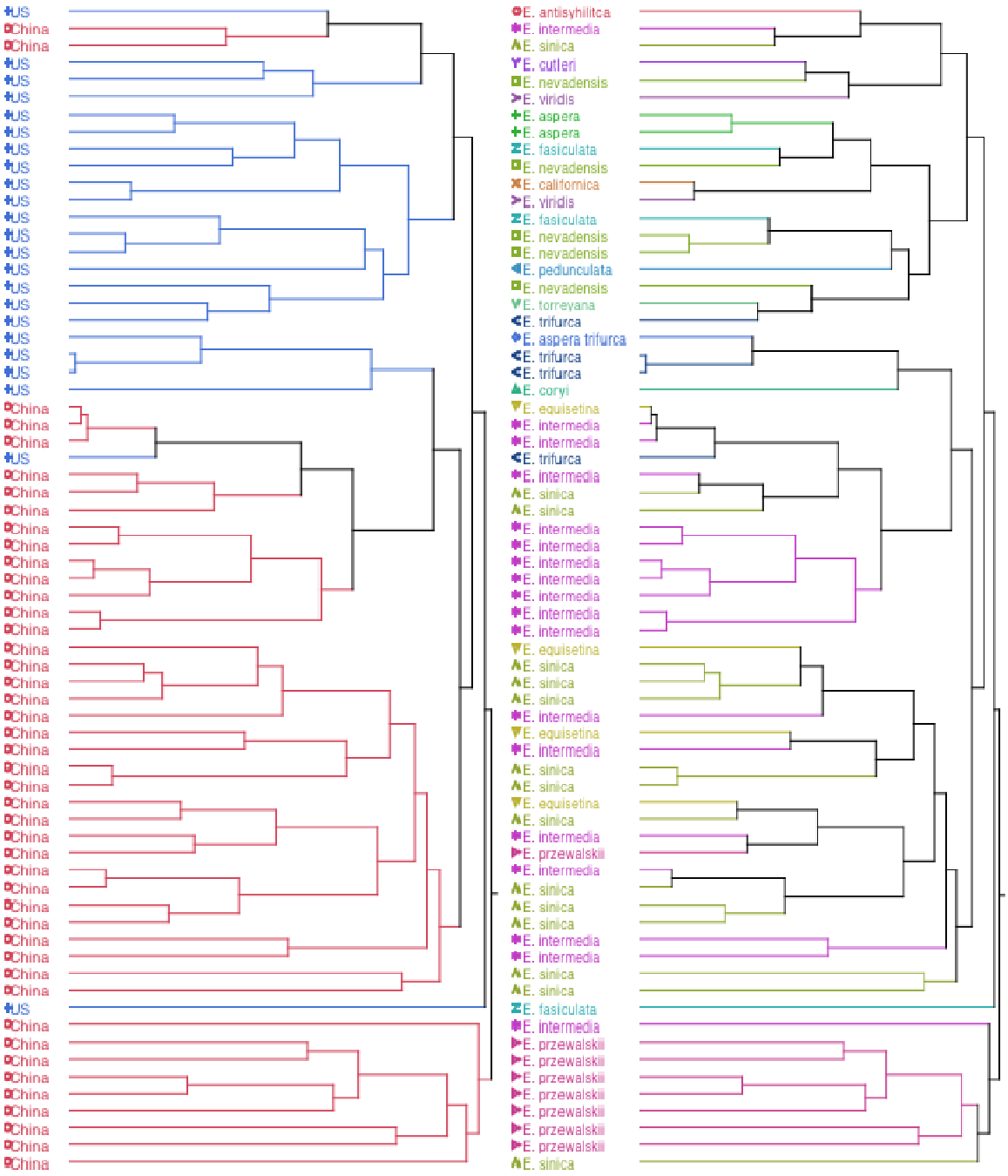


Figure 5.12. Dendogram obtained from HCA for elemental fingerprints of different ephedra species collected from the US and China. The left dendogram is showing samples represented by its geographic source while the right dendogram showed samples represented by species.

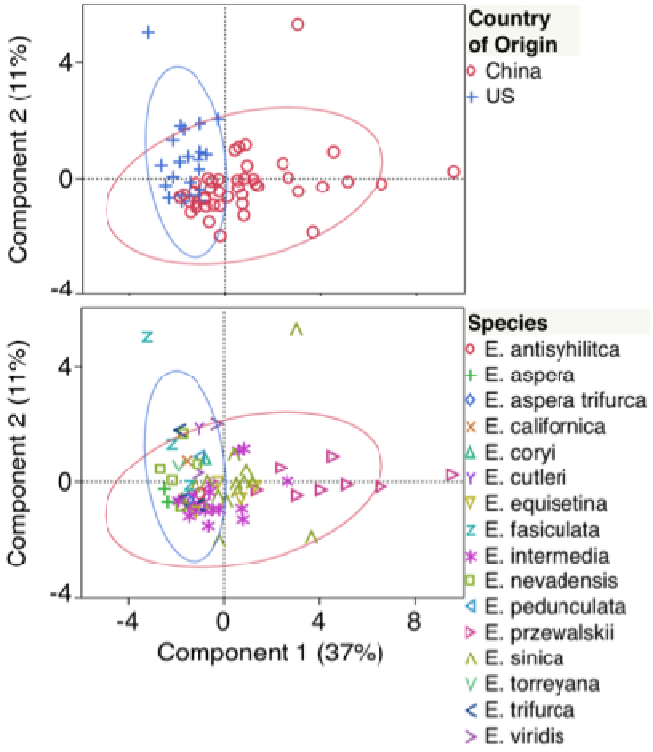


Figure 5.13. PCA score plot of the elemental profile of ephedra from the U.S. and China.

5.5 Conclusions

Elemental composition of ephedra from the U.S. and China are sufficiently different to make elemental fingerprinting feasible as a means to distinguish the two. Iron, V, and U are the key elements responsible for that discrimination; ephedra from China tend to have higher concentration of these elements. Elemental profiles can also be used to distinguish *E. przewalskii* from the rest of the species included in this study regardless of geographic origin. However, species other than *E. przewalskii* were generally indistinguishable.

5.6 ACKNOWLEDGEMENTS

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5.7 LITERATURE CITED

[1] Halberstein, R.A. Medicinal Plants: Historical and Cross-Cultural Usage Patterns. *AEP* **2005**, 15:686-99

[2] Newman, D.J., Cragg, G.M., Snader, K.M. Natural Products as Sources of New Drugs Over the Period 1981 – 2002. *J. Nat. Prod* **2003**, 66:1022-1037

[3] Newman, D.J., Cragg, G.M., Snader, K.M. The Influence of Natural Products upon Drug Discovery. *Nat Prod.* **2000**, Rep 17:215-234

[4] Jones, W.P., Chin Y., Kinghorn, A.D. The Role of Pharmacognosy in Modern Medicine And Pharmacy. *Current Drug Targets* **2006**, 7:247-264

[5] Cassileth, B.R.,Heitzer, M., Wesa, K. The Public Health Impact of Herbs and Nutritional Supplements. *Pharmaceutical Biology* **2009**, 47(8): 761 – 767

[6] Joshi, K. et al. Molecular Markers in Drug Herbal Technology. *Current Science* **2004**, 87: 159-165

[7] Anon. WHO Guidelines on Good Agricultural and Collection Practices for Medicinal Plants, Geneva. World Health Organization **2003**

[8] Khan, I.A. Issues Related to Botanicals. *Life Sciences* **2006**, 78:2033-2038

[9] Cui, J., T. Zhou, J. Zhang, and Z. Lou. Analysis of Alkaloids in Chinese Ephedra Species by Gas Chromatography. *Phytochemical Analysis*, **1991**, 2, 116-119

[10] Palamar, J. How ephedrine escaped regulation in the United States: A Historical review of misuse and associated policy. Health Policy **2011**, 99 pp 1-9

[11] Caveney, S., Charlet, D.A., Freitag, H., Maier-Stolte, M., Strarratt, A.N., New observations of the secondary chemistry of world Ephedra (Ephedraceae). *Am. J. Bot.* 2**001**, 88 pp. 1199–1208.

[12] Final Rule declaring dietary supplements containing ephedrine alkaloids adulterated because

they present an unreasonable risk. Federal Register **2004**, 69

[13] Martin WR, Sloan JW, Sapira JD, et al. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine and methylphenidate in man. *Clinical Pharmacology and Therapeutics* **1971**,12 pp. 245–58.

[14] Frank RS. The clandestine drug Laboratory situation in the United States. *Journal of Forensic Science* **1983**, 28(1) pp. 18–31.

[15] Schaneberg, B.T., S. Crockett, E. Bedir, and I.A. Khan. The role of chemical fingerprinting: application to Ephedra. *Phytochemistry* **2003**, 62, 911–918

[16] Ross, J.J., Pelders, M.G., De Smeet, P.A., A case of positive doping associated with a botanical food supplement. *Pharm. World Sci*. **1999**, 21, 44–46.

[17] Rader, J.I., P. Delmonte, M.W. Trucksess. Recent studies on selected botanical dietary supplement ingredients, *Anal Bioanal Chem*, **2007**, 389, 27–35

[18] Food and Drug Administration. *Federal Register*, **2007,** 62 (121)

[19] Chinaka, S., S. Tanaka, N. Takayama, K. Komai, T. Ohshima, K. Ueda. Simultaneous chiral analysis of methamphetamine and related compounds by capillary electrophoresis. *J. Chrom. B* **2000**, 749, 111–118.

[20] Betz, J.M., M.L. Gay, M.M. Mossoba, S. Adams. Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing Ma huang. *J. AOAC Int.* **1997**, 80, 303–315.

[21] Gurley, B.J., P. Wang, S.F. Gardner. Ephedrine-type alkaloid content of nutritional supplements containing Ephedra sinica (mahuang) as determined by high performeance liquid chromatography. *J. Pharm. Sci*. **1998**, 87, 1547–1553.

[22] Gay, M.L., K.D. White, W.R. Obermeyer, J.M. Betz, S.M. Musser. Determination of ephedrine-type alkaloids in dietary supplements by LC-MS using stable-isotope labeled internal standard. *J. AOAC Int*. **2001**, 84, 761–769.

