



# FIRMS

Forensic Isotope Ratio  
Mass Spectrometry



## 7<sup>th</sup> Conference of the Forensic Isotope Ratio Mass Spectrometry Network

16-19 September 2019

San Michele all'Adige (TN)

Italy



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Forensic Isotope Ratio  
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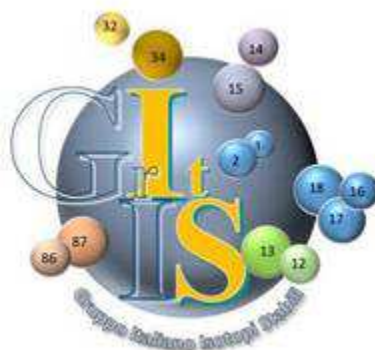
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## WELCOME

The 7<sup>th</sup> Conference of the Forensic Isotope Ratio Mass Spectrometry Network (FIRMS 2019) will be held in San Michele all'Adige (Italy), in region Trentino-Alto Adige/Südtirol which is located in the heart of the Italian Alps, well renowned for its excellent wine, famous fruits, and beautiful Dolomites mountains.

FIRMS 2019 is an opportunity for scientists, technicians, and experts interested in forensic application of stable isotope analysis to discuss a variety of topics (including illicit drugs; explosives; packaging materials; hair, fingernails and other human remains; foods and beverages as well as more general aspects of forensic isotope analysis), and to connect and share research in a welcoming forum.



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## SCIENTIFIC COMMITTEE:

**Federica Camin** FIRMS and FEM

**Jim Carter** FIRMS and Queensland Health

**Lesley Chesson** FIRMS and DPAA Laboratory

**Max Coleman** FIRMS and JPL

**Philip Dunn** FIRMS and LGC Ltd

**John Howa** FIRMS and IsoForensics Inc.

**Luana Bontempo** FEM



## PROGRAM:

### 17 SEPTEMBER

9.00 - 9.30 Registration and Poster installation

9:30 - 10.00 Welcome address

#### **Session 1: Isoscape/origin traceability**

10.00 - 11.00 *Jason West* (Department of Ecosystem Science and Management, Texas A&M University) **Progress and opportunities for the use of isoscapes in forensic science**

11.00 - 11.30 Coffee break

11.30 - 11.50 *Gabriel Bowen* (Department of Geology & Geophysics and Global Change and Sustainability Center; University of Utah)

#### **Groundwater isoscapes for the contiguous USA**

11.50 - 12.10 *Sarah Magozzi* (Department of Geology and Geophysics, University of Utah)

#### **Combining models of environment, behavior, and physiology to predict tissue hydrogen and oxygen isotope variance among individuals**

12.10 - 12.30 *Fábio José Viana Costa* (Brazilian Federal Police)

#### **Pinpointing timber origin with assignment tests using foliar isoscapes of Amazonian trees**

12.30 - 12.50 *Florian Cueni* (University of Basel, DUW – Botany)

#### **Predicting the Geographic Origin of Berries Using Mechanistic Oxygen and Hydrogen Stable Isotope Models**

12.50 - 13.10 Discussion

13.10 - 14.00 Lunch

#### **Session 2: Human Remains**

14.40 - 15.00 *Lesley A. Chesson* (PAE, DPAA Laboratory, Joint Base Pearl Harbor-Hickam)

#### **Development of the DPAA's isotope testing program for unidentified human remains**

15.20 – 15.40 *Gregory Eugene Berg* (DPAA Laboratory, Joint Base Pearl Harbor-Hickam)

#### **Classification(s) of Isotope Signatures in Unidentified Human Remains**

14.00 - 14.40 *Clement P. Bataille* (University of Ottawa)

#### **Sulphur Isotopes in Human Hair: Diet vs. Provenance**

15.00 - 15.20 *Christy J. Mancuso* (Department of Biology, University of New Mexico)

#### **Using Amino Acid Specific Stable Isotope Ratios in Human Scalp Hair for Residency Determination**

15.40 - 16.10 Coffee break

16.10 - 16.30 *Melanie Marie Beasley* (Department of Anthropology, Purdue University)

#### **Using $\delta^{15}\text{N}$ from Soldier Fly Larvae for Postmortem Interval (PMI) Estimation**

16.30 - 16.50 *Gabriela Bielefeld Nardoto* (Department of Ecology, University of Brasília)

#### **New perspectives in human forensic anthropology: linking social dimensions and diet through the stable isotopes in fingernails**

16.50 - 17.10 *Tiffany B. Saul* (Forensic Institute for Research and Education, Middle Tennessee State University)

#### **Postmortem Preservation of Isotope Ratios of Human Hair**

17.10 - 17.30 Discussion

17.30 Poster session and Aperitif

18 SEPTEMBER

**Session 3: Food**

8.30 - 9.00 Registration

9.00 - 9.40 *Laura Gambino* (Department of Central Quality Inspection and Fraud Repression of Agri-Food Products (ICQRF) Italian Ministry of Agricultural, Food, Forestry and Tourism Policies (MIPAAFT) Laboratory of Catania)

**Forensic application of stable isotope ratios analysis in official food control: Activity carried out by the ICQRF-Laboratory**

9.40-10.00 *Pavel A. Solovyev* (Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach)

**IRMS, SNIF-NMR and Proton NMR in Wine Analysis**

10.00 - 10.20 *Doris Potočník* (Department of Environmental Sciences, Jožef Stefan Institute)

**Development of new food matrix reference materials for stable isotope analysis**

10.20 - 10.40 *Jose Manuel Moreno-Rojas* (Department of Food Science and Health. Andalusian Institute of Agricultural and Fisheries Research and Training, IFAPA)

**The use of stable isotope ratios for the authentication of agrifood industry products in Andalusia**

10.40 - 11.10 Coffee break

11.10 - 11.30 *Syahidah Akmal Muhammad* (Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia)

**Addressing the unfulfilled codex standard for honey for stingless bee honey through dehumidifying process**

11.30 – 11.45 Discussion

12.00 - 13.00 Visit of the lab

**Session 4: QC method/sponsors**

14.00 - 14.40 *Kylie Jones* (Forensics, Specialist Operations, Australian Federal Police)

**Isotopic Databases for Operational Forensic Casework - Current Focus, Casework Examples and Future Plans for IRMS within the Australian Federal Police Laboratory**

14.40 - 15.00 *Philip J. H. Dunn* (National Measurement Laboratory, LGC Ltd)

**The FIRMS PT Scheme: What can be learned about inter-laboratory performance?**

15.00 - 15.20 *Gwyneth Gordon* (School of Earth & Space Exploration, Arizona State University)

**From hydrogen to uranium and back: QA/QC and reporting for radiogenic and heavy mass-dependent isotopes**

15.20 - 15.40 *Jim Ehleringer* (School of Biological Sciences, University of Utah)

**Stable isotope evidence introduced into U.S. courts**

15.40 - 16.10 Coffee break

16.10-16.30 *Mario Tuthorn* (Thermo Fisher Scientific)

**Advances in multi-element isotopic bulk analysis for forensic applications**

16.30-16.50 *Mike Seed* (Elementar UK Ltd)

**Introducing new quality control features for stable isotope laboratories using Elementar instruments and software**

16.50-17.10 Discussion

18.00

Social dinner

19 SEPTEMBER

**Session 5: Drugs /environmental contamination**

8.30 - 9.00 Registration

9.00 - 9.20 *Frank Hülsemann* (German Sport University Cologne, Institute for Biochemistry)

**Atypical  $\delta^{13}\text{C}$  values of urinary norandrosterone in doping control analysis**

9.20 - 9.40 *Loredana Iannella* (Laboratorio Antidoping, Federazione Medico Sportiva Italiana)

**The IRMS anti-doping analysis: applications and current challenges**

9.40 - 10.00 *Carlos Eduardo Souto de Oliveira* (EFS Pesquisa e Inovação, Brasil)

**Application of traditional (Pb, Sr, Nd, C) and non-traditional (Zn, Cu, Fe, Cl) isotopes to discriminate and investigate pollutant sources in environmental contamination**

10.00 - 10.20 *Thomas P. Doherty* (Picarro Inc)

**A Spectral Analysis Method to Eliminate Methanol and Ethanol Interference with CRDS Measurements of Water Isotope Ratios**

10.20 - 10.50

Coffee break

10.50 - 11.10 *Denis Werner* (Ecole des sciences criminelles (School of criminal justice, University of Lausanne)

**Source inference of gasoline traces: implementation of a multiblock method to combine data from MS and IRMS**

11.20 - 11.40 *Concetta Pironti* (Department of Chemistry and Biology, University of Salerno)

**Biodegradation of commercial surfactants: identification of degradations products by stable isotope carbon composition**

11.40 - 12.00 *Lisette M. Kootker* (Geology & Geochemistry cluster, Vrije Universiteit Amsterdam,)

**Spatial variations in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in modern human dental enamel and tap water from the Netherlands: implications for forensic provenance studies**

11.50 - 12.10

Discussion and Closing remarks



# ORAL PRESENTATION ABSTRACTS



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# **Progress and opportunities for the use of isoscapes in forensic science**

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**Jason B. West**

Dept. of Ecosystem Science & Management Texas A&M University

Since the publication of the first text on isoscapes in 2010, there has been a rapid increase in the development of data sets, modeling and applications of spatial variation in stable isotope ratios. Across a large number of disciplines efforts to describe and in some cases quantitatively model spatial variation have yielded new maps of the variation, insight to processes underlying it and approaches to using these new surfaces to infer important aspects of various systems. This work combines knowledge of processes driving isotope variation across landscapes and spatially explicit sampling that permits interpolation approaches. I will discuss the expanding role isoscapes have come to play in inferring the geographic origin and other aspects of materials and highlight examples relevant to forensics where the development of both mechanistic model predictions and spatially explicit sampling are useful for the development of isoscapes. Finally, I will discuss areas where additional research is needed and suggest potential constructive paths forward.