[23] Joshi, V.C., and I. Khan. Macroscopic and Microscopic Authentication of Chinese and North American Species of Ephedra. *Journal of AOAC International* 2**005**, 88, 707-713

[24] Nikdel, S. et al. Trace metals: Defining geographical origin and detecting adulteration of orange juice. In Adulteration of Fruit Beverages; Nagy, S., Attaway, J.A., Rhodes, M. Eds.; Marcel Dekker: New York, **1988**, pp 81-105

[25] Schwartz, R.S., Hecking, L.T. Determination of geographic origin of agricultural products by multivariate analysis of trace element composition. *J. Anal. At. Spectrosc*. **1991**, 6 pp 637-642

[26] Anderson, K.A. et al. Determining the geographic origin of potatoes with trace metal analysis using statistical and neural network classifiers. *J. Agric. Food Chem*. **1999**, 47 pp. 1568-1575

[27] Anderson, K.A., and Smith, B.W. Chemical profiling to differentiate geographic growing origin of coffee. *J. Agric. Food Chem*. **1999**, 50 (7) pp 2068-2075

[28] Samsoe-Petersen, L. et al. Uptake of trace elements and PAHs by fruits and vegetables from contaminated soils. *Environ. Sci. Technol*. **2002**, 3 (14) pp 3057-3063

[29] J.D. Ingram, J.C. Lin, Geochemcial tracers of sediment sources to San Francisco Bay, *Geology* **2002**, 30, 575–578.

[30] K. Pye, S.J. Blott, D.J. Croft, S.J. Witton, Discrimination between sediment and soil samples for forensic purposes using elemental data: an investigation of particle size effects, *Forensic Sci. Int*. **2007**, 167, 30–42.

[31] K. Pye, D. Croft (Eds.),Forensic Geoscience: Principles, Techniques, and Applications Geol. Soc. London Spec. Publ. 232, **2004**, 318.

[32] Esechie, H. Distribution of chemical constituents in the plant parts of six tropical-origin forage grasses at early anthesis. *J. Sci. Food Agric*. **1992**, 58, 435-438.

[33] Anderson, K., and B.W. Smith. Use of Chemical Profiling to Differentiate Geographic Growing Origin of Raw Pistachios. *J. Agric. Food Chem*. **2005**, 53, 410-418

[34] Broadley, M.A., N.J. Willey, J.C. Wilkins, A.J.M. Baker, A. Mead, P.J. White. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytologist* **2001**, 152, 9-27.

[35] Baker, A.J.M. Accumulators and excluders"Strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **1981**, 3, 643-654.

[36] Harada, H., T. Hatanaka. Natural background levels of trace elements in wild plants: variation and distribution in plant species. *Soil Science & Plant Nutrition* **2000**, 46, 117-125.

CHAPTER SIX

MERCURY AND HEAVY METALS IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES FROM ARTISANAL SMALL-SCALE GOLD MINING COMMUNITIES IN MOZAMBIQUE

6.1 ABSTRACT

Artisanal small-scale gold mining (ASGM) is considered one of the largest anthropogenic sources of mercury to the atmosphere. ASGM workers typically combine elemental mercury (Hg) with crushed ore and mineral deposits to amalgamate gold and silver, and subsequently heat (burn) the amalgam to drive-off the Hg and isolate the gold. This has resulted in environmental and health concerns for artisanal mining camps worldwide. The Manica District in Mozambique has several such mines, including Munhena, Tsetsera, and Clean Tech. The latter is unique among the mines in that it utilizes centrifugation and magnet technology instead of Hg to extract gold. In this study we evaluated Hg levels in soil obtained near these mines, and determined 15 metals (Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Rb, Sr, and V) in hair and fingernails (biomonitors for metals) of miners from each site. Samples were microwave digested and analyzed using sector-field ICPMS. Mean Hg levels in the soil near the Clean Tech mine were considerably lower (0.25 µg/g) than upper Munhena (7.1 µg/g), lower Munhena (12.0 µg/g), and Tsetsera (3.1 µg/g). Clean Tech also tended to have lower levels of metals in fingernails. Miners from Munhena had considerably higher metal concentrations in fingernails, whereas Tsetsera miners had higher metal concentrations in hair. Several statistical tests are not amenable to the current data set due to the limited number of samples, nevertheless it appears the Hg-free practices employed at Clean Tech are leading to lower levels of metals in the local environment and in the miners themselves. Overall, the heavy metal concentration in fingernail and hair from

miners were much higher than non-mining populations, which suggests high metal exposure in artisanal mining communities.

6.2 INTRODUCTION

Artisanal and small-scale gold mining (ASGM) activity uses rudimentary methods to extract minerals and metals from an ore. About 25% of the estimated 3000 tons of gold produced globally comes from ASGM [1]. Poor economies and the high price of gold ($1300 per ounce as of April 2014) [2] have dramatically increased artisanal gold mining in developing countries [3-4]. The majority of gold miners use mercury, amalgamation to extract gold from an ore [5-6]. Miners add elemental mercury to amalgamate gold particles from a ground ore, and the amalgam routinely contains between 40 and 60% mercury by mass. The amalgam is typically “burned” to separate gold from the amalgam. This burning process volatilizes the Hg in the amalgam releasing it into the environment and the atmosphere [7]. ASGM and coal burning are the major contributors of anthropogenic emission of Hg to the air [8].

Mercury emission from artisanal mining has raised a lot of concerns due to environmental and health impacts to the miners, and due to its large contribution to the pool of atmospheric Hg globally [9]. Mercury is toxic and volatile and readily dispersed through atmospheric pathways. It is well-known to cause harmful effects to the nervous system and can cause headaches, memory loss, and cognitive and motor impairment [9].

In addition to mercury, ASGM miners are also exposed to other potential toxic metals [10]. Toxic metals may be released during mining processes such as grinding, crushing, milling, and amalgamation of ores and mineral deposits [11]. A study of the concentration of Hg and As

in water, soil, and human nail samples in Nicaraguan mining community, revealed that uptake of Hg occurs via drinking water and soil exposure [12]. The same study found that residential proximity to the mine, and Hg concentration in drinking water and soil, were correlated to the Hg concentration in fingernails. Another study found that the area around gold washing and milling were the main sources of As, Ti and Sr in hair from Mongolian miners [13]. 57% of 448 ASGM miners in the Brazilian Amazon had concentration of Pb in blood that exceeded the US Central for Disease Control and Prevention guideline [14]. Others found high concentrations of Cr, As, Al, Cu, Mn, Ni, Se, and Zn in urine of ASGM miners in Ghana [15]. Saunders et al. reported that nail concentrations of Hg, Pb, Al, Mn, and As exceeding reference levels, and that young adults presented neurological symptoms and poor hearing, further suggesting that high metal levels in artisanal mining communities present a health hazard [16].

6.2.1 Metals in hair and nails

Hair and nails are considered metabolic end products, which can reflect the metabolism of metal in the body [17]. Metals can be incorporated into their structure during the growth process [18]. The amount of heavy metals in hair and fingernails can be used to monitor heavy metal exposure [10, 15, 19]. There are several advantages of using hair and nail over other biological specimens: (1) simple, non-invasive, and less costly collection, (2) ease of transport and storage, and (3) generally stable for a longer periods of time [20-21]. Compared to blood, hair and nail represents a longer exposure time as it is accumulated over longer periods [18, 22].

6.2.2 Purpose of this study

The purpose of this study is to assess the heavy metal exposure to ASGM in the Manica District of Mozambique, and to evaluate the impact of a “mercury-free” mine (Clean Tech) on the local environment (i.e., soil Hg concentrations) and on metal levels in hair and fingernails of the miners, compared to other mines in the District that use mercury.

6.3 MATERIALS AND METHODS

6.3.1 Sample sites

ASGM mines located in Manica District of Mozambique, a country located in southeastern Africa, were studied (Figure 6.1). Agriculture and mining are the main sources of income in the District. Approximately 8% of the population (estimated at about 156,000) are involved in ASGM. The majority of the miners use Hg amalgamation in extracting gold from mineral deposits and ore (Figure 6.2) [3]. To assess the heavy metal exposure to gold miners, four ASGM mines in Manica District were chosen: the Clean Tech, Tsetsera, Lower Munhena, and Upper Munhena (Figure 6.1).

All but Clean Tech uses Hg in the mining process [23]. Clean Tech mine had a history of being mined with mercury but the owner was the only one to amalgamate and burn. Clean Tech is a privately owned mine located in Penhalonga region in Manica District. For the past several years, Clean Tech stopped using mercury in gold mining operation. At Clean Tech, gold is extracted by centrifugation and magnetic removal of gangue minerals [23]. This process creates a safer environment for the miners. In addition, miners at Clean Tech were given worker benefits such as 2 meals a day, transportation, and some health benefits. Miners were given uniforms and safety equipment and follows strict regulations [24].

Munhena is a region in Manica district, where mining is the chief source of income and most of the population is economically dependent on mining activities [3]. Gold mines in

Munhena are divided into two distinct entities, Upper Munhena and Lower Munhena, based on location and mining practices. Although both mines are essentially in the same location, Upper Munhena is located near the mountain peak while Lower Munhena is at the base of the peak. Upper Munhena is industrialized and employs about 100 miners. Upper Munhena uses Hg in gold amalgamation but has improved its safety practices by burning amalgam at specific times of the week, in specific places. They also use retort technology to minimize Hg exposure to miners and limit contamination in the environment [24].

Lower Munhena, on the other hand, is unorganized and all artisanal. Here, mining is poverty-driven. Approximately 25-50 miners worked independently with no safety regulations. Miners work wearing rags and mostly without shoes. Some miners work around women and children. They also perform mining processes inside their huts, where they live and do all their household chores (Figure 6.2) [24].

Tsetsera is similar to Lower Munhena's mining practices where the miners have significantly less exposure to safer mining technology and techniques. The mining operation was located in an open pit at the bottom of a 40-meter deep unreinforced mining shaft. More women and children are present in the mine. Some are even participating in the mining procedure. At the time of sampling, around 80 miners were working independently and most of them resisted participating in the project. Because of this, only few samples were collected from this mine [24].

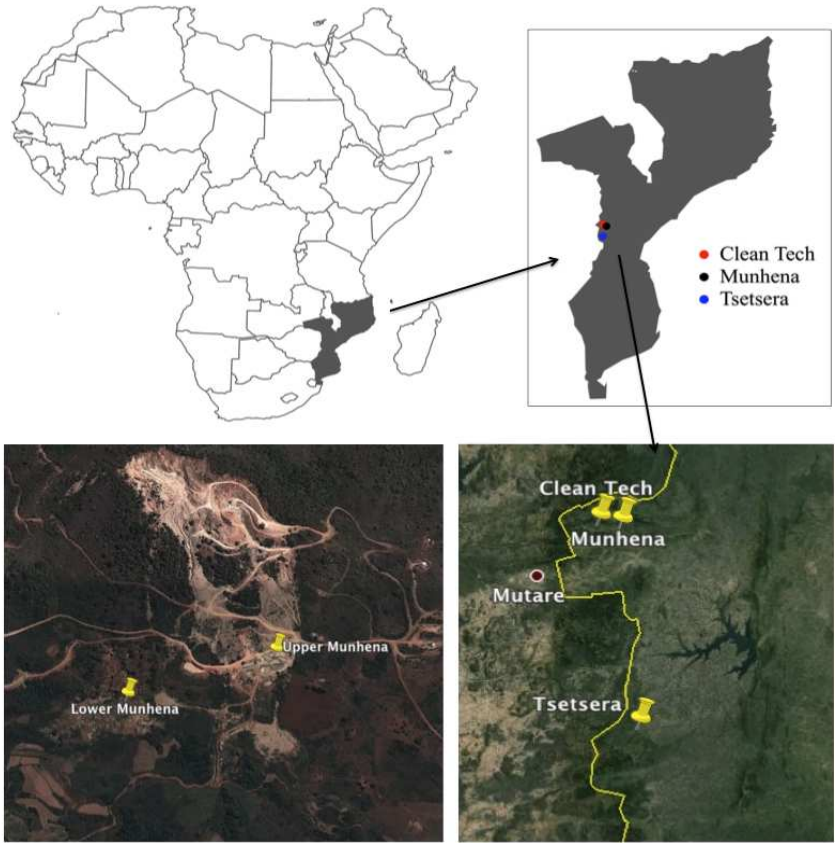

Figure 6.1. Location Clean Tech, Tsetsera, and Lower and Upper Munhena mines in the Manica District of Mozambique

Figure 6.2. A miner from Manica District, Mozambique burning off Hg from an amalgam to retrieve the gold (left). This process is sometimes done inside the hut where they live (right). Photos courtesy of Dr. Adam Kiefer (Mercer College)

6.3.2 Samples

Soil samples were collected from each mining site (Table 6.1). Hair and fingernail samples from miners were also collected; detailed information about these miners is given in Appendix B (Table 6.7) Miners who participated in this project have ages ranging from 21 to 49 years. The amount of time spent working in the mining operations ranges from 1 to 12 years. Most of the miners are male but 1 female from Clean Tech mine participated in this project. Information about their smoking habit was collected and memory tests were performed.

Table 6.1. Location and description of soil samples collected from artisanal gold mines in Manica, Mozambique

Sample ID	Location	Sample Site Description
C1	Clean-Tech	Entrance of mine, sloped surface
C2	Clean-Tech	new mine #3
M1	Upper Munhena	Parking lot dividing upper and lower Munhena
M2	Upper Munhena	Tailing Pit 1
M3	Upper Munhena	Base of ball mill
M10	Upper Munhena	Tailing Pit 2
M11	Upper Munhena	Tailing Pit 4
M12	Upper Munhena	Alternative tailing pit
M13	Upper Munhena	Alternative tailing pit 2
M4	Lower Munhena	Lower Munhena mine, close to water
M5	Lower Munhena	In between two ball mills, close to water
M6	Lower Munhena	By hut near ball mill and live chickens
M7	Lower Munhena	Next to man panning
M8	Lower Munhena	Processing site
M9	Lower Munhena	By Burning hut
T1	Tsetsera	Taken from access road on mountainside
T2	Tsetsera	Inside rock pit
T3	Tsetsera	Village side street 1
T4	Tsetsera	In front of hut/stove
T5	Tsetsera	beside washing/grinding area and ball mill (edge of water)
T6	Tsetsera	beside washing pit; water's edge
T7	Tsetsera	beside washing area
T8	Tsetsera	between burning and ball mill
T9	Tsetsera	sediment from new washing pit
T10	Tsetsera	on path leaving Tsetsera

6.3.3 Mercury in Soil

Approximately 0.1 gram samples of soil were leached with 5 mL each of high purity HNO_3 and HCl acids (Seastar Chemicals Inc., Sidney, BC, Canada) using a microwave digestion system (Ethos; Milestone Inc., Shelton CT, USA) equipped with a multi-prep rotor (41 pfa vessels). The digestion program consisted of a 30 min ramp to 120^0C, 20 min at 120^0C, followed by a 60 min ramp to 180^0C where the temperature was held for 20 more minutes. The resulting digest was transferred to centrifuge tube and diluted to 50-mL with deionized water (≥18.2M Ω). The digests were allowed to sit overnight to let any particles settle before transferring 10-mL to another 50-mL tube. Then 500 µL of 0.2N BrCl was added into each tube and the sample was further diluted to 50-mL with DI water as before. The resulting solutions contained 2% HNO_3, 2% HCl and 0.002 N BrCl.

A series of Hg standards (0.1 ppb, 0.5 ppb, 1 ppb, 5 ppb, and 10 ppb) was prepared in 2% HNO_3, 2% HCl, and 0.002N BrCl by dilution of a Hg standard obtained from Spex Certiprep (Metuchen, NJ, USA). The rinse (wash) solution contained the same acid matrix; we found that this eliminated carryover between samples. Before analysis 5 µL of a 10 ppm internal standard solution (also from Spex) was added to all samples, standards and blanks; ^{209}Bi was used as an internal standard. Mercury was determined using a quadrupole-ICPMS (X-Series 2; Thermo Fisher Scientific, Waltham, MA, USA). Instrument parameters were optimized for sensitivity before Hg analysis.

6.3.4 Multi-element analysis of hair and nail samples

The limited amount of samples required the use of very clean centrifuge tubes (15mL). The tubes were cleaned in two steps. The first step involved soaking the tubes for at least 24

hours in a 15% HNO_3 acid bath and then rinsing it with DI water after. The second step was heating the acid-washed tubes, containing 4mL concentrated HNO_3 and 1 mL concentrated HCl, for 2 hours at 80^0C. Finally, tubes were rinsed with DI water and air-dried in a clean laminar flow hood.

Hair and fingernail samples were washed prior to analysis. Washing involved sonicating the samples in (in chronological order) Triton X-100, DI water and acetone for 10 minutes. Samples were washed with DI water every after sonication. Samples are air-dried in a clean laminar flow hood after a final rinse of DI water. Approximately 0.01 g sample was weighed into the acid-cleaned tubes. About 5 mL each of high purity HNO_3 and HCl acids (Seastar Chemicals Inc., Sidney, BC, Canada) were added to the tube. To the reference material, an additional 100µL of concentrated HF were added to the tubes to allow complete digestion. The samples were allowed to sit for ~30 minutes prior to digestion. The tubes, loosely capped, were then heated at 80^0C for 2 hours. Digests were diluted to 2% HNO_3 using a diluent 1 ng/g of Rh as the internal standard.

The resulting solution was introduced into a high-resolution SF-ICPMS (Element-XR; Thermo Scientific) for multi-element analysis using a PFA micro-flow nebulizer with a HF resistant sample introduction system (ESI, Omaha, NE, USA). The instrument was tuned to optimize sensitivity and stability prior to analysis. Instrumental and data acquisition parameters are given in Table 6.2. A series of multi-element standards (0.01-50 n/g) were prepared such that it contains the same acid make-up and Rh concentration with that of the sample solution.

Table 6.2. Data acquisition and instrument parameters used during the multi-element analysis

Plasma	
Auxiliary gas flow	1.15 L min^{-1}
Sample gas flow	1.270 L min^{-1}
Cool gas flow	16.00 L min^{-1}
RF power	1450 W
Data acquisition	
Isotopes monitored in LR	^{37}Ba, ^{111}Cd, ^{133}Cs, ^{204}Hg, ^{208}Pb, ^{85}Rb, ^{88}Sr
Isotopes monitored in MR	^{27}Al, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{60}Ni, ^{51}V, ^{66}Zn,
Integration time	10ms for LR 30ms for MR
Sample per peak	50 for LR 20 for MR

6.3.5 Evaluation of Data

Results for the elemental concentrations in hair and fingernails are presented in Box-Whisker plots. The plot shows the range, median, the first quartile, the third quartile of data set. Whiskers, lines extending from each end of the box, represent the range of the data points. A horizontal line within the box represents the median. The ends of the box represent the first and third quartiles, which correspond to 25 and 75 percentile of the data set, respectively. To evaluate the effect of age and time spent involved in mining operation, elemental concentrations were evaluated using Simple Pearson correlation.

6.4 RESULTS AND DISCUSSION

6.4.1 Hg in soil

The concentration of Hg in soil normally ranges from 0.05-0.08 µg/g [25]. Most of the soil samples obtained in this study are higher (Table 6.3). Soil from two sites near the Clean Tech mine had Hg concentrations of 0.018 and 0.492 µg/g, with a mean concentration of is 0.250 µg/g. This is 36 times lower than soil samples from Munhena, which had a mean Hg concentration of 8.97 µg/g. Soil from Tsetsera mines had a mean concentration of 3.1±5.2 µg/g. Sample M12 collected from a tailing pit at the Munhena mine had the highest Hg concentration (39.1 µg/g). Mercury levels in the soil varied depending on the proximity and land use but generally soil closest to the processing sites (burning, milling, and panning) had higher levels. Mercury levels in the soil from Clean Tech were relatively low compared to Munhena and Tsetsera, indicating that the Hg-free Clean Tech operation is indeed more environmentally friendly.

6.4.2 Hg in hair and fingernails of miners

Although the calibration curve for Hg was good (r^2>0.99), the percent recovery in the reference material (GBW07601) was unacceptably high. Because of this, the accuracy of Hg concentrations are suspect. Unfortunately there was too little sample to re-analyze. Thus the Hg concentrations in hair and fingernails presented here do not represent accurate values. However,

given that all samples were subject to the same digestion procedure we can examine the data on a relative basis (Figure 6.3). Comparative analysis of Hg concentration in hair and fingernail shows that miners working at Clean Tech have lower Hg levels relative to their counterparts at Munhena and Tsetsera. Samples from the Munhena miners were very high in Hg concentration with respect to other miners. The data suggests that miners in Clean Tech have relatively low Hg exposure from mining operations.