## **Combining models of environment, behavior, and physiology to predict tissue hydrogen and oxygen isotope variance among individuals**

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<sup>1</sup>Sarah Magozzi, <sup>2</sup>Hannah B. Vander Zanden, <sup>3</sup>Michael B. Wunder, <sup>4</sup>Clive N. Trueman, <sup>1</sup>Gabriel J. Bowen

<sup>1</sup>Department of Geology and Geophysics, University of Utah, 84112 Salt Lake City, UT, United States

<sup>2</sup>Department of Biology, University of Florida, 32611 Gainesville, FL, United States

<sup>3</sup>Department of Integrative Biology, University of Colorado Denver, 80204 Denver, CO, United States

<sup>4</sup>Ocean and Earth Science, University of Southampton Waterfront Campus, SO14 3ZH Southampton, United Kingdom

Variations in stable hydrogen and oxygen isotope ratios in animal tissues are commonly used to reconstruct origin and migration. The underlying premise is that tissues grown at the same site are isotopically similar. However, large variation in tissue isotopic compositions often exists even among conspecific individuals within local populations, which complicates inference of origin and movement. Although field data and correlation analyses have enabled speculation about the underlying drivers of inter-individual variation, few testable hypotheses or conclusive insights have emerged from such work. Here we develop a process-based modeling framework that provides explicit predictions of the amount and mechanisms of isotopic variation. The model toolbox includes isoscape models of environmental isotopic variability, an agent-based model of behavior and movement, and a physiology-biochemistry

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model of isotopic incorporation into tissues. We then use the model to test whether predicted isotopic variation for co-located individual birds reflects observed variance in hatch-year Spotted Towhees from Red Butte Canyon, Utah, and to identify the major drivers of such variability. Models that include environmental isotopic variability predict isotopic variation of the same order of magnitude as observed. We find that the dominant driver of among-individual isotopic variation in the model is difference in nesting habitat: birds nesting in and near the riparian zone access isotopically lighter resources and have a higher total water flux, which results in more negative body water and tissue isotopic ratios than individuals nesting away from the stream. In addition to providing testable hypotheses regarding the mechanistic drivers of inter-individual variation, predictions of isotopic variance from our models can be used to improve estimates of uncertainty in assignment analyses, and/or used in designing optimal isotope and metadata collection for origin and migration research.

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## **Pinpointing timber origin with assignment tests using foliar isoscapes of Amazonian trees**

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<sup>1</sup>Fábio J. V. Costa, <sup>2</sup>João Paulo Sena-Souza, <sup>2</sup>Glauber Neves,  
<sup>3</sup>Luiz A. Martinelli, <sup>2</sup>Gabriela B. Nardoto

<sup>1</sup>Brazilian Federal Police, Brasília, DF, Brazil, 70610-200

<sup>2</sup>Department of Ecology, University of Brasília, Brasília, DF, Brazil, 70910-900

<sup>3</sup>CENA, University of São Paulo, Piracicaba, SP, Brazil, 13416-000

Illegal logging is a significant threat to biodiversity conservation in the Amazon region, as well as a trigger for further deforestation and forest degradation. Some regulatory efforts have enhanced the detection of some forms of illegal logging but are susceptible to more subtle methods that can mask the origin of illicit timber. Stable isotopes in plants are known to reflect geographical variations. The well-established carbon and nitrogen biogeochemical patterns of the Amazonian forests are related to the precipitation gradient from western to eastern and soil fertility gradient from southwestern to northeastern. Such a pattern subsidized the development of both foliar carbon and nitrogen isoscapes of Amazonian trees. We used a georeferenced library of more than 3000 leaf carbon and nitrogen values for individual Amazonian trees, located in 65 plots in the Amazon Basin, and rasters of environmental variables from Worldclim to build multivariate regression models. We selected the best model using Akaike Information Criterion to build the foliar isoscape. We performed the assignment tests for the most four high standing tree prices of commercial timber species as well as the most abundant sampled species using a bivariate normal distribution for foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . We delimited our probabilistic results in 50% boundaries for testing the percentage of corrects assignments

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laying inside these boundaries. More than 70% of the individuals were correctly assigned. Taking that Amazonian trees species are not randomly distributed across the basin, but, instead, they follow natural distribution patterns, combining foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  revealed to be an endogenous marker for the geographical provenance. It showed to be a promising tool at the basin-wide variation level in the Amazon to support control agencies to fight against illegal logging and to tight and strength conservation actions in logging activities to a more accurate and precise geographical provenance.

## **Predicting the geographic origin of berries using mechanistic oxygen and hydrogen stable isotope models**

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<sup>12</sup>Florian Cueni, <sup>1</sup>Daniel B. Nelson, <sup>2</sup>Markus Boner, <sup>1</sup>Ansgar Kahmen

<sup>1</sup>University of Basel, DUW – Botany, 4056 Basel, Switzerland

<sup>2</sup> Agroisolab GmbH, 52428 Jülich, Germany

In food quality control, stable isotopes are routinely used for origin analysis, typically relying on time-consuming and expensive collection of reference data sets that require yearly updates. In contrast, applications using mechanistic plant physiological water isotope models may provide a faster, cheaper, and seasonally dynamic alternative. However, such applications require detailed knowledge of fractionation processes and water fluxes through a given plant. Here we present results from an experiment designed to quantify these factors for strawberries and raspberries in order to facilitate the application of a mechanistic plant physiological oxygen and hydrogen isotope model.

We grew strawberry and raspberry plants in climate-controlled growth chambers at 30%, 50%, and 70% relative humidity. This caused varying degrees of leaf water isotopic enrichment and permitted the direct comparison of the oxygen and/or hydrogen isotope fractionation between source water and leaf/berry tissue water, bulk material, cellulose, sugars, and lipids (n-alkanes). For all three treatments, our results revealed that the isotopic enrichment of berry water was weaker than that of leaf water. This effect was explained using the two-pool-modified Craig & Gordon model to demonstrate that there was a

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greater contribution from unenriched xylem water ( $f$ ) in the tissue water of berries compared to leaves. Further results suggest constant fractionation for post-source water biosynthetic effects across treatments with exchange effects (e.g.,  $px_{pex} = 0.5$ ;  $fH = 0.3$ ) that can be applied to model the isotopic composition of different organic tissues. Validation tests of these models with data from 196 field samples collected across Europe since 2007 confirm the findings.

Our study shows that if the model relevant parameters ( $f$ ,  $px_{pex}$  and  $fH$ ) for a given berry can be quantified, mechanistic models are a novel and adaptive way of producing precise prediction models for samples of unknown origin.



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## Groundwater isoscapes for the contiguous USA

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Gabriel J. Bowen, Jessica Guo, Scott Allen

Department of Geology & Geophysics and Global Change and Sustainability Center; University of Utah, Salt Lake City, Utah, USA

Water extracted from subsurface aquifers has been and continues to be a major source of drinking and irrigation water in locations around the world. Regional studies and global compilations have demonstrated that the stable H and O isotopic composition of groundwaters often differs from that of local precipitation and/or surface water resources due to the old age, subsurface migration, or seasonally biased recharge of aquifer water. Together, these factors suggest the need for improved quantitative understanding of the distribution of groundwater isotope ratios in support of forensic provenance applications. We report the results of an ongoing effort to develop multi-layer spatial models representing the isotopic composition of subsurface freshwater resources at different depths across the United States. Building from a compilation of ~40,000 public-domain data and targeted sampling, we are able to demonstrate coherent patterns of isotopic variation both geographically and with depth and show that multiple, isotopically distinct pools of groundwater exist and are exploited at many places in the country. Failure to recognize and consider these patterns represents a potential source of error in isotope-based provenance analyses, which can be reduced or eliminated where adequate groundwater isoscape data products are available and are integrated in robust isotope data analysis workflows.

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## **Sulphur isotopes in human hair: diet vs. provenance**

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Clement P. Bataille, Michelle M.G. Chartrand, Francis Raposo,  
Gilles St-Jean

University of Ottawa, Department of Earth and Environmental Sciences, 25 Templeton Street,  
ARC building Room 419, Ottawa ON K1N 6N5, Canada

Isotope analysis in human hair are becoming increasingly useful in forensic applications. While the principles governing carbon and nitrogen isotope variability in human hair are well-understood, the controls of sulphur isotope variations remain largely unexplored. Here, we analyze the carbon, nitrogen, and sulphur isotopes in hair from 590 local Canadian volunteers and paired those analyses with detailed geographic, life, dietary and environmental data. We then use a series of machine-learning regressions to identify the dominant predictors of isotopic variability for each of this isotopic system. Nitrogen isotope variations in hair primarily reflect the amount of animal protein consumed by individuals, with higher nitrogen isotope signatures consuming more animal proteins. Carbon isotope variations in Canadians' hair broadly track the organization of the sugar and meat production industry on the landscape. Sulphur isotope signatures in Canadians' hair are not primarily controlled by seafood consumption rate. Rather their variations display a predictable spatial distribution that follow the variable atmospheric deposition of marine sulphates to local agricultural systems, and track the amount of local food consumed by individuals. We develop a spatial isoscape of sulphur isotope variations in hair across Canada and show that sea salt deposition and soil pH can explain 55% of the variance. Our best model including sea salt deposition, soil pH and sea food consumption rate explains 62% of the variance with large residuals associated with individuals that traveled in the recent past and expressed non-local sulphur isotopic signatures. The high

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predictability of sulphur isotope variations is very promising to further develop this isotopic system in provenance applications in forensics and archeological applications.

## **Development of the DPAA's isotope testing program for unidentified human remains**

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<sup>1</sup>Lesley A. Chesson, <sup>2</sup>Gregory E. Berg, <sup>3</sup>Amelia J. Edwards,  
<sup>3</sup>Thuan H. Chau, <sup>4</sup>Eric J. Bartelink

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<sup>3</sup>SNA International, Contractor, DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI, USA

<sup>4</sup>Department of Anthropology, California State University – Chico, Chico, CA, USA

Forensic isotope testing of unidentified human remains can help investigators determine the origin of an unknown individual because isotopic profiles recorded in tissues, such as bone and teeth, are related to sources of diet and drinking water. Capitalizing on this, the Defense POW/MIA Accounting Agency (DPAA) is developing an isotope testing program to support the identification of missing US Service Personnel.

This presentation provides an overview of work in progress to establish sample preparation and analysis capabilities at the DPAA for “bio” elements (C, N, O, and eventually S). Buildout is focusing on several questions:

[1] Is it possible to isotopically differentiate populations of interest?