Table 6.3. Concentration of mercury in soil from artisanal gold mines in Manica, Mozambique

Sample ID	Location of mine	Sample site description	Hg (µg/g)
C1	Clean-Tec	Entrance of mine, sloped surface	0.018
C2	Clean-Tec	new mine #3	0.49
M1	Upper Munhena	Parking lot dividing upper and lower Munhena	0.57
M2	Upper Munhena	Tailing Pit 1	1.68
M3	Upper Munhena	Base of ball mill	5.23
M10	Upper Munhena	Tailing Pit 2	2.04
M11	Upper Munhena	Tailing Pit 4	0.61
M12	Upper Munhena	Alternative tailing pit	39.1
M13	Upper Munhena	Alternative tailing pit 2	0.38
M4	Lower Munhena	Lower Munhena mine, close to water	7.42
M5	Lower Munhena	In between two ball mills, close to water	19.4
M6	Lower Munhena	By hut near ball mill and live chickens	12.2
M7	Lower Munhena	Next to man panning	10.3
M8	Lower Munhena	Processing site	12.6
M9	Lower Munhena	By Burning hut	10.2
T1	Tsetsera	Taken from access road on mountainside	0.078
T2	Tsetsera	Inside rock pit	0.076
T3	Tsetsera	Village side street 1	1.01
T4	Tsetsera	In front of hut/stove	0.746
T5	Tsetsera	beside washing/grinding area and ball mill (edge of water)	0.485
T6	Tsetsera	beside washing pit; water's edge	0.333
T7	Tsetsera	beside washing area	14.0
T8	Tsetsera	between burning and ball mill	11.7
T9	Tsetsera	sediment from new washing pit	2.00
T10	Tsetsera	on path leaving Tsetsera	0.083

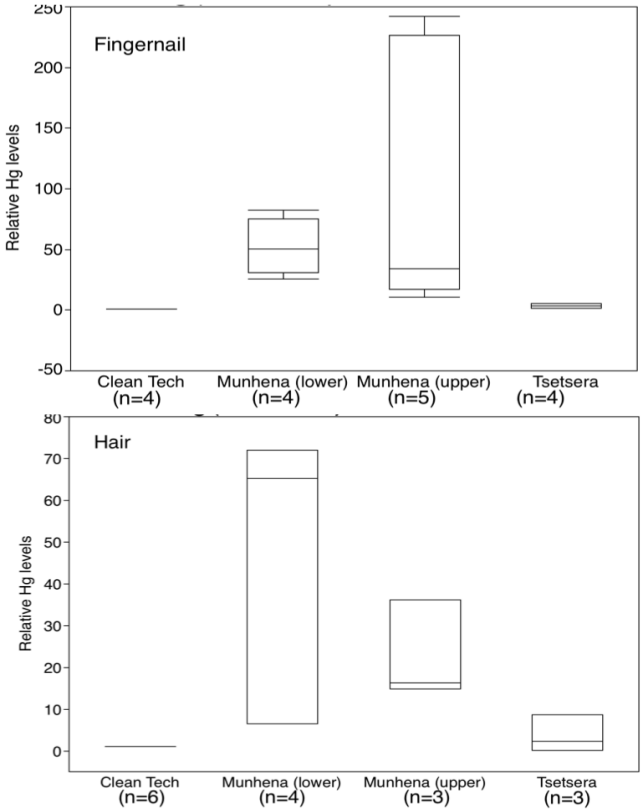

Figure 6.3. Mercury concentration in hair and fingernails of miners from Manica District, Mozambique, normalized to the concentration at Clean Tech. For example, the mean Hg level in fingernails from lower Munhena miners is ~50 times the level found for Clean Tech miners.

6.4.3 Metals in hair and fingernails of miners

Results for metals in the hair and fingernails are presented in two datasets. The first compares the concentration of Al, Ba, Cd, Co, Cr, Cu, and Fe in hair and fingernails from the four mines (Figure 6.4). The second focuses Mg, Mn, Ni, Pb, Rb, Sr, and V (Figure 6.5). Appendix 6.2 provides detailed elemental information for each sample and includes the participant's age and years working in the mine. The mean metal concentration for hair and fingernails is summarized in Table 6.4. For hair, Rb had the lowest mean concentration of 0.091±0.079 µg/g (average of all samples), while Pb had the highest 327±588 µg/g (average of

all samples). Rubidium was also lowest in fingernails (mean 0.241±0.127 µg/g), while Fe was highest (mean 1508±1137 µg/g).

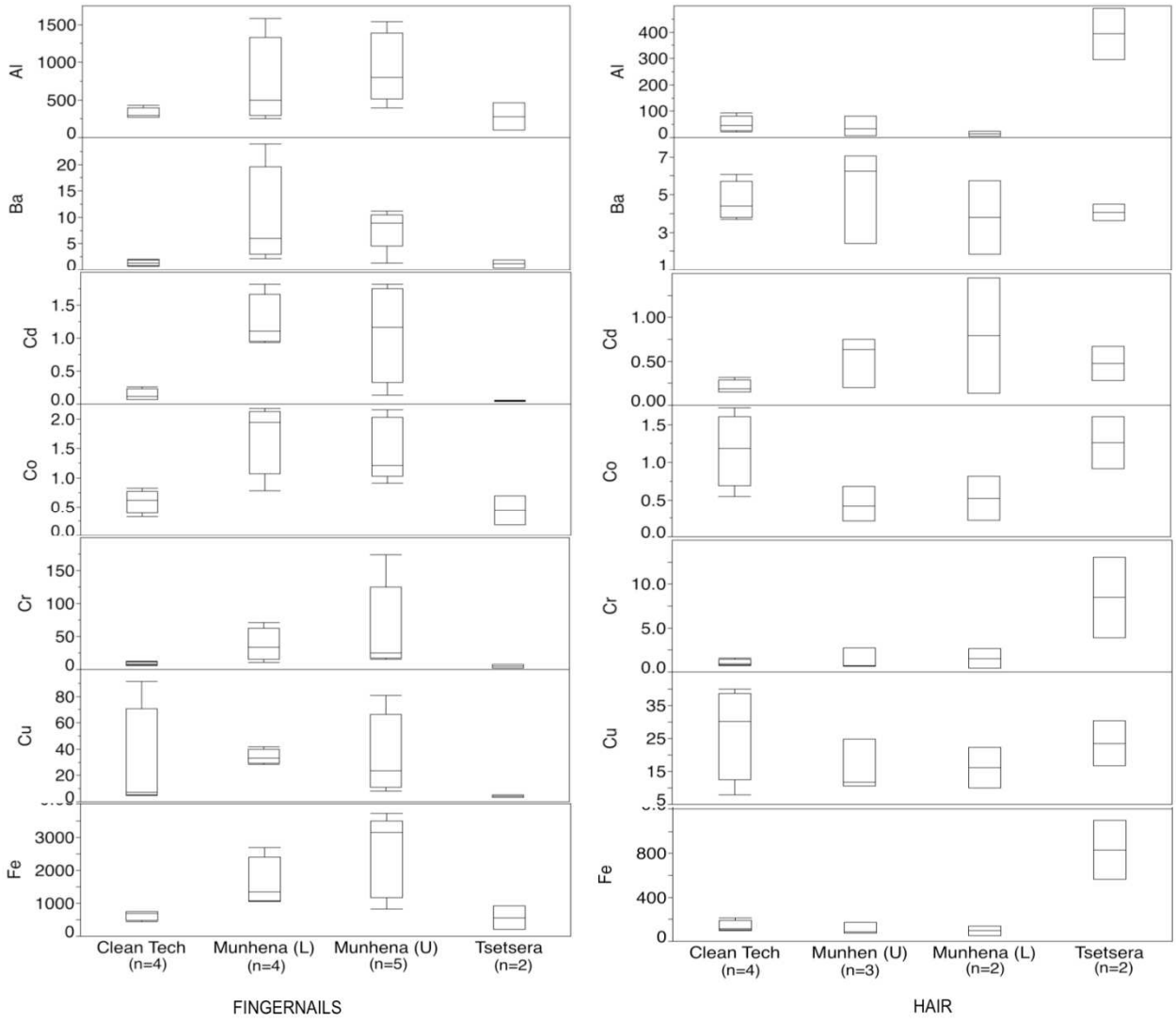

Figure 6.4. Concentration (µg/g) of Al, Ba, Cd, Co, Cr, Cu, and Fe in fingernails (left) and hair (right) for ASGM miners from Clean Tech, Lower Munhena (L), Upper Munhena (U), and Tsetsera.

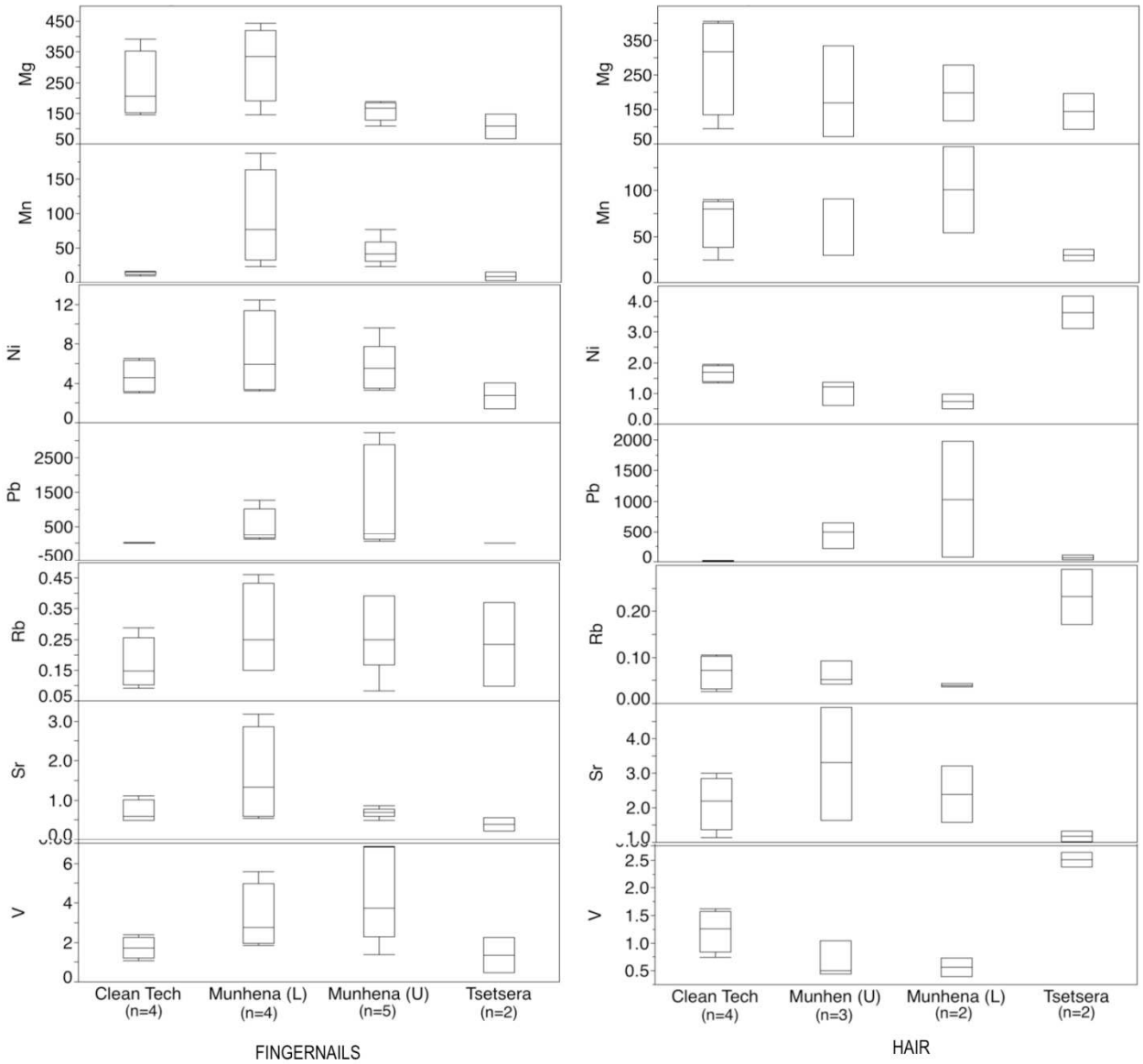

Figure 6.5. Concentration (µg/g) of Mg, Mn, Ni, Pb, Rb, Sr, and V in fingernails (left) and hair (right) for ASGM miners from Clean Tech, Lower Munhena (L), Upper Munhena (U), and Tsetsera.

Table 6.4. Average of metal concentration in hair and fingernails from ASGM miners in Manica District, Mozambique

Element	Fingernail				Hair			
	Clean Tech (n=4)	*Lower Munhena (n=4)*	*Upper Munhena (n=5)*	*Tsetsera* (n=2)*	*Clean Tech (n=6)*	*Lower Munhena* (n=2)*	*Upper Munhena (n=3)*	*Tsetsera (n=3)*
Rb	0.168 ± 0.084	0.277 ± 0.154	0.273 ± 0.128	0.234	0.069 ± 0.038	0.039	0.062 ± 0.027	0.232 ± 0.084
Sr	0.693 ± 0.294	1.60 ± 1.22	0.682 ± 0.130	0.391	2.13 ± 0.777	2.389	3.28 ± 1.64	1.18 ± 0.219
Cd	0.138 ± 0.087	1.24 ± 0.399	1.07 ± 0.729	0.049	0.212 ± 0.075	0.796	0.530 ± 0.289	0.474 ± 0.278
Ba	1.34 ± 0.582	9.50 ± 9.79	7.77 ± 3.81	1.16	4.64 ± 1.04	3.79	5.24 ± 2.48	4.06 ± 0.622
Pb	6.38 ± 7.15	466 ± 537	1258 ± 1503	3.90	12.4 ± 8.12	1026	452 ± 219	68.9 ± 54.5
Mg	237 ± 110	315 ± 124	157 ± 31.9	108	284 ± 143	199	192 ± 133	144 ± 72.5
Al	321 ± 75	706 ± 597	921 ± 463	282	51 ± 30	15	41 ± 37	394 ± 137
V	1.72 ± 0.548	3.24 ± 1.66	4.39 ± 2.40	1.36	1.23 ± 0.380	0.562	0.664 ± 0.328	2.511 ± 0.186
Cr	8.81 ± 2.604	37.1 ± 25.4	62.0 ± 67.3	4.67	1.07 ± 0.378	1.55	1.36 ± 1.18	8.49 ± 6.49
Mn	14.13 ± 2.84	91.01 ± 70.40	44.1 ± 19.7	9.00	68.56 ± 29.87	101	70.39 ± 35.27	29.92 ± 8.8
Fe	641 ± 145	1609 ± 755	2499 ± 1258	564	137 ± 53	96	112 ± 52	833 ± 380
Co	0.592 ± 0.207	1.716 ± 0.634	1.468 ± 0.534	0.441	1.159 ± 0.483	0.518	0.438 ± 0.239	1.264 ± 0.488
Ni	4.689 ± 1.662	6.893 ± 4.323	5.612 ± 2.504	2.732	1.659 ± 0.273	0.739	1.064 ± 0.398	3.638 ± 0.745
Cu	27.72 ± 42.46	34.21 ± 5.68	35.55 ± 30.27	4.30	27.13 ± 13.97	16.18	15.69 ± 7.89	23.48 ± 9.65

*No standard deviation; (n=2)

Generally, the concentration of metals in fingernails is higher than in hair (Figures 6.2 and 6.3). Simple Pearson correlation between metal concentration in hair and fingernails is poor and not significant except for Ni and Sr (Table 5). Most elements are negatively correlated except for Sr, Cd, Ba, Mg, and Mn. Age and time spent in mining operation showed poor correlation with the metal concentration in fingernails (r<0.42) and hair (r<0.64).

Table 6.5. Simple Pearson correlation of the elemental concentration in hair versus elemental concentration in fingernails

Elements	r	p value
Ba	0.243	0.561
Rb	-0.274	0.510
Al	-0.530	0.561
Cr	-0.277	0.506
Mn	0.510	0.197
Fe	-0.484	0.224
Co	-0.443	0.272
Ni	-0.748	0.032
Cu	-0.0282	0.947
Sr	0.703	0.0518
V	-0.629	0.095
Pb	-0.0527	0.990
Mg	0.557	0.152
Cd	0.586	0.127

Most of the metal level (µg/g) in hair is relatively similar for miners from each site except for Tsetsera (Figures 6.2 and 6.3). Miners from this site have relatively higher level of Al, Cr, Fe, Rb, and V in their hair. This result was driven by a 39-year old miner, ALS 1-2-4, from Tsetsera, who had very high concentration of most of the metals. He spent 9 years in the mining operation in this location. He also smoked more than 10 cigarettes a day. In addition, he only remembered 1 word from the memory test. However, it is not clear which factor affected this result. Some miners served more time than he in the mining operation and some miners smoke more than 10 cigarettes a day but their metal concentration are not as high as his.

Metal level in fingernails is very high for miners in Upper and Lower Munhena compare to Tsetsera and Clean Tech, which have relatively similar concentration for most metals. This may indicate that miners from Munhena are exposed to high level of these metals. However, due to limited number of samples, correlation between mining location and high metal level cannot be verified by statistical analysis.

Since health-related agencies can't agree on what levels of metals in hair and fingernail reflects safe-body burdens, the levels found in the miner's can't be directly assessed from a health perspective. However, heavy metal levels in hair and fingernail were compared with non-mining populations using literature data [18]. The miner's hair and fingernail heavy metal levels data was compared with the hair and nail samples of male adults from West Aswan, Egypt and Participants age ranges from 30 to 65 years old. The miner's hair metal level was also compared to hair samples obtain from a non-industrialized region in Poland, Silesian Beskid, where majority of the population are involved in agriculture. Hair samples were collected from participants with ages ranging from 0-80 years old [26]. Comparison is given in Table 6.6. Metal level for miner's hair is highly elevated especially for Cr, Cu, Fe, Mn, and Pb. Concentration of Pb in miner's hair and fingernails are strikingly high compared to non-miners from Egypt and Poland. Concentration of elements Cd in miner's hair and Polish participants are relatively similar. The average concentration of Cu in hair may be relatively the same for miner's Egyptian participants but the standard deviation for miner's Cu is very large. Concentration of Cu in miner's hair ranged from 7.97-40.06 µg/g.

Table 6.6. Comparison of metal levels (µg/g) in hair between miners (this study) and non-mining populations from Egypt [18] and Poland [26].

Element	**Hair**			**Fingernail**	
	Miner	*Egypt*	*Poland*	*Miner*	*Egypt*
Cd	0.452±0.401	0.20±0.08	0.61±1.13	0.729±0.685	0.62±0.19
Co	0.865±0.518		0.44±0.72		
Cr	2.59±3.65		0.60±1.13		
Cu	21.4±10.8	18.2±1.62	7.96±0.12	28.9±27.7	15.34±3.11
Fe	249±3156		45.70±34.73		
Mn	67.9±38.4		2.41±2.24		
Ni	1.69±1.08		0.75±1.15		
Pb	327±588	7.61±1.36	4.99±3.90	546±1008	11.32±2.01

6.5 CONCLUSIONS

The heavy metal concentration in fingernail and hair from miners were higher than non-mining populations, which suggests high metal exposure in artisanal mining communities. Mean Hg levels in the soil near the Clean Tech mine were considerably lower (0.25 µg/g) than upper Munhena (7.1 µg/g), lower Munhena (12.0 µg/g), and Tsetsera (3.1 µg/g). Clean Tech also tended to have lower levels of metals in fingernails. Miners from Munhena had considerably higher metal concentrations in fingernails, whereas Tsetsera miners had higher metal concentrations in hair. Several statistical tests are not amenable to the current data set due to the limited number of samples, nevertheless it appears the Hg-free practices employed at Clean Tech are leading to lower levels of metals in the local environment and in miners.

6.6 ACKNOWLEDGMENTS

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6.7 LITERATURE CITED

[1] Spiegel, S.J., Veiga, M.M. International guidelines on mercury management in small-scale gold mining. *Journal of Cleaner Production* **2010**, 18, 375-385

[2] Anon. Gold Price. Gold Price Pty Ltd. http://goldprice.org/ (accessed April 9, 2014)

[3] United Nations Industrial Development Organization (UNIDO), 2005. Pilot Project for the Reduction of Mercury Contamination Resulting from Artisanal Gold Mining Fields in the Manica District of Mozambique, Final Report, **2005** 1-43. http://www.unites.uqam.ca/gmf/intranet/gmp/countries/mozambique/Moz_Final_Report_Aug_4.pdf (accessed April 9, 2014)

[4] Shandro, J.A., Veiga, M.M., Chouinard, R. Reducing mercury pollution from artisanal gold mining in Munhena, Mozambique. *Journal of Cleaner Production* **2009**, 17, 525-532.