[2] How isotopically variable is an individual (i.e., intra-person variation)?

[3] Has isotopic fractionation affected samples since collection (e.g., during preparation, analysis)?

[4] Are measurement results for a sample prepared and analyzed at different times and locations comparable?

We have previously demonstrated it is possible to isotopically differentiate U.S. Americans from residents of northeast and southeast Asia; reference datasets

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for other regions are needed, and data collection is underway. We tested multiple bones within a skeleton—and repeated this for multiple skeletons—to better understand intra-person isotopic variation. We are investigating isotopic fractionation during sample preparation, particularly the influence of water used during bioapatite extraction. Finally, we are assessing data comparability by analyzing samples prepared in different years and at different facilities, starting with enamel bioapatite and bone collagen.

These efforts will generate validation data needed for accreditation of the DPAA's isotope testing program. Results will be used to build databases and characterize population variation. Results will also help us define measurement uncertainty and interpretative differences for sample comparisons made during casework.

## **Classification(s) of isotope signatures in unidentified human remains**

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<sup>1</sup>Gregory E. Berg, <sup>1</sup>Michael W. Kenyhercz, <sup>2</sup>Lesley A. Chesson

<sup>1</sup>DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI, USA

<sup>2</sup>PAE, Contractor, DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI, USA

The Defense POW/MIA Accounting Agency (DPAA) has recently established an isotope analysis capability in order to test unidentified human remains for forensic identification. While isotope signatures in human bone and teeth vary by population, segregation frequently is done via interpretation of bi-plots or comparisons of sample values against population means. Forensic statistical interpretation of isotope data from unidentified remains leapt forward when Bartelink et al. (2014) showed population-level classification ability via discriminant function analysis that included posterior and typicality probabilities. This type of analytical structure fits well within the model of optimal forensic science practices advocated by the National Academy of Sciences (NAS, 2009).

Following Bartelink et al. (2014), Berg and Kenyhercz created Isolocate© (2017), an R-based graphic user interface (GUI) that provides a variety of classification packages for “bioelement” isotope data (C, N, O). Currently, Isolocate© includes the following populations: US, Southeast Asia, Northeast Asia, Japan, and Unidentified Border Crossers (Mexico/Central America/South America countries). This program is free for use via links at [www.anthropologyapps.com](http://www.anthropologyapps.com).

Here we will briefly discuss the underlying theory, coding, statistical classification options (e.g. discriminant functions, likelihood ratios, odds ratios, etc.), and the visual outputs of Isolocate©. Further, guidelines for general use

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of the GUI within an identification framework will be presented. Discussion of expansion plans, new datasets, data sharing (to include data comparability), and future goals will also be presented in this first introduction of Isolate© to the forensic community. We will show that the use of this program allows for testable and repeatable isotope data analysis for the DPAA, as well as the forensic community as a whole, following the NAS (2009) recommendations.

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## Using amino acid specific stable isotope ratios in human scalp hair for residency determination

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<sup>1</sup>Christy J. Mancuso, <sup>2</sup>James R. Ehleringer, <sup>1</sup>Seth D. Newsome

<sup>1</sup>Department of Biology, MSC03-2020, University of New Mexico, Albuquerque, NM 87131

<sup>2</sup>University of Utah, School of Biological Sciences, 257 South 1400 East, Salt Lake City, UT 84112

Hydrogen isotope ( $\delta^2\text{H}$ ) analysis of scalp hair tissue has been used as a diagnostic tool to determine an individual's residency location for over a decade. Hair tissue has been useful because the chemical signature does not change over time and it is strongly related to local drinking water. This relationship has been used to create predictive geolocation models; however, the prediction areas are broad and limit their precision. Our study focuses on developing an analytical tool that could increase the predictive power for residency determination by examining the influence of food and drinking water on  $\delta^2\text{H}$  values of essential (AAESS) and non-essential (AANESS) amino acids in scalp hair. We examined hair samples that were collected from local barbershop and salons along a north to south gradient from Dillon, MT to Big Springs, TX in the USA. The  $\delta^2\text{H}$  values of bulk hair increased along this transect and were consistent with precipitation gradients. We found that (a)  $^2\text{H}$  values of certain AAESS and AANESS increased along the north to south gradient and (b)  $\delta^2\text{H}$  values of AANESS were more significantly correlated with local tap water relative to  $\delta^2\text{H}$  values of AAESS. Specifically, ~36-52% of the hydrogen in the AANESS alanine, glutamic acid, and proline are derived from tap water, greater than the bulk  $^2\text{H}$  contribution (~32%).  $\delta^2\text{H}$  analysis of these AANESS could provide more consistent patterns with local drinking waters,

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improving spatial predictions for unidentified human remains. Specific AAESS (e.g., leucine) were also influenced by local tap water sources (~20%); however, these values appear to be more faithful indicators of diet rather than drinking water. These AA-specific  $\delta^2\text{H}$  patterns suggest that regional or local dietary proteins have a larger influence on hair  $^2\text{H}$  values than previously recognized. The application of our findings has the potential to improve the geospatial resolution of isotope analysis as a tool for law enforcement agencies.

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## Using $\delta^{15}\text{N}$ from soldier fly larvae for postmortem interval (PMI) estimation

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<sup>1,3</sup>Melanie M. Beasley, <sup>2</sup>Julie Lesnik, <sup>3</sup>Hayden McKee, <sup>3</sup>Anielle Duncan, <sup>3</sup>Dawnie W. Steadman

<sup>1</sup>Department of Anthropology, Purdue University, West Lafayette, IN, USA

<sup>2</sup>Department of Anthropology, Wayne State University, Detroit, MI, USA

<sup>3</sup>Department of Anthropology, University of Tennessee, Knoxville, TN, USA

This presentation will provide an application of stable isotope analysis of  $\delta^{15}\text{N}$  ratios of soldier fly larvae collected from decomposing human bodies to use as an alternative to traditional PMI estimation techniques. This research was conducted at the Anthropology Research Facility in Knoxville, TN, an outdoor laboratory for the study of human decomposition. A total of twenty donors were enrolled in the study, and muscle and associated maggot samples were collected for as long as each were present on a donor. Donors were placed on the surface in two trials with ten donors each in January (winter) and July (summer) to test differential seasonality effects to the method as a PMI estimator. Soldier fly larvae were present from June through December, with some donors never experiencing fly activity. A total of six donors from the winter trial and all ten of the summer donors had collectable larvae. Regardless of whether raw  $\delta^{15}\text{N}$  ratios or  $\Delta^{15}\text{N}$  ( $\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{Day X larvae}} - \delta^{15}\text{N}_{\text{Day 1 muscle}}$ , with Day 1 as the initial fresh tissue sample to normalize the baseline of different donors) were compared the summer and winter trials were significantly different ( $p = 0.00$ ). The winter trial ( $n = 43$ ) had larvae arrive after five months of decomposition with a mean  $\delta^{15}\text{N}$  of 27.4‰ (1SD = 6.4) and range of 18.0 to 43.2‰. The summer trial ( $n = 66$ ) had larvae arrive after three

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weeks of decomposition with a mean  $\delta^{15}\text{N}$  of 18.0‰ (1SD = 4.0) and range of 10.7 to 30.0‰, however there are only four samples above 23.3‰. With a sectioning point of 21.7‰ calculated from the mean  $\delta^{15}\text{N}$  of the larvae, the overall accuracy rate for the sample was 79.8%. For conservative PMI estimates, it is our recommendation that  $\delta^{15}\text{N}$  values be used as general indicators of winter vs summer decomposition when values are above 24‰ vs below 19‰, respectively. This pilot study indicates that the significant influence to  $\delta^{15}\text{N}$  values of larvae is time of decomposition prior to arrival of the larvae.

## **New perspectives in human forensic anthropology: linking social dimensions and diet through the stable isotopes in fingernails**

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<sup>1</sup>Gabriela Bielefeld Nardoto, <sup>1</sup>João Paulo Sena-Souza, <sup>2</sup>Jim Ehleringer, <sup>3</sup>Luiz Antonio Martinelli

<sup>1</sup>Department of Ecology, University of Brasília, Brasília, DF, Brazil, 70910-900

<sup>2</sup>SIRFER, University of Utah, Salt Lake City, UT, 84112

<sup>3</sup>CENA, University of São Paulo, Piracicaba, SP, Brazil, 13416-000.

Several works have used the multi isotope approach to examine the geographical origin of human remains as well as human movements in forensic anthropology while some studies have used it associated with socioeconomic status and dietary behavior changes. These studies have demonstrated the progressive substitution of local staples for industrialized processed foods in the developing regions of the world (the so-called nutrition transition process). This process has been increasingly recorded in their rural areas. Taking this scenario, we brought here a new proxy for tracking human movements under the worldwide “global supermarket” diet trend. We associated the spatial distribution of  $\delta^{13}\text{C}$  in contemporary human fingernails, representing the food sources and dietary practices in a region, with the globally well-recognized human development index (HDI) from the United Nations Organization. We measured the  $\delta^{13}\text{C}$  of fingernails from about 4000 Brazilian inhabitants living in rural and urban areas spatially distributed in the territory. We used the HDI at the municipality level and associated it with the average  $\delta^{13}\text{C}$  of fingernails for

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the corresponding municipality we have data. There was a positive spatial correlation between the  $\delta^{13}\text{C}$  and HDI (0.67;  $p < 0.01$ ). The highest  $\delta^{13}\text{C}$  and HDI was found in the Southeast and South Brazilian regions, followed by the Center-West region. The increase in the number of food items derived from C4 plant resources and the replacement of food items derived from C3 plant resources was associated with higher ultra-processed food purchases as a proportion of overall household food purchases. On the other hand, the  $\delta^{13}\text{C}$  from the North and Northeastern regions had the strongest link to C3 food sources, especially in their rural areas. This integrated approach proved to be useful as a new tool to be used in human nutrition studies but especially in tracking human movements in forensic anthropology studies in the contemporary world.