[5] Robins, N.A., N.A. Hagan. Mercury Production and Use in Colonial Andean Silver Production: Emissions and Health Implications. *Environmental Health Perspectives* **2012**, 120 (5), 627-631

[6] Telmer, K.H., Veiga, M.M., World Emissions of Mercury from Artisanal and Small Scale Gold Mining. In: Mercury Fate and Transport in the Global Atmosphere. Springer, New York, **2009** 131-172.

[7] Kiefer, A.M., Drace, K., Gottlieb, S., Coursey, S., Veiga, M.M., Marrumber, P.N., Chapo, M.A.J. Evaluation of mercury content in amalgams from Munhena mine, Mozambique. Journal of Cleaner Production, **2013** (Available online: doi:10.1016/j.jclepro.2013.09.039)

[8] UNEP, 2013. Global Mercury Assessment 2013: Sources, Emissions, Releases and Environmental Transport. UNEP Chemicals Branch, Geneva, Switzerland

[9] World Health Organization (WHO). Mercury and Health. http://www.who.int/mediacentre/factsheets/fs361/en/ (Accessed April 10, 2014)

[10] Saunders, J.E., B.G. Jastrzembski, J.C. Buckey, D. Enriquez, T.A. MacKenzie, and M.R. Karagas. Hearing Loss and Heavy Metal Toxicity in a Nicaraguan Mining Community:Audiological Results and Case Reports. *Audiol Neurotol* **2013**,18, 101–113

[11] United Nations Environment Programme. Reducing mercury use in artisanal and small-scale gold mining: A Practical Guide. **2012.** http://www.unep.org/chemicalsandwaste/Portals/9/Mercury/Documents/ASGM/Techdoc/UNEP%20Tech%20Doc%20APRIL%202012_120608b_web.pdf (Accessed: June 6, 2014)

[12] Wickre JB, Folt CL, Sturup S, Karagas MR: Environmental exposure and fingernail analysis of arsenic and mercury in children and adults in a Nicaraguan gold mining community. *Arch Environ Health* **2004**, 59, 400–409.

[13] Oyuntsetseg, B., K. Kawasaki, M. Watanabe, and B. Ochirbat. Review Article Evaluation of the Pollution by Toxic Elements around the Small-ScaleMining Area, Boroo, Mongolia. *ISRN Analytical Chemistry* **2012**, Article ID 153081, 1-99

[14] Barbosa Jr., F., M. Fillion, M. Lemire, C.J. Passos, J.L. Rodriguez, A. Philibert, J.R. Guimarães, D. Mergler. Elevated blood levels in a riverside population in the Brazilian Amazon. *Environ. Res*. **2009**, 109, 594-599

[15] Basu, N., D. Nam, E. Kwansaa-Ansah, E.P. Renne, J.O. Nriagu Multiple metals exposure in a small-scale artisanal gold mining community. *Environ. Res*. **2011**, 111, 463-467

[16] Saunders, J.E., B.G. Jastrzembski, J.C. Buckey, D. Enriquez, T.A. MacKenzie, and M.R. Karagas. Hearing Loss and Heavy Metal Toxicity in a Nicaraguan Mining Community: Audiological Results and Case Reports. Audiol Neurotol 2013, 18, 101–113

[17] Abdulrahman, F.I., J.C. Akan, Z. M. Chellube, M. Waziri Levels of Heavy Metals in Human Hair and Nail Samples from Maiduguri Metropolis, Borno State, Nigeria. *World*

Environment **2012**, 2(4), 81-89

[18] Rashed, M.N., and F. Hossam. Heavy Metals in Fingernails and Scalp Hair of Children, Adults and Workers from Environmentally Exposed Areas at Aswan, Egypt. *Environmental Bioindicators,* **2007**, 2, 131–145

[19] Shan, U., and N. Ikram. Heavy metals in human scalp hair and nail samples from Pakistan: influence of working and smoking habits. *IJCBS,* **2012**, 1, 54-58

[20] Hayashi, M., K. Yamamoto, M. Yoshimura, H. Hayashi, and A. Shitara. Cadmium, Lead, and Zinc Concentrations in Human Fingernails. *Bull. Environ. Contam. Toxicol*. **1993**, 50, 547-553

[21] Kosanovic, M., and M. Jokanovic. Quantitative analysis of toxic and essential elements in human hair. Clinical validity of results. *Environ Monit Assess*, **2011**, 174, 635–643

[22] Wenning R. Potential problems with the interpretation of hair analysis results. *Ensic Sci Int*, **2000** 107, 5–12.

[23] Drace, K, A.M. Kiefer, M.M Veiga, M.K. Williams, B. Ascari, K.A. Knapper, K.M. Logan, V.M. Breslin, A. Skidmore, D.A. Bolt, G.G. Geist, L. Reidy, J.V. Cizdziel. Mercury-free, small-scale artisanal gold mining in Mozambique: utilization of magnets to isolate gold at clean tech mine. *Journal of Cleaner Production* **2012**, 32, 88-95

[24] Drace, D., and A. Kiefer. Mercer Mission: Mozambique 2011 Summary Report. Mercer University. **2011**, 1-7

[25] World Bank Group. Mercury Pollution Prevention and Abatement Handbook. **2008**. http://www.ifc.org/wps/wcm/connect/0b94c30048855447b21cf26a6515bb18/HandbookMercury.pdf?MOD=AJPERES&CACHEID=0b94c30048855447b21cf26a6515bb18 Accessed: April 10, 2014.

[26] Nowak, B. Contents and relationship of elements in human hair for a non-industrialised population in Poland. *The Science of the Total Environment* **1998**, 209, 59-68

APPENDICES

APPENDIX A: ELEMENTAL PROFILES OF EPHEDRA FROM U.S. AND CHINA

Table 5.5. Elemental profiles of ephedra from U.S. and China

Species	Province/ State	Country	Cd	Ba	Pb	U	Rb	Sr	V	Mn	Fe	Co	Ni	Cu	Zn	Mg
E. equisetina	Hebie	China	0.220	20.2	0.725	0.012	1.232	73	0.606	22.5	351	0.126	0.99	2.76	21.43	3984
E. equisetina	Nei Mengol	China	0.096	8.9	0.164	0.022	0.705	130	0.688	13.1	337	0.123	0.65	2.33	7.52	2797
E. equisetina	Nei Mengol	China	0.073	12.3	0.547	0.036	2.935	185	0.979	23.1	485	0.288	2.00	3.79	13.73	4352
E. equisetina	Xinjiang	China	0.074	11.9	0.562	0.035	1.450	95	1.181	27.1	547	0.220	1.06	2.64	15.16	2691
E. intermedia	Beijing	China	0.036	7.1	0.157	0.017	1.762	138	0.374	19.2	224	0.094	0.59	2.70	10.39	1946
E. intermedia	Gansu	China	0.050	21.2	0.346	0.020	1.213	130	0.334	11.2	228	0.074	0.48	2.73	7.30	2970
E. intermedia	Gansu	China	0.059	9.3	0.276	0.009	0.702	199	0.284	14.7	193	0.067	0.53	3.31	11.37	3597
E. intermedia	Gansu	China	0.146	25.0	1.044	0.032	1.127	230	0.944	24.3	454	0.189	1.14	2.37	12.44	6099
E. intermedia	Gansu	China	0.102	22.9	0.584	0.018	0.974	202	0.557	17.5	310	0.114	0.90	3.37	19.39	3699
E. intermedia	Gansu	China	0.064	9.5	0.263	0.014	1.251	131	0.658	16.9	353	0.118	0.62	3.19	11.32	2989
E. intermedia	Gansu	China	0.065	9.6	0.202	0.007	0.920	218	0.221	14.4	151	0.050	0.43	2.87	9.11	2923
E. intermedia	Gansu	China	0.056	6.1	0.388	0.019	1.125	179	0.520	19.3	318	0.164	1.39	2.41	8.81	2983
E. intermedia	Hebei	China	0.061	6.3	0.248	0.014	0.964	104	0.675	17.9	413	0.143	0.79	3.10	25.67	2258
E. intermedia	Nei Mengol	China	0.083	8.8	0.243	0.022	0.649	111	0.609	13.8	297	0.126	1.10	2.32	5.36	2970
E. intermedia	Nei Mengol	China	0.149	11.8	0.459	0.040	1.196	158	1.067	18.1	478	0.228	3.35	4.06	8.82	3343
E. intermedia	Ningxia	China	0.052	35.4	1.660	0.029	1.063	196	0.918	23.2	407	0.164	1.11	2.59	10.02	4504
E. intermedia	Ningxia	China	0.123	20.8	0.652	0.016	1.199	153	0.725	33.5	414	0.830	27.43	3.24	6.07	2833
E. intermedia	Ningxia	China	0.064	10.3	0.357	0.019	1.320	100	0.659	21.8	422	0.168	1.17	3.41	11.09	2638
E. intermedia	Ningxia	China	0.091	9.1	0.366	0.018	1.061	145	0.785	16.0	505	0.170	0.94	2.90	7.99	3138
E. intermedia	Qinghai	China	0.067	7.8	0.373	0.049	1.792	116	1.387	17.4	639	0.243	1.14	2.78	10.09	1425
E. intermedia	Qinghai	China	0.041	9.8	0.243	0.025	1.547	172	0.621	26.7	335	0.139	0.83	2.54	8.54	2115
E. intermedia	Shanxi	China	0.078	7.4	0.195	0.015	0.605	89	0.501	14.6	251	0.118	0.97	2.32	6.57	2467
E. przewalskii	Gansu	China	0.108	22.4	0.806	0.053	2.075	133	2.192	31.1	930	0.377	1.76	4.56	8.32	1516
E. przewalskii	Nei Mengol	China	0.071	24.1	1.164	0.113	4.233	102	5.305	57.3	1955	0.871	3.98	4.92	11.43	2582
E. przewalskii	Nei Mengol	China	0.116	30.9	1.096	0.048	1.622	105	2.043	31.3	893	0.333	1.60	2.52	6.52	2226
E. przewalskii	Nei Mengol	China	0.102	23.1	1.443	0.058	2.195	105	2.781	29.1	1214	0.444	1.90	3.23	9.07	1684
E. przewalskii	Nei Mengol	China	0.003	39.0	2.145	0.061	2.145	107	2.556	35.5	1106	0.417	1.99	2.95	9.33	2271
E. przewalskii	Qinghai	China	0.175	19.5	1.334	0.131	3.313	146	3.047	32.1	1472	0.540	2.31	4.18	16.22	3222
E. przewalskii	Shanxi	China	0.062	22.6	1.254	0.076	2.851	91	3.497	35.2	1258	0.551	2.36	2.98	9.21	1771
E. przewalskii	Xinjiang	China	0.092	15.1	0.477	0.045	2.027	131	1.466	31.3	661	0.255	1.12	3.17	7.96	1805
E. sinica	Beijing	China	0.089	20.1	0.558	0.012	1.489	108	0.660	19.9	363	0.139	0.62	3.78	9.25	2894
E. sinica	Hebei	China	0.079	28.5	1.028	0.017	1.960	191	0.785	26.0	497	0.164	0.81	4.46	11.03	3603
E. sinica	Hebei	China	0.053	30.7	0.461	0.009	1.057	94	0.609	21.1	328	0.131	0.91	3.11	9.32	2442
E. sinica	Hebei	China	0.082	14.4	0.407	0.039	3.245	30	2.237	26.3	1101	0.467	2.18	6.19	15.18	931