## **Postmortem preservation of isotope ratios of human hair**

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The application of isotope analysis by forensic anthropologists to assist with the identification of unknown human remains has increased in recent years. Isotope ratios of hair can be used to provide information about diet, as well as applied to isotopic landscape “isoscape” models for predicting travel and residence histories. Previous studies to develop isoscapes have used pristine hair samples from salons and do not reflect conditions typically associated with forensic contexts. This study was conducted in order to understand the impact of prolonged outdoor exposure during and after the decomposition process on the isotopic composition of human hair.

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Two outdoor research laboratories for the study of human decomposition were used in this investigation: the Anthropology Research Facility in Knoxville, TN (USA) and the Forensic Anthropology Research Facility in San Marcos, TX (USA). Body donors with known residence histories (n=8 TN and n=2 TX) were enrolled in the study and placed in both surface and burial conditions. Hair samples were collected over one year to determine the effects of decomposition and outdoor exposure upon isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) over time.

It was determined that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were consistent over time as opposed to  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ , which were impacted by exposure. To test the accuracy of geographic predictions made from the isotopic analysis of hair, measured  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values were compared to isoscape models. Predictions from  $\delta^{18}\text{O}$  values fell within 250 km of last known residence of donors 81% of the time. In contrast,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios predicted regions within 250 km of last known residence 100% of the time. This research revealed that isotope ratios of hair can be affected postmortem by exposure during and after the decomposition process. However, the observed changes did not significantly impact geographic predictions made for each donor.

## **Forensic application of stable isotope ratios analysis in official food control: Activity carried out by the ICQRF-Laboratory**

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Catania

Measurement of stable isotope ratios of H, C and O for foodstuff has been adopted as the official method by the European Union (EU) regulations, OIV (The International Organization of Vine and Wine) and AOAC International (association of analytical communities), in order to detect the illegal practices of watering, the addition of exogenous sugars and mislabelling (e.g. stating a different geographical origin). According to Italian law, an illegal practice can be punished either as a crime or as an administrative penalty. In this work, a general overview of the fraudulent cases detected by stable isotope ratio analysis in the frame of ICQRF official quality control is reported. ICQRF, the Italian Law Enforcement Body, represents one of the major European agri-food control bodies, acting in several agriculture and food fields such as wine, oil, fruit juice, honey and sugars. Among these areas, wine plays the most relevant role.

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## **IRMS, SNIF-NMR and Proton NMR in Wine Analysis**

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Wine is an important element of world food industry; last year the wine production in EU was over 180 million hl (over 60% share of the world) and contributed over € 7 billion to EU trade balance. Such large markets always attract fraudulency and wine is no exception: in 2012 to 2015 counterfeit goods have cost € 59 billion, of these spirits and wine being € 2.7 billion.

Therefore, effective control of such adulterations requires use of robust and reliable analytical methods for authentication of wines.

One of these is isotope ratio mass spectrometry (IRMS), via which it is possible to measure ratios of nature stable isotopes of carbon, hydrogen and oxygen in wine. They depend on the origin of the compounds containing them and, therefore, allow for determining geographical origin of the samples and addition of water and exogenous sugar.

Another approach is Site-specific Natural Isotopic Fractionation Nuclear Magnetic Resonance Spectroscopy (SNIF-NMR). By this method, the site-specific D/H isotope ratio in methylic and methylenic sites of ethanol is measured, and the values aid in determining the origin of ethyl alcohol in wine. In 1990s European Commission and International Organization of Vine and Wine (OIV) has adopted this method as a certified official method for analysis of wines. It is the only method based on NMR recognized as official standard.

IRMS and SNIF-NMR methods are robust and reliable, but have some disadvantages, such as use of expensive standards, prolonged sample preparation, expensive equipment maintenance; also, the analysis is destructive in many cases.

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Over the recent years, a new approach for wine analysis emerged: proton NMR spectroscopy, which can be used for either targeted analysis, or non-targeted profiling. Compared to classical methods, proton NMR requires less sample preparation, less time to record the spectrum and has cheaper equipment maintenance costs. However, it has not yet been officially approved for food authentication.

## **Development of new food matrix reference materials for stable isotope analysis**

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Stable isotope analysis in food forensics is gaining importance as a sophisticated multi-dimensional tool encompassing isotopes of different elements. However, although some methods have been officially validated and acknowledged as AOAC or EU methods, there are still numerous approaches which are not yet standardised and where the lack of certified isotopic Reference Materials (RMs) limits the mutual comparability of data. The fundamental analytical principle of identical treatment of samples and RMs requires chemical similarity of samples and RMs, which demands the

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development of suitable food matrix RMs. Two-point normalisation of isotopic raw data along isotopic scales is mandatory and requires at least two isotopically different RMs. Most laboratories have prepared their own food matrix laboratory standards for day-to-day quality control. However, proprietary food matrix standards are typically stored in air that over time may cause slow oxidation and drifting chemical and isotopic characteristics. The aim of our work is to develop, characterise, and preserve new RMs of chemically complex agricultural plant and animal-derived food matrix materials including: (i) honey RMs from Vietnam and Canada spanning different climates, (ii) flours from C3 and C4 plants, (iii) vegetable oils from C3 and C4 plants, and (iv) collagen powders from marine and terrestrial animal origins. Long-term storage of food in contact with atmospheric oxygen invariably causes food becoming stale via slow oxidation, even during storage at low temperatures. Each 'mother supply' of our new food matrix RMs has been divided into multiple aliquots that have been flame-sealed in round-bottom glass flasks under vacuum to exclude oxygen and to extend the shelf life to decades when RMs are stored frozen in the dark. The end-user will be able to obtain 0.5 to 1 g or mL aliquots of RMs with recommended/certified isotopic compositions for testing and verification of food authenticity and provenance.

## **Establishing a $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio landscape of Austria for food provenancing**

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The establishment of isoscapes based on the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio has been identified as powerful tool for a variety of applications (e.g. past human migrations, ecological questions like fish origin and migration, origin of primary food products). For food provenancing the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio has been recognized as a main indicator of geographical origin, as it is incorporated into plants and animals without any significant fractionation. The assignment of food products to a certain geographical area or origin can be accomplished via two methods: either by matching the chemical fingerprint a) to a set of reference samples ("database approach") or b) to a map showing the geographical distribution isotopes and elements ("isotope approach"). The major advantage of using a distribution map of  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratios in soils and water bodies

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is, that it can be applied independently of the type of plant or fish and can be considered as time-stable (a major advantage in comparison to databases based e.g. on light isotopes of N, O, C). Therefore the spatial distribution of  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratios (as well as the multielement pattern) of the bioavailable fraction in soils and water of the main production areas in Austria were systematically mapped. Soil, water, fish and vegetable samples were collected across Austria. The samples were analysed using (multi collector) inductively coupled plasma mass spectrometry ((MC) ICP-MS). First results showed a distinct distribution of the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratios across different regions in Austria. Selected primary food commodities could be discriminated with low error rates using  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in combination with elemental concentrations of Sr, Ca, Mg, Mn, Cu, Zn, Rb, Ba and the Sr/Ca ratio by discriminant analysis. Acknowledgements: COMET-K1 competence centre FFoQSI, funded by the Austrian ministries BMVIT, BMDW and the Austrian provinces Niederoesterreich, Upper Austria and Vienna within the FFG COMET programme.

# **The use of stable isotope ratios for the authentication of agrifood industry products in Andalusia**

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The work of the Department of Food Science and Health at IFAPA in Cordoba regarding food traceability and fight against fraud in focused on the organic food production and the Iberian pig sectors.

On one hand, Organic producers claim to the administration to do continuous efforts to increase the controls and to avoid fraud. At present, the main problem of this sector is the absence of analytical techniques to verify the origin of the products. Among the numerous attempts the S-IRMS technique demonstrated to have a great potential with some drawbacks.

Our study was focused in the organic horticultural Andalusian production using stable isotopes involving 686 samples cultivated in 37 (conventional and certified-organic) farms (2016-2018). The analyses ( $\delta^{15}\text{N}$ ) and relations under a new holistic perspective considering soil, fertilizers and fruits improved the verification of a unique food-based value of the farms opening a new framework in the organic food sector and the organic-food chain verification. Moreover, an isotopic databank of fertilizers was built.

On the other hand, the Iberian pork products have earned a reputation due to their exceptional organoleptic characteristics based on the feeding regime (acorns) followed by the animals at the end of their fattening period related to the dehesa ecosystem. The legislation defines the quality categories of Iberian

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pigs as bellota, cebo and cebo de campo, where animals are fed acorns, concentrates or a combination of both, respectively.

The only control method is the supervision of the farms and fields by authorized controlling enterprises. Therefore, it is necessary to assure the traceability of these products to protect consumers against fraud.

In this sense, this research shows the isotopic ratios of stable isotopes from different pig tissues in order to evaluate the impact of the feeding regime on those values. Our results showed differences among the isotopic signatures, due to a clear impact of the feeding regime



## **Addressing the unfulfilled codex standard for honey for stingless bee honey through dehumidifying process**

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Floral honey has long been sold in Malaysia as a premium food supplement, but recently stingless bee honey (SBH) is gaining traction to also become a premium food supplement. SBH or its local name 'madu kelulut' is produced by stingless bees, which can be classified into two genera, namely, the Melipona and the Trigona. The distinctive feature of this honey is that it is stored naturally in the pot (cerumen), thus contributing to its nutritional values which some studies found to be similar if not more than the normal honey. This has prompted the Malaysian government to promote SBH as a superfood and efforts are being made for it to be exported and marketed overseas. However,

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progress in this regard is now hampered by the SBH not fulfilling the CODEX Standard for Honey (CODEX STAN 12-19811) in terms of moisture content and the lack of protein to be analysed for Internal Standard Carbon Isotope Ratio Analysis (ISCIRA). ISCIRA is the method to measure the carbon-13 content of the protein extracted from the whole honey and compare it the carbon-13 content of the whole honey to detect C4 sugar adulteration. Hence, in this study we propose a water removal step using freeze drying process prior to ISCIRA of SBH which may serve a dual purpose: 1) to decrease the moisture content to below 20%; 2) to increase the amount of protein extracted as the presence of water contributes unnecessary weight to the sample prior to protein extraction. We found, once moisture content was decreased to below 20% for 11 SBH samples, the percentage increment of protein extracted from the samples varied between 6 to 385% with 4 samples found to be adulterated. Conclusively, the removal of water from SBH to a level below 20% is now necessary not just for fulfilling the moisture content criterion but to also increase the possibility of getting enough protein for ISCIRA, whereby, in serving both needs, it addresses the setbacks of SBH not satisfying the standards outlined in CODEX.

# **Isotopic Databases for Operational Forensic Casework - Current Focus, Casework Examples and Future Plans for IRMS within the Australian Federal Police Laboratory**

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The implementation of a technique such as Isotope Ratio Mass Spectrometry (IRMS) into a traditional forensic science setting is not without its challenges. Developing and adapting procedures, collecting data about the expected variation of a material and ensuring that quality assurance benchmarks are met are considerable efforts for operational laboratories that are typically run with little time or funding for novel project work. However, the impact on forensic casework and the justice system, may be significant when the targeted material and/or casework situation to be investigated is one that is typically considered of low or no evidentiary value.

This presentation will discuss a range of activities being undertaken within the Australian Federal Police laboratory in support of the use of IRMS within forensic casework. The selection of materials of interest that the AFP is seeking to implement IRMS for will be discussed and current work to build or update paper and polymer databases will be presented. For both materials, in excess of 150 samples have now been collected and analysed from across Australia.

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Complimentary to this are homogeneity studies that will be used to examine and demonstrate sample consistency within polymers. Future work will also be discussed which will include a survey of household white powders and materials commonly encountered in hoax events within threatening and anonymous postal mail.

Finally, a number of cases will be discussed, focusing on how IRMS was crucial in providing a level of analysis previously unavailable which supported or informed an investigation.

## **The FIRMS PT Scheme: What can be learned about inter-laboratory performance?**

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Demonstrating the comparability of isotope-delta values between different laboratories is vital for the validation of methods and for combining datasets. Comparability is usually demonstrated by participation in inter-laboratory comparisons (ILCs) and the FIRMS Network has provided a non-accredited ILC program for its members since 2004. Provision of a proficiency testing (PT) scheme accredited to ISO/IEC 17043:2010 became possible in 2013 when administration was outsourced to the external provider LGC Standards. The FIRMS PT scheme was included in LGC's scope of accreditation to ISO17043:2010 in 2015 and it remains the only accredited PT for isotopic analyses.

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Each round of the PT scheme comprises two materials that are of interest to forensic science, each with up to four isotope ratios ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ) as measurands. One material is selected to be “easy” to analyse, the other to be more “challenging.” Participants report a single isotope-delta value for each material and isotope ratio, ideally obtained from ten replicate analyses. The results of the PT scheme are interpreted using z-scores or z'-scores calculated using a standard deviation of performance assessment (SDPA). Since 2015, the SDPAs have been fixed by members of the FIRMS PT steering group as being both statistically valid and achievable - 0.15 ‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and since 2018 at 0.25 ‰ for  $\delta^{18}\text{O}$  and 1.5 ‰ for  $\delta^2\text{H}$  values

Results for the FIRMS ILCs and PT scheme will be presented. Such data are invaluable to investigate inter-laboratory comparability not just for individual laboratories but also for particular materials that have been analysed in multiple rounds (e.g. vanillin and caffeine). These also include cases where the same batch of material has been distributed to participants many years apart (e.g. glycine and phenacetin). These data can also be used to investigate patterns in laboratory engagement such as the relationship between participation and acceptable performance.

## **From hydrogen to uranium and back: QA/QC and reporting for radiogenic and heavy mass-dependent isotopes**

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Forensic isotope ratio mass spectrometric (IRMS) analysts are expanding the axes of information for geolocation and source differentiation by including radiogenic (Sr, Pb, Nd) isotopes and mass-dependent isotope information for elements heavier than sulfur (Ca, Fe, Cu, Zn, Mo, Sr, U, among others). Recent articles in anthropology and forensics provide guidelines for quality control in IRMS sample preparation, analysis, and reporting, but similar authoritative guidance for other isotope systems in general is not available.

Many basic concepts (analytical blanks, process blanks, matrix-matched certified standards) are similar, but there are fundamental differences in elemental chemistry, sample preparation procedures, and analytical techniques between traditional IRMS analyses and measurements by MC-ICP-MS or TIMS. MC-ICP-MS is a solution or laser ablation technique using high-temperature plasma. TIMS is a solid source thermal ionization technique, while IRMS analyzes gases with sample preparation automated in front-end peripherals. Instrumental mass fractionation corrections can include internal normalization, sample-standard bracketing, element spiking, and double spiking, depending on the isotope system. A lack of understanding of these

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underlying differences in ionization, sample preparation, standardization, and normalization can lead to poor quality data with incorrect inferences.

For instance, radiogenic isotope analysis is much more sensitive to contamination compared to IRMS analysis, but chemical recoveries (yields) are far less important. This is because a) many radiogenic isotopes are typically trace elements that are only a small component in a complex matrix and 2) due to internal normalization, the precision possible in radiogenic isotopes is several orders of magnitude more precise compared to IRMS. Examples of proposed best practices text for journal publication for  $^{87}\text{Sr}/^{86}\text{Sr}$ , Pb isotopes,  $\delta^{88/86}\text{Sr}$ ,  $\delta^{98/95}\text{Mo}$ , and  $\delta^{238/235}\text{U}$  values will be provided.



## **Stable isotope evidence introduced into U.S. courts**

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We provide a summary of the first court case in the United States where the judicial system has allowed the introduction of stable isotope evidence. Carbon and nitrogen isotopes of sequences of human hair were provided as evidence in establishing that a decedent had experienced episodes of starvation prior to death. As this case established legal precedence within the U.S. judicial system, it is worth reviewing the factors that went into this judicial decision. We discuss the basis of the court's decision to allow stable isotope measurements to be introduced as evidence in a trial, the analytical methodology expected and approved by the court, and of factors considered in describing an expert witness.

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## **Isotope fingerprints and criminal forensics: how we detect point of origin or track movements.**

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Christopher Brodie, Oliver Kracht, Dieter Juchelka, Mario Tuthom, Maddalena Bonanomi, Jens Griep-Raming  
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In this presentation we explain how stable isotope fingerprints identify the origin of material, such as drugs of abuse and human provenance, and track movements, such as human movements, in the context of criminal forensic investigations. Data are shown that illustrate how isotope fingerprints of carbon, nitrogen, sulfur, oxygen and hydrogen offer conclusive answers on questions associated with how similar or different materials are, or to identify the origin of the material. Here, we focus on drugs of abuse, human provenance and explosives. An overview of the interpretation of isotope fingerprints and the technology used to detect the isotope fingerprint of a material is also provided. Isotope fingerprints in any sample material are related to natural processes and geographical regions and can therefore be used to define differences in those sample materials. This means that, in criminal forensic investigations, sample materials can be put into a geographical context, so that their origin can be traced, or understood with respect to a specific process or set of processes in nature, such as botanical, dietary and food web variations and geographical location, to provide some information on their movement in the environment.

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## **Introducing new quality control features for stable isotope laboratories using Elementar instruments and software**

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Mike Seed, Michael Sudnik  
Elementar UK Ltd

Quality control is a crucial requirement for all stable isotope laboratories. Through the development of ionOS software for the control and acquisition of our instrumentation and ArDB software to construct, visualize and manage your analytical results in a database, our customers now have access to a comprehensive range of tools to help ensure that their data integrity is of the highest order.

This presentation will demonstrate the unique capabilities of the Elementar IRMS instrumentation and how we intuitively implement complex functionalities such as multi-point, multi-isotope calibration and long-term data quality evaluations to ensure the instrumentation is working at peak analytical performance.

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## Atypical $\delta^{13}\text{C}$ values of urinary norandrosterone in doping control analysis

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In routine doping control analysis IRMS has been established to identify an exogenous origin of 19-norandrosterone (NorA) in urine samples. Analysis of the carbon isotope ratio is necessary, as low amounts of urinary NorA might be of endogenous origin. Traces of NorA can be naturally found in human urine samples, with higher concentrations during pregnancy. Additionally, NorA can be formed in urine by in-situ microbial degradation of androsterone.

Since 2003 the Cologne laboratory analysed approximately 200 doping control samples with NorA concentrations below 15 ng/ml by IRMS. Negative samples show similar  $\delta^{13}\text{C}$  values for NorA and the corresponding endogenous reference compounds, whereas NorA originating from pharmaceutical preparations usually show  $^{13}\text{C}$  depleted values. However, in 2016 a routine doping control sample did not fit into the usual pattern as it revealed an atypical  $^{13}\text{C}$  enriched value for urinary NorA, compared to the corresponding endogenous reference compounds of the athlete.

It has been mentioned in literature, that beside endogenous production, traces of urinary NorA may originate from the consumption of offal of intact, non-castrated pigs. In order to check if this could be a potential cause for atypical or even adverse analytical findings in doping control analysis, different excretion studies were conducted. After meals of wild boar offal or meat some volunteers showed urinary NorA with  $\delta^{13}\text{C}$  values, which did not correspond to the consumer's endogenous isotopic signature. Further analysis of boar's tissue revealed a high fluctuation of the animal's  $\delta^{13}\text{C}$  values over the year.

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Thus, the consumption of wild boar offal in the hours preceding a doping control test may result in an atypical or even positive test result, and both athletes as well as anti - doping laboratories and authorities should be aware of this aspect.

## **The IRMS anti-doping analysis: applications and current challenges**

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The GC-C-IRMS analysis is an anti-doping mandatory requirement to disclose the exogenous or endogenous origin of urinary steroids through their <sup>13</sup>C composition determination. It is a confirmatory investigation performed on urinary samples in which the concentration and/or the ratio concentrations of specific markers are higher than the limits defined by the WADA. The finding of prohibited compounds in urine may be attributed, if no exogenous administrations occurred, to an ex vivo microbial formation from endogenous naturally excreted steroids. The urine samples collection and transport operations take place in not sterile conditions, resulting in a normal or pathogen microbial flora contamination able to change urine specimens' composition and steroid profile parameters. A new GC-C-IRMS method was developed and validated to establish the origin of prednisolone and prednisone at urinary concentrations between 30 and 60 ng/mL, as specifically required by the WADA. Prednisolone and prednisone are two synthetic glucocorticoids banned "in competition" when administered by systemic routes, but they can be also produced in situ from cortisol and cortisone physiologically present in urine by the  $\Delta^1$ -steroid-dehydrogenase ( $\Delta^1$ -SDH). The prednisolone and prednisone pharmaceutical preparations are synthesized by a combination of microbial and

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chemical processes. The natural precursors used in their industrial manufacturing mainly derive from C3 plants and display delta values more negative than the endogenous ones. Sixteen different pharmaceutical prednisolone and prednisone formulations commercially available in Europe were analysed. They are all characterized by typical  $\delta^{13}\text{C}$  inside the exogenous range, except for one Belgian product. It displayed an uncommon delta value overlapping the endogenous American reference delta values range. The presumable use of natural sources different from the C3 plants issues a new critical challenge to the IRMS anti-doping analysis.