Table 5.5. Elemental profiles of ephedra from U.S. and China (continued)

Species	Province/ State	Country	Cd	Ba	Pb	U	Rb	Sr	V	Mn	Fe	Co	Ni	Cu	Zn	Mg
E. sinica	Nei Mengol	China	0.045	20.4	0.368	0.009	5.857	61	0.463	22.1	303	0.144	1.24	4.98	10.33	2644
E. sinica	Neimeng	China	0.085	32.2	0.802	0.015	2.464	183	0.697	29.6	326	0.139	0.68	3.89	10.59	3243
E. sinica	Neimeng	China	0.096	20.5	0.784	0.043	1.009	147	0.975	24.5	437	0.217	1.91	2.54	10.89	2453
E. sinica	Neimeng	China	0.075	57.6	0.611	0.051	8.661	156	0.589	71.8	278	0.124	0.73	3.40	13.00	4163
E. sinica	Ningxia	China	0.184	25.4	0.782	0.035	1.579	114	1.199	24.4	629	0.212	1.06	3.47	20.37	3079
E. sinica	Ningxia	China	0.117	20.0	0.659	0.019	0.872	115	0.780	20.9	465	0.150	0.60	3.76	21.02	2941
E. sinica	Ningxia	China	0.140	23.5	0.615	0.040	1.092	109	0.965	20.4	452	0.160	0.80	3.67	15.71	3529
E. sinica	Ningxia	China	0.047	20.7	0.446	0.028	0.612	151	0.328	14.9	216	0.061	0.28	2.52	6.25	3930
E. sinica	Shanxi	China	0.061	10.3	0.540	0.016	0.349	167	0.393	12.6	210	0.066	0.31	1.58	5.73	3216
E. sinica	Shanxi	China	0.034	18.7	0.553	0.010	1.393	52	0.500	17.8	314	0.101	1.03	2.75	10.55	1749
E. sinica	Xining	China	0.046	7.6	0.506	0.015	1.014	62	0.676	17.6	395	0.121	0.25	4.26	26.46	1157
E. antisyhilitca	TX	US	0.217	27.4	0.346	0.015	0.783	115	0.549	16.9	192	0.106	2.30	2.92	35.27	1818
E. aspera	CA	US	0.078	27.4	0.081	0.007	0.312	62	0.138	16.3	55	0.034	0.51	1.77	8.73	1338
E. aspera	TX	US	0.027	9.1	0.110	0.005	0.701	70	0.117	23.3	73	0.029	0.42	1.40	9.98	1400
E. aspera trifurca	TX	US	0.036	2.4	0.161	0.014	0.861	275	0.405	25.4	118	0.121	0.27	2.80	5.21	1483
E. californica	CA	US	0.045	34.8	0.251	0.015	0.706	94	0.402	28.6	170	0.080	0.47	1.62	7.14	1581
E. coryi	NM	US	0.070	24.7	0.602	0.012	1.639	383	0.543	22.7	240	0.211	1.52	2.73	6.54	1719
E. cutleri	NM	US	0.095	53.3	0.230	0.019	0.644	128	0.329	39.3	154	0.082	0.66	2.22	25.79	1703
E. fasiculata	AZ	US	0.071	23.8	0.137	0.010	1.135	105	0.291	24.8	125	0.089	0.94	2.65	15.22	1364
E. fasiculata	AZ	US	0.071	55.3	0.174	0.010	1.119	137	0.318	13.2	127	0.066	0.62	1.58	5.10	1227
E. fasiculata	AZ	US	2.733	50.1	0.116	0.006	0.644	170	0.133	22.1	60	0.026	0.22	0.93	2.76	1608
E. nevadensis	AZ	US	0.048	8.1	0.091	0.022	1.124	116	0.134	17.3	81	0.070	1.16	1.80	18.24	2188
E. nevadensis	AZ	US	0.033	42.3	0.074	0.004	0.490	104	0.101	10.2	86	0.025	1.12	1.63	6.14	2065
E. nevadensis	CA	US	0.048	53.1	0.080	0.004	1.577	121	0.214	25.2	114	0.046	0.75	2.19	11.87	2523
E. nevadensis	CA	US	0.081	29.3	0.081	0.007	0.523	163	0.112	38.3	77	0.160	2.09	3.07	25.67	2304
E. nevadensis	NM	US	0.049	41.5	0.174	0.007	0.603	88	0.235	8.0	121	0.057	2.93	2.33	5.44	1491
E. pedunculata	TX	US	0.103	56.4	0.103	0.005	1.608	68	0.242	18.6	112	0.114	5.41	4.19	5.83	1699
E. torreyana	NM	US	0.043	31.9	0.121	0.007	0.815	199	0.260	22.0	110	0.060	0.55	2.54	8.05	2388
E. trifurca	NM	US	0.089	13.0	0.164	0.010	0.752	126	0.222	17.4	132	0.131	0.65	2.16	5.70	2173
E. trifurca	TX	US	0.081	2.7	0.114	0.008	1.040	241	0.266	29.1	106	0.239	0.25	3.80	5.86	1339
E. trifurca	TX	US	0.108	42.9	0.116	0.007	1.122	220	0.236	32.5	117	0.050	0.66	1.82	10.74	1852
E. trifurca	TX	US	0.083	2.6	0.123	0.010	1.026	240	0.253	27.8	101	0.245	0.22	3.59	5.61	1347
E. viridis	CA	US	0.051	30.3	0.271	0.015	0.814	109	0.727	22.7	305	0.131	0.88	2.00	5.92	2293
E. viridis	CA	US	0.113	41.0	0.230	0.014	0.572	139	0.632	50.8	278	0.160	1.48	2.30	19.04	3430

APPENDIX B: INFORMATION ABOUT THE MINERS FROM MANICA DISTRICT, MOZAMBIQUE, WHO PARTICIPATED AND PROVIDED FINGERNAIL AND NAIL SAMPLES USED IN THIS STUDY

Table 6.7. Detailed information about the miners from Manica District, Mozambique, who participated and provided fingernail and nail samples used in this study

Sample Name	Location	Age	Gender	Weeks in mine	Job in mine	time spent in mining	time in this mine	jobs before
ALS 1-1-1	lower Munhena	30	M	3 weeks/month	everything	3 years	3 years	Farming
ALS 1-1-2	lower Munhena	46	M	7 months	everything	4 years	4 years	Security
ALS 1-1-3	lower Munhena	27	M	3 weeks/month	everything	3 years	1 year	Seller
ALS 1-1-4	lower Munhena	26	M	3 weeks/month	everything	6 years	6 yars	None
ALS 1-1-5	lower Munhena	45	M	6 months/yr	everything	3 years	3 years	Farming
ALS 1-1-6	lower Munhena	45	M	12 months	everything	6 years	6 years	Plumbing
ALS 1-1-7	lower Munhena	35	M	3 weeks/month	everything	5 years	5 years	Farming
ALS 1-1-8	Upper Munhena	49	M	4 weeks	everything	12 years	7 years	Farming
ALS 1-1-9	Upper Munhena	23	M	2 weeks/month	everything	4 years	4 years	Farming
ALS 1-1-10	Upper Munhena	36	M	All	everything	6 years	4 years	None
ALS 1-1-11	Upper Munhena	51	M	2 weeks/month	everything	11 years	1 years	None
ALS 1-1-13	Upper Munhena	30	M	1 week/month	everything	5 years	5 years	Farming
ALS 1-1-14	Upper Munhena	41	M	1 month/year	everything	6 years	5 years	Farming
ALS 1-1-15	Upper Munhena	35	M	7 months	everything	5 years	5 years	Seller
ALS 1-1-16	Upper Munhena	21	F	Always	everything	5 years	5 years	None
ALS 1-2-1	Tsetsera	22	M	12 months	everything	2 years	2 years	None
ALS 1-2-2	Tsetsera	25	M	3 months/year	digger	11 years	10 years	Seller
ALS1-2-4	Tsetsera	31	M	6 months/year	everything	9 years	9 years	Farming
ALS1-2-5	Tsetsera	42	M	All	everything	8 years	8 years	None
KAK 1-7-1	Clean Tek	30	M	3 weeks/month	emulcifier	3 years	3 weeks	None
KAK 1-7-2	Clean Tek	23	M	52 weeks	Rock extraction	6 years	6 years	Domestic worker
KAK 1-8-1	Clean Tek	27	M	4 months/year	demolition hammer operator	1 yr 8 mon	1 yr 8 mon	Random
KAK 1-8-2	Clean Tek	25	M		demolition hammer operator	1 year	1 year	driver
KAK 1-8-2	Clean Tek	25	M		demolition hammer operator	1 year	1 year	driver

Table 6.7. Detailed information about the miners from Manica District, Mozambique, who participated and provided fingernail and nail samples used in this study (continued)