## **Application of traditional (Pb, Sr, Nd, C) and non-traditional (Zn, Cu, Fe, Cl) isotopes to discriminate and investigate pollutant sources in environmental contamination**

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Environmental pollution imposes serious health risks to populations exposed to contaminants, therefore current investigation of the main pollutant sources in contamination cases are very important to forensic approach in order to support make decision and appoint responsibilities. In this work, we review the principles, applications and perspectives of isotope techniques in

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environmental studies. To this end, radiogenic (Pb, Sr, Nd) and stable (Zn, Cu, Fe, C, Cl) isotopic systems has been successfully applied to discriminate and trace sources in soil, water, atmosphere and sediments considering different environment pollution contexts (metallurgy, urban pollution, drain acid mine, etc.). Isotope sample preparation is conducted in clean room conditions followed by measurements in high-resolution mass spectrometers (MC-ICP-MS and TIMS). In the last years, improvements of the analytical and methodological for isotope determinations have been allowed the combined use of these isotope systems as a technique labelled Multi Isotopic Systems (MIS). This technique, using complementary information of each element, have been improving the source discrimination resolution and source apportionment in many studies. Pollutant sources of particulate material from São Paulo, London, Beijing and Paris megacities were discriminated using Pb, Cu, Zn, Sr and C isotopic systems. In São Paulo, vehicular traffic (exhaust and non-exhaust), industrial emissions and construction activities were differentiated by combination of Pb, Cu and Zn isotopes. In many works, Pb, Cu, Zn and Fe isotopes were applied to trace contribution and dispersion of pollutant sources on the soil, sediment and water of contaminated areas by industrial, mining or other anthropic activities. In the topic of organic pollutants, C and Cl isotopes can be used by researchers, environmental agencies and policy makers to assess degradation and distinguish plumes of organic compounds in contaminated groundwater and soils.

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## **A spectral analysis method to eliminate methanol and ethanol interference with CRDS measurements of water isotope ratios**

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The application of infrared spectroscopy for measurement of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in natural water samples has been limited by the fact that organic compounds often interfere either directly or indirectly with the target absorption spectra of pure water. In the current study, we evaluated the performance of a mechanistic spectral fitting algorithm ('organic fitter') combined with a statistical correction of residual errors ('post-processing correction') for analysis of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in organic-contaminated waters via cavity ring-down spectroscopy (CRDS).

We generated two sets of test samples: a set of simple synthetic mixtures of methanol, ethanol, and waters of different isotopic compositions ( $n = 24$ ), and a set of more complex natural mixtures of waters and organic contaminants extracted from plant leaf and stem samples ( $n = 48$ ). We then measured the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of the synthetic and natural mixtures using three methods: (i)

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CRDS with organic fitter alone (two different schemes, based on different regions of the water spectrum); (ii) CRDS with organic fitter and post-processing correction; and (iii) temperature conversion/elemental analyzer isotope ratio mass spectrometry (TC-EA-IRMS).

For the synthetic mixtures with the highest levels of alcohols, the organic fitters alone made a significant improvement in minimizing large positive biases for both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , compared to when no fitter was used. Even greater agreement with the true values was obtained when adding the post-processing correction. When compared to TC-EA-IRMS results, post-processing correction of the CRDS measurements did little to minimize the difference between the two methods for the plant extracts, especially for  $\delta^{18}\text{O}$ . Potential causes for the differences and their implications will be discussed.

## **Source inference of gasoline traces: implementation of a multiblock method to combine data from MS and IRMS**

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Gasoline is an ignitable liquid often found in arson cases. Gas Chromatography – Mass Spectrometry (GC-MS) analysis is currently used as the gold standard to determine the presence of gasoline in debris sampled at the fire scene or on suspects. Under certain circumstances, a link between non-degraded gasoline samples and evaporated specimens can be established.

Analysing gasoline samples by Gas Chromatography coupled with Mass Spectrometry and Isotope Ratio Mass Spectrometry (GC MS/IRMS) can improve the clustering of these samples. This research aims to study the contribution of the IRMS to the MS. The contribution of carbon isotopic compositions ( $\delta^{13}\text{C}$ ) of gasoline traces analysed by IRMS has been studied in order to understand if these additional isotopic data add value to the source inference of gasoline traces. Besides, the IRMS contribution was also compared to the chemical structural information already obtained with the MS used routinely.

The Common Component and Specific Weight Analysis (CCSWA) multiblock method was also used to investigate the combination of MS and IRMS data in the context of gasoline traces common source inference. CCSWA is a powerful chemometric tool for combining different data types from a same set of samples analysed by various techniques. The use of multiblock method is applied to combine MS with IRMS data to analyse simultaneously the structure

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and the relation between these two datasets. The interpretation of the results is also facilitated given the potential of discrimination between the samples.

In this talk, we would like to highlight the issues related to a complementary approach based on MS and IRMS, the chemometric aspects of such approach and its impact on the discrimination of gasoline samples coming from different sources (gas station, manufacturers).

## **Biodegradation of commercial surfactants: identification of degradations products by stable isotope carbon composition**

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In the last years surfactants and surface active agents are widely used and the problems associated with their environmental, social and economic impacts have stimulated research and development of eco-friendly and biobased formulations. Specifically, renewable surfactants are considered as promising alternatives to obtain green detergents from biomass with reduction of waste and emission.

Biobased surfactants derived from fatty acids and vegetal raw material continue to grow in their employment and interest, due mainly to their good surfactant properties, biodegradability, biocompatibility, and their potential replacement of fossil fuel-derived surfactants, which is of interest to many consumers because of the linkage of fossil fuels to environmental pollution. However, undesirable effects of surfactants in wastewater include the reduction of the concentration of dissolved oxygen because of foam formation and concomitant destruction of

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flora and fauna on surface water brought about by enhanced eutrophication. In fact identification of biodegradation products of commercial surfactants are the most important parameter to characterize total impact of products on environment and health. This study focuses on the innovative application of isotope analysis to identify surfactants in commercial products and their biodegradation products.

The  $^{13}\text{C}/^{12}\text{C}$  carbon isotope ratio is a chemical parameter with many important applications in several scientific areas and the technique of choice currently used is the isotope ratio mass spectrometry (IRMS). This study focused on the determination of the ratio of stable carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ ) to identify, innovatively, the origin of surfactants in commercial detergent formulations and their biodegradation products: commercial bio-sourced and synthetic homecare products were degreaser, liquid handwash, dishwash, multisurface cleaner.

# **Spatial variations in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in modern human dental enamel and tap water from the Netherlands: implications for forensic provenance studies**

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Strontium isotope research is an established tool in archaeology to infer information about human and faunal geological provenances. The accuracy of the interpretations, however, strongly depends on the quality and quantity of bioavailable Sr background data. In recent years, variations in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in either modern biosphere or archaeological background data have been published for different parts of Europe (e.g., United Kingdom, the Netherlands, France, Sweden) and America. Due to the continental 'supermarket diet', however, the range of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in modern humans are not expected to display a high correlation with the local biosphere, but rather to represent a mix of local (water), national and international food sources. Consequently, the applicability of these isoscapes for forensic investigations is debatable. This study aims to evaluate this issue through an extensive isotope study of modern human dental enamel ( $n=97$ ) and tap water ( $n=127$ ) samples from the Netherlands. The range of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in human dentition is relatively limited (0.70853-0.70997), while the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of tap water samples have greater variation (0.70839-0.71278). Moreover, no strong relationship was found between the geographical location of the individuals sampled and the

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geology they lived during enamel mineralisation, pointing towards a random distribution of modern human  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in the Netherlands. The Sr isotope ratios of tap water samples, however, are strongly dependent on the water source. As a result, there is a distinct correlation between geographical location of the water source and tap water  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. In conclusion, the range of Dutch human and tap water Sr isotope ratios is more variable than expected, making an accurate determination of geolocation of human individuals within the Netherlands challenging. Nevertheless, the presented database is highly valuable in determining a Dutch or non-Dutch provenance in forensic contexts.

**POSTER  
PRESENTATION  
ABSTRACTS**



FONDAZIONE  
EDMUND  
MACH



**FIRMS**

Forensic Isotope Ratio  
Mass Spectrometry

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## **Uncertainty from sampling in C and N isotope analysis in sediment samples**

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There has been an increasing concern with quality control in analytical techniques results that implies analytical uncertainties reporting. However, uncertainty associated with sampling and sample preparation was still not fully taken into consideration. This is particularly unsettling when dealing with environmental samples where systematic and standardized procedures are more difficult to be carried on. The main purpose of most measurements is to support decisions based on them. For this, the awareness of the 'range of certainty', where the results of the determinations lie, is essential for forensic purposes and very useful for most kinds of environmental investigations. To obtain this 'range of certainty', the assessment of the uncertainty of the measurement, including the uncertainty derived from sampling, is mandatory. In environmental forensics, pollution investigations should comprise sampling the source and sink of the suspect contaminants. In this study, the sediments of a stream were the sink of a hazardous waste landfill site leachate contamination and were the subject of metal, PAH and C and N isotope analyses to investigate the pollution sources. These analyses were performed in eight duplicate samples to reliably estimate the uncertainty from sampling, and this work is focused on the Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope

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ratios results. C and N relative content in the samples and C/N ratio were also determined to assist the interpretation of the results. The samples collected at reference site S1, at the stream's headwaters, displayed the least enriched values for  $\delta^{13}\text{C}$  (-26.2 ‰). Carbon and nitrogen isotope ratios revealed a clear distinction between S1 and the other sites downstream pollution sources. The uncertainty for the  $\delta^{13}\text{C}$  analysis within this data set, was estimated in 3.7 % and for the  $\delta^{15}\text{N}$  determinations was 16.8 %. The applied approach demonstrated the importance of the uncertainty from sampling in the forensic context.

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## **Characterisation of European and extra-European olive oils using bulk and compound specific stable isotope ratio analysis**

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European law requires a designation of origin for virgin and extra virgin olive oils (at least in terms of EU/non- EU provenance). Stable isotope ratios have been successfully applied to determine the geographical origin of olive oils, but never to distinguish EU and non-EU oils.

In this study  $^2\text{H}/^1\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  ratios were analysed in bulk olive oils using Isotope Ratio Mass Spectrometry (IRMS) as well as  $^{13}\text{C}/^{12}\text{C}$  and  $2\text{H}/1\text{H}$  in the four main fatty acids (linoleic, oleic, palmitic and stearic acids). In particular, a method to determine  $\delta^{13}\text{C}$ , and for the first time  $\delta^2\text{H}$  of the four main fatty acids extracted from olive oil triglycerides was developed. Triglycerides were subjected to base-catalysed transesterification with methanol to obtain the corresponding fatty acid methyl esters (FAMES) in a one-step reaction. Then they were analysed through Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-C\Py-IRMS).

The isotopic composition of olive oils was successfully used to distinguish samples originating in the two areas. Specifically, when bulk data were

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combined with fatty acid isotopic data the differentiation power of the method improved clearly. This separation is due to the specific isotopic fingerprint of the individual countries making up the EU and non-EU samples.

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## **Preliminary results on Italian industrial hemp traceability**

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Industrial hemp (*Cannabis sativa* L.) is becoming more and more important due to its thousands of uses, such as in the food, fibres, pharmaceutical and building sectors.

Here we present the first results obtained from the stable isotopes and metal trace analyses performed on *Cannabis* samples collected in 2018 in Veneto, Emilia Romagna and Friuli Venezia Giulia regions.

For the Friuli Venezia Giulia specimens, the whole plant was collected (root, stem and inflorescence), while for Veneto and Emilia Romagna only inflorescence samples have been considered. This study has been performed in the framework of a PhD thesis whose main target is defining a method to trace and guarantee the geographic origin of the Italian *Cannabis sativa* and its derived products.

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$\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of these preliminary samples have been measured by means of EA-IRMS and a first screening on trace metals has been performed by ICP-MS and by ICP-OES methods.

The  $\delta^{13}\text{C}$  results, here reported, suggest that our samples follow the Benson-Calvin photosynthesis pathway (C3 plant) having  $\delta^{13}\text{C}$  values between -24 ‰ and -35 ‰ (V-PDB).

At the moment, the  $\delta^{15}\text{N}$  results show a large range of variability among the three sampling areas taken into account, with their values ranging between -0.34‰ and 14.04‰ (Air). This is probably linked to the different cultivation methods (organic or conventional) and kinds of fertilizer used (synthetic or organic).

Further sampling campaigns are planned this year and in 2020, starting from early Summer (Southern Italy) to Autumn (Northern Italy). Two specific sites will be considered for the so called “Study of Hemp Factory”: one site is located in the Friuli Venezia Giulia region (Udine) and another one in Sicily (Caltagirone). The two sites have been chosen because of their difference in climatic conditions and geological background. A further campaign will be carried out in other areas of Italy (North, Centre and South) to define spatial variability maps.



## **Strontium and lead isotope ratios for differentiating commingled human remains – An investigation using calcined bone fragments**

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Isotope analysis is a technique used in forensic investigations of human remains to provide information on the potential origin of unidentified individuals. It may be useful when traditional investigative methods, such as DNA analysis, have been exhausted. The Defense POW/MIA Accounting Agency, which works to provide the fullest possible accounting for missing US Service Personnel, is beginning to use isotope testing as part of the identification process.

In this study, we explored the utility of Sr and Pb isotope analysis to separate commingled remains suspected to represent two individuals from a Vietnam War era aircraft crash. Fifteen fragments of calcined bone were selected for isotope analysis. Fragments were sub-sampled and submitted for Sr and Pb isotope analysis to two analytical facilities, the University of Florida (UF) and University of Utah (UU). Paired comparisons of the measured isotope ratios from UF and UU found no significant differences between the facilities. Assuming the individuals involved in the crash had isotopically distinct diets and drinking water, a non-normal distribution of isotope data could suggest there

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was more than one individual within the assemblage, while normally distributed data may suggest that the fragments were from a single individual.

All isotope analysis results from UF were normally distributed. In contrast, only  $^{204}\text{Pb}/^{208}\text{Pb}$  and  $^{207}\text{Pb}/^{208}\text{Pb}$  ratios from UU were normally distributed. Visual inspection of the non-normally distributed data from UU showed a bimodal distribution for  $^{87}\text{Sr}/^{86}\text{Sr}$  while the distribution of  $^{206}\text{Pb}/^{204}\text{Pb}$  was skewed. Since the UF and UU data suggest different interpretations on the makeup of the assemblage, sample analysis at a third facility would be required to validate any conclusions drawn from these analytical results. Additional sampling of the commingled remains may also help generate better statistical inferences on the potential number of individuals represented in the assemblage.

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## **Comparability of carbon and nitrogen isotope ratios of bone collagen following sample preparation at two facilities**

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Isotope analysis can be a useful tool to discriminate unidentified human remains from different populations. Application of the technique requires reference datasets that are sometimes compiled from measurement results generated at different times in different laboratories. The Defense POW/MIA Accounting Agency (DPAA) previously published reference isotope data of bone collagen samples from U.S. Americans and Asians that were prepared by California State University, Chico (CSU-Chico); as the DPAA now develops its own sample preparation and analysis capabilities, comparability to these established databases must be demonstrated. We sampled 30 long bone elements from 30 individuals of known geographic origin. Samples were halved and prepared as collagen at the DPAA and CSU-Chico. To control potential variability due to analytical technique, the Stable Isotope Facility at the

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University of California, Davis analyzed all samples (for this study, and the DPAA databases). Mean differences for collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were -0.1‰ (paired t-test;  $p = 0.0005$ , 95% confidence interval = -0.15 to -0.05‰) and -0.2‰ ( $p = 0.0031$ , 95% CI = -0.24 to -0.07‰), respectively. Results suggest a systematic bias, whereby collagen prepared at the DPAA had slightly higher  $\delta$  values. However, while isotopic differences between the samples were statistically significant, these differences are not meaningful. As evidence of this,  $\delta$  values were used to predict likely population of origin using linear discriminate function analysis and previously published population data. Predictions were the same—regardless at which lab samples were prepared—and all fit known origin. In sum, we found that samples prepared at the DPAA generated isotope analysis results comparable to those samples prepared at CSU-Chico. Based on the similar results and non-meaningful differences, in-house preparation of bone collagen at the DPAA can be confidently used as an investigative lead for unidentified human remains.

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## **Carbon isotope evaluation of the claims in MillerCoors, LLC v. Anheuser Busch, LLC**

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Carbon isotope analysis can estimate the contribution of C4 vs C3 carbon sources in foods, because corn is isotopically enriched in  $^{13}\text{C}$  compared to rice or barley. MillerCoors (MC) is suing Anheuser-Busch (AB) over a recent AB advertising campaign highlighting that Miller Lite and Coors Light beers contain corn syrup. MillerCoors (MC) is claiming false and misleading advertising and trademark dilution. MC assert 1) there is no corn syrup in the final beer product of Miller Lite or Coors Light; 2) they never use high fructose corn syrup (HFCS); 3) AB uses corn syrup as a fermentation aid in several of their other products; and 4) AB adds HFCS to some of its other product lines. Plaintiffs claim consumer fears about potential health impacts of corn syrup, and HFCS in particular, economically damage their brands.

This study measured  $\delta^{13}\text{C}_{\text{VPDB}}$  values for 27 beers, including the top eight best-selling US beers covering more than 50% of the US market. It included 11 beverages from AB, three beers from MC, other craft and mass market beers, corn syrup and HFCS. EA-IRMS measurements followed previously developed methods, with a standard uncertainty (uc) of 0.16 ‰. Miller Lite ( 24.6 ‰) and Coors Light (-24.5 ‰) had between 0 and 15% carbon from C4 (corn) sources. In comparison, Bud Light ( 27.3 ‰) was depleted in  $^{13}\text{C}$ , suggesting little to no C4 carbon. However, other AB products use more C4 carbon sources than Miller Lite or Coors Light, including Bud Light Platinum (-22.0 ‰, 27 ±5% C4), Natural Light (-22.1 ‰, 26 ±5% C4), Category 5 Malt Hurricane ( 21.1 ‰, 33

$\pm 5\%$  C4), Rolling Rock Extra Pale (20.7 ‰,  $37 \pm 5\%$  C4), and Lime-A-Rita (-11.9 ‰,  $98 \pm 4\%$  C4). A variety of craft beers showed no contribution from C4 sources within error. Corn syrup and HFCS could not be isotopically distinguished from each other. This study supports claim 3, but can not address claims 1, 2, and 4, highlighting both the utility and limitations of  $\delta^{13}\text{C}$  measurements in ingredient identification.

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## **Distinguishing eggs of different origin through statistical prediction model, using oxygen isotope ratios of liquid albumen and farm water**

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Rules on food labelling applicable to all foods are laid down in 'Article 26' of Regulation (EU) No 1169/2011 of the European Parliament and of the Council. Hence, the Department for Environment and Rural Affairs (Defra, UK) funded project FA0159 'The development of isotopic and fingerprinting techniques to verify the production origin and geographical origin of food and feed, phase 1' and aspects of the work conducted on eggs will be presented.