Sample Name	Alcohol today?	Cigarettes per day	fish per week	dental fillings?	Mentallic taste in mouth?	Mouth water?	Headaches?	Words remembered?
ALS 1-1-1	No	1 to 3	1 to 3	Yes	No	Yes	Yes	1-table
ALS 1-1-2	Yes	>10	>10	No	No	No	Yes	2-table, cloud
ALS 1-1-3	No	0	4 to 6	No	No	Yes	Yes	2-table, green tree
ALS 1-1-4	No	0	1 to 3	No	No	No	Yes	0
ALS 1-1-5	No	0	1 to 3	Yes	No	No	Yes	0
ALS 1-1-6	Yes	4 to 6	>10	Yes	Yes	No	Yes	3-table, cloud, green tree
ALS 1-1-7	No	4 to 6	1 to 3	Yes	No	Yes	Yes	1-table
ALS 1-1-8	No	0	0	Yes	Yes	Yes	Yes	3-table, cloud, green tree
ALS 1-1-9	No	0	1 to 3	No	No	No	No	1-table
ALS 1-1-10	No	0	0	Yes	No	No	Yes	3-table, cloud, green tree
ALS 1-1-11	No	7 to 10	4 to 6	Yes	No	No	Yes	0
ALS 1-1-13	No	0	1 to 3	Yes	No	Yes	No	1-green tree
ALS 1-1-14	No	4 to 6	0	Yes	No	Yes	Yes	0
ALS 1-1-15	No	7 to 10	1 to 3	Yes	Yes	Yes	No	0
ALS 1-1-16	No	0	1 to 3	Yes	Yes	Yes	Yes	1-green tree
ALS 1-2-1	No	0	4 to 6	No	No	No	No	2-cloud, green tree
ALS 1-2-2	Yes	0	1 to 3	Yes	Yes	Yes	Yes	0
ALS1-2-4	Yes	>10	1 to 3	No	No	No	Yes	1-table
ALS1-2-5	No	7 to 10	1 to 3	Yes	No	Yes	No	1-green tree
KAK 1-7-1	No	0	1 to 3	No	No	No	No	N/A
KAK 1-7-2	No	0	1 to 3	No	No	No	N0	N/A
KAK 1-8-1	No	0	1 to 3	No	No	No	No	3-table, cloud, green tree
KAK 1-8-2	No	1 to 3	1 to 3	No	No	No	No	3-table, cloud, green tree

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EDUCATION

University of Mississippi
Ph.D in Chemistry (GPA: 3.78; August 2014)
University, MS 38677
Dissertation: Elemental Fingerprinting Using Inductively Coupled Plasma Mass Spectrometry and Chemometrics: Application to Environmental Science and Provenance Studies.
Advisor: Dr. James V. Cizdziel

Central Mindanao University
B.S. in Chemistry (May 2006)
Musuan, Bukidnon, 8709 Philippines
Thesis: Physico-chemical and MPN analysis of the drinking water of San Martin, Malaybalay City, Bukidnon
Advisor: Dr. Lordino Cabigon

RESEARCH EXPERIENCE

University of Mississippi, University, MS 38677
Graduate Researcher, 2009 – 2014

- Developed and validated robust and accurate methods for analyzing fish muscle plugs, botanicals, human hair and fingernails, drywalls, gypsum, and soil for fingerprinting by using Inductively-Coupled Plasma Mass Spectrometry (ICPMS)
- Determined the feasibility and discriminatory power of elemental profiling for distinguishing farm-raised catfish and ephedra from various geographic locations in the US and elsewhere
- Evaluated the feasibility of using elemental fingerprinting as a reliable forensic tool in distinguishing soil samples from different geographic locations.
- Evaluated the feasibility of several methods (elemental fingerprinting, FTIR, and GC-MS) to identify and provenance contaminated drywall
- Evaluated data using univariate and multivariate statistics such as Discriminant Analysis and Principal Component analysis
- Determined the amount of mercury in soil using ICPMS to assess the mining industry in Mozambique
- Experience in troubleshooting and maintenance of instruments

Central Mindanao University, Musuan, Bukidnon 8709 Philippines
Undergraduate Thesis, 2005-2006

- Analyzed the physico-chemical characteristic of drinking water in the Philippines
- Examined the microbiological properties of drinking water

SKILLS AND TECHNIQUES

- Digestion of various biological samples using open-vessel and microwave-digestion systems
- Expertise in elemental analysis using quadrupole and sector-field ICPMS
- Proficient in the use of MALDI-MS, FTIR, Fluorescence Spectroscopy, GCMS, UV-Vis spectroscopy, and DMA
- Proficient in univariate and multivariate statistical analysis

TEACHING EXPERIENCE

University of Mississippi, University, MS 38677
Teaching Assistant, 2009 – 2013

- **Instrumental Analysis** (Spring 2010, 2011, 2012)
  - Taught various methods of analysis using instruments such as Matrix Assisted Laser Desorption Ionization Mass Spectroscopy (MALDI-MS), Fourier Transformed Infrared Spectroscopy (FTIR), Ultraviolet–Visible Spectroscopy (UV-Vis), Fluorescence Spectroscopy, Gas Chromatography-Mass Spectroscopy (GC-MS) and Direct Mercury analyzer (DMA)
  - Taught analytical processes such as sample preparation, sample analysis, data analysis, data interpretation and report writing
- **Quantitative Analysis** (Fall 2010)
  - Taught a variety of quantitative analytical techniques such as titration, gravimetry, iodometry, potentiometry, spectrophotometry and the use of an ion-selective electrode.
  - Taught data analysis and interpretation from such analyses
- **General Chemistry** (Spring and Fall 2009)
  - Taught basic chemistry laboratory skills and analytical techniques

RELATED PROFESSIONAL EXPERIENCE

Krispy Foods Incorporated, Tablon, Cagayan de Oro City, Philippines
Quality Control / Quality Assurance Analyst, Aug. 2006 – May 2007

- Analyzed the critical control points of raw and in-process materials
- Evaluated in-process materials and the final products
- Managed and coordinated duties of the production staff

Busco Sugar Milling Corporation, Quezon, Bukidnon, Philippines
Chemistry Internship, April – May 2006

- Analyzed critical control points of the raw and in-process materials
- Performed boiler-water and microbiological analysis

PUBLICATIONS

- Kevin Drace, Adam M. Kiefer, Marcello M. Veiga, Matt K. Williams, Benjamin Ascari, Kassandra A. Knapper, Kaitlyn M. Logan, Vanessa M. Breslin, Ashley Skidmore, Daniel A. Bolt, Grant Geist, Lorlyn Reidy, James V. Cizdziel. (2012) Mercury-free, Small-scale Artisanal Gold Mining in Mozambique: Utilization of Magnets to Isolate Gold at Clean Tech Mine. *Journal of Cleaner Production* **32:** 88-95
- Kaixuan Bu, James Cizdziel, and Lorlyn Reidy. (2012) Analysis of Herbal Supplements for Selected Dietary Minerals and Trace Elements by Laser Ablation and Solution-Based ICPMS. *Microchemical Journal* (In press: DOI 10.1016/j.micro.2012.07.011)
- Lorlyn Reidy, Kaixuan Bu, Murrell Godfrey, and James Cizdziel. Elemental Fingerprinting of soils using ICPMS and multivariate statistics: A study for- and by- forensic chemistry majors. *Forensic Science International* (Accepted for publication on August 19, 2013)
- Lorlyn Reidy, Rachel Williams, Derek Bussan, James V. Cizdziel. Elemenal Fingerprinting of Gypsum Drywall using SF-ICPMS and Multivariate Statistics: Identifying "Chinese Drywall" *International Journal of Environmental Analytical* Chemistry (In preparation)
- Lorlyn P. Reidy, James V. Cizdziel, Michael Ketterer, John S. Brewer, Menghe Li, Claude Boyd, Craig Tucker. Fingerprinting of Farm-Raised Catfish (*Ictalurus punctatus*) using Trace Elements and $^{87}Sr/^{86}Sr$ Ratios Determined by ICPMS. *Journal of Agricultural and Food Chemistry* (In preparation)

SEMINARS / PRESENTATIONS

Talks

- "Fingrprinting of Farm-Raised Catfish (*Ictalurus Punctatus*) using Trace Elemental Profiles and Strontium Isotope Ratios Determined by ICPMS" – 39th NOBCChE National Meeting (Washington, DC, September 2012)
- "Determining the Origin of Foodstuffs by Elemental Fingerprinting Using ICPMS and Chemometrics: Preliminary Results from a Catfish Study" - 2011 Mid-South ICP-MS Symposium (University of Mississippi, August 2011)
- "Authentication and Tracing Geographical Origin of Samples Using Isotope Ratios and Elemental Profiles" – Department of Chemistry and Biochemistry Seminars (University of Mississippi, March 2010)

Poster Presentations

- "Elemental Fingerprinting of Soils using ICPMS and Multivariate Statistics" - The National Center for Natural Products Research Annual Poster Session (University of Mississippi, November 2012)
- "Fingerprinting of Farm-raised Catfish Using Elemental Profiles Determined by Inductively Coupled Plasma Mass Spectrometry and Chemometrics" - Annual Symposium of Mississippi Academy of Science (Hattiesburg, MS, February 2012)
- "Fingerprinting of Fish using Elemental Profiles Determined by Inductively Coupled Plasma Mass Spectrometry and Chemometrics" - The National Center for Natural Products Research Annual Poster Session (University of Mississippi, October 2011)
- **"**Fingerprinting of Catfish Using Elemental Profiles Determined by Inductively Coupled Plasma Mass Spectrometry and Chemometrics" - Poster and Research Day 2011 held by Graduate Student Council (University of Mississippi, April 2011)

AWARDS

- Dissertation Fellowship – University of Mississippi (Fall 2013)
- Advancing Science Award – NOBCChe (2012)
- Graduate Assistantship – University of Mississippi (January 2009 – present)
- Travel Grant – University of Mississippi (2012)
- Graduate Student Council Research Award – University of Mississippi (2011)
- Licensed Chemist – Philippines (September 2007 – present)
- Dean's List / Academic Scholar – Central Mindanao University (2002 – 2005)

LEADERSHIP/SERVICE

- **Senator** – Graduate Student Council, University of Mississippi (March 2012 – May 2013)
- **Committee Member** – Student Affairs, Graduate Student Council, University of Mississippi (March 2012 – May 2013)
- **Vice President** – Central Mindanao University Chemical Society (2005 – 2006)
- **Associate Secretary** – Central Mindanao University Chemical Society (2004 – 2005)

PROFESSIONAL AFFILIATIONS

- American Chemical Society (September 2012 – present)
- National Organization for the Advancement of Black Chemists and Chemical Engineers (NOBCChe) (February 2012 – present)
- Mississippi Academy of Science (February 2012 – present)