The geographical origin of feed used by poultry farms may vary, depending on cereal prices within the UK and elsewhere. Therefore, the local proxy of a farm may fluctuate significantly, which might provide a challenge for traditional stable isotope databases of solid food products (e.g. chicken). These are based on the data of the freeze-dried and defatted extract = protein (HCNS isotope ratios) alone, using EA-IRMS. To overcome this, we took the novel approach to use CO<sub>2</sub>-eq-IRMS, which is commonly applied to aqueous samples (e.g. wine, juice). We measured <sup>18</sup>O/<sup>16</sup>O isotope ratios of liquid albumen, without any sample treatment, alongside the water on the egg farms. In this way generated  $\delta^{18}\text{O}$  data allowed to develop an egg/water model, which can detect if eggs originate from different sources, based on a statistically robust minimum

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sample size, assuming a sufficient  $\delta^{18}\text{O}$  difference between sources (e.g. 1.78 ‰).

The advantage of this methodology is, that a database of samples is not required to determine mixed origin.



## **An isotopic water survey of the Australian capital territory**

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The potential to use isotopic abundances to indicate prior location or travel of humans is desirable for law enforcement. Although we are able to measure the stable isotopic values quite easily, there are limitations which are yet to be fully defined to enable robust and repeatable interpretation of the isotopic abundances of human samples. This survey of tap water within the Australian Capital Territory (ACT) is the first step in understanding urban water systems and the isotopic values of hair in an Australian context.

Tap waters from 50+ premises within the ACT have been sampled, measured for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  and mapped using Elementar ArDB software with a view to determining the variability of the tap water collected within a ~50km radius over a 10 day period. Any differences observed will be discussed and the overall variability determined for the region. To complement this, a number of sites were sampled as both tap and “filtered water” for comparison.

This poster will also outline the next steps in this project including periodic sampling of a sub-set of locations from the ACT, an expansion to other Australian capital cities and hair sampling of persons within the locations sampled. It is hoped that studies of this kind will strengthen the basis upon which we are able to utilise examinations of hair within forensic investigations of persons of interest.

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## **Nitrogen isotope fractionation from ammonia gas to ammonium in particulate ammonium chloride**

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Atmospheric particulate matter (PM) impacts human health, environmental pollution, and climate change. In general, the main components of PM are elemental carbon, organic carbon, secondary compounds and metals. The secondary inorganic nitrogen ions in PM include ammonium ( $\text{NH}_4^+(\text{p})$ ) and nitrate ( $\text{NO}_3^-(\text{p})$ ). Recently, the nitrogen stable isotope ratios of  $\text{NH}_3$  gas ( $\delta^{15}\text{N}-\text{NH}_3(\text{g})$ ) and particulate  $\text{NH}_4^+$  ( $\delta^{15}\text{N}-\text{NH}_4^+(\text{p})$ ) have been used to determine the sources and elucidate the environmental dynamics of  $\text{NH}_3(\text{g})$  and  $\text{NH}_4^+(\text{p})$ . Although the nitrogen isotope fractionation value ( $\Delta\delta^{15}\text{N}$ ) needs for source apportionment of  $\text{NH}_4^+(\text{p})$  in the ambient environment, studies of  $\Delta\delta^{15}\text{N}$  are limited and estimated the  $\Delta\delta^{15}\text{N}$  by theoretical calculation or laboratory experiments in closed system.

Here, we produced actual submicron size particles and exposed them to  $\text{NH}_3(\text{g})$  in a dynamic chamber under various experimental conditions of temperature and  $\text{NH}_3(\text{g})$  turnover rate.  $\Delta\delta^{15}\text{N}$  was lower when temperature was higher. For low turnover rates (about 0.9 /day), the  $\Delta\delta^{15}\text{N}$  was  $31.6\text{‰} \pm 2.0\text{‰}$  (mean  $\pm$  1SD), almost equal to the  $\Delta\delta^{15}\text{N}$  from theoretical calculations and laboratory tests in closed systems. Also, there was an inverse relationship between  $\Delta\delta^{15}\text{N}$  (y) and the turnover rate (x):  $y=27.5e^{(-0.01x)}$  ( $r^2 = 0.93$ ). This relationship can help to explain the discrepancies between  $\Delta\delta^{15}\text{N}$  values in closed and open systems. The present study could help to eliminate

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these discrepancies by estimating  $\Delta \delta^{15}\text{N}$  under different temperatures and turnover rates in a dynamic chamber using actual produced particles. This is the first such attempt. The results of this study could help in determining the sources and elucidating the mechanisms of particle formation.

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## Isotope profiling in wine authentication

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Product Authentication Laboratory, a new laboratory located in Lodz, is mostly involved in the food control by employing isotopic profiling. Two methods for the isotope ratio measurement are routinely used for this purpose: isotope ratio mass spectrometry (IRMS) and nuclear magnetic resonance spectroscopy (SNIF-NMR). Our laboratory is equipped with isotope ratio mass spectrometer (MAT 253) with an elemental analyzer (Flash 2000 HT) and nuclear magnetic resonance spectrometer (Bruker 500 MHz) for analysis of liquids (with <sup>19</sup>F lock channel probe dedicated to the deuterium measurements).

Product Authentication Laboratory has just obtained an international accreditation according to the norm ISO/IEC 17025:2005, confirming its competence in the area of testing the authenticity of wines and thus becoming the first Polish center that specializes in detecting adulteration in wines as a result of the illegal addition of sugar and/or water. Based on isotopic ratios, it is also possible to confirm the geographical origin of a wine. For this purpose it is necessary to compile a large set of data relating isotopic composition to geographic origin. The aim of this study was, therefore, collecting of data of isotopic ratios for wines published over the last thirty years and to evaluate the

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usefulness of the appropriate statistical techniques to discriminate between different countries and climatic regions of wine origins. Preliminary results indicate the discriminatory power of the oxygen isotopic ratios of the water content of wines.

## **Carbon isotope ratios of cannabis to describe growth conditions, in Cyprus: A preliminary study**

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Cannabis remains the most commonly used illicit drug among the general population in Cyprus. However, current understanding of its cultivation practices, geographic sources and distribution routes remains poor. Stable isotope ratio analysis has the potential to improve our understanding of cannabis production and trafficking since stable isotopes are natural probes, revealing characteristics regarding the geographic origin and growth environment of a plant. Stable carbon and nitrogen isotope analytical techniques have been used as forensic tools for the identification of cannabis source.

In this preliminary study, the stable carbon isotopic ratios were measured in cannabis samples, seized by the law enforcement officers in Cyprus, to predict their growth environment. Forty-six cannabis samples, including plants (either hemp or marijuana) and flowers from either small-quantity seizures or large ones were analysed. All samples were seized in Cyprus, but most of them are of an unknown cultivation location.

A small amount of each sample (approx. 100 mg) was washed with deionized water under sonication for 30 min and left to dry overnight at 40 °C. Afterwards, the dried samples were ground and  $260 \pm 41$  µg of powdered material was loaded into tin capsules and analyzed by EA-IRMS, EUROVECTOR.

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The samples exhibited  $\delta^{13}\text{C}$  of -38.64‰ to -25.89‰. One sample was found to have a remarkably low  $\delta^{13}\text{C}$  values, indicating indoor growth. Twelve samples were found to have  $\delta^{13}\text{C}$  values between -32‰ and -29‰ and 33 samples between -29‰ and -22‰, indicating shade or indoor and outdoor growth environmental conditions, respectively.

Further studies including the measurement of different isotopic ratios such as nitrogen and hydrogen will be carried out in the near future in order to create a database of isotopic signature of cannabis cultivated or seized in Cyprus. The aforementioned database will be eventually applied for the control of cannabis trafficking in Cyprus.

## **Authenticity testing of Cypriot alcoholic drinks, by using Stable Isotopes & Chemometrics**

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The authenticity of alcoholic beverages remains a big chapter in the authenticity of foodstuffs. The defining of the parameters required by Regulation 787/2019 on the definition, description, presentation and labeling of spirit drinks and their compliance with the more general food Regulation 1169/2011, requires the creation of databases from various parameters combined with high-tech techniques.

The work presented here is a part of the development of the necessary methodology for the characterization of spirits, aiming at the protection of Cypriot traditional products. At the same time, it aims at the creation of a useful tool for the competent authorities, for market control and consumer protection.

In the framework of “AgroFOOD” and “Black Gold” projects funded by the INTERREG V-A Greece- Cyprus 2014-2020 and the University of Cyprus, a total of 241 samples were analyzed. The samples included traditional Zivania and innovative Cypriot “Ceratonía” carob drinks and various alcoholic beverages such as tsipouro, rum, vodka, gin, sambucca, grappa, baccardi, tequila, whiskey, brandy, liqueur, ouzo, “eau-de-vie”, wine-alcohols, molasses and samples of various alcohols used in beverages or as substitutes.

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The isotopic ratios of  $^{13}\text{C}/^{12}\text{C}$ ,  $^{18}\text{O}/^{16}\text{O}$  and D/H obtained by IRMS and SNIF-NMR respectively, have been statistically analyzed with the use of an array of chemometric techniques. PCA and PLS-DA have discriminated the carob drinks as a separate group, similar to the group of Cypriot traditional zivánias, close to tsipouro and distinct from the other beverages. Zivánias were classified according to their geographical origin with a correct classification rate of >95%. The classification of all samples based on their type gave 82.2% overall success.

The combined information of stable isotopes appears to create a unique isotopic fingerprint for the two Cypriot beverages. Hence, the establishment of a European Database is definitely of great importance.

## **The Heul Girl murder case: a new step towards identification through stable and radiogenic isotope research**

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In 1976, the partially skeletonised remains of a female adolescent were found in former parking lot “the Heul” along the A12 near Maarsbergen, the Netherlands. For 30 years, she was misidentified as 18-year old Monique Jacobs, who was reported missing the previous year: Jacobs contacted her family in 2006. After exhumation in 2012, conventional human identification and DNA research failed to identify the girl. Although the Dutch statute of limitations on murder had expired, a stable (C-N-H-O) and radiogenic (Sr-Pb) isotope study was commissioned (dental enamel, mandible and femur bone, and hair). The dental enamel and bones display significantly lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (0.7077-0.7086) than the modern Dutch reference dataset (avg.  $0.7093 \pm 0.0003$ ), indicative of geolocations with a component of young volcanism or limestone. The Pb isotope data point towards a possible eastern European origin, but residence in western Europe during the last period of life (third

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molar). The  $^{87}\text{Sr}/^{86}\text{Sr}$ , and  $\delta^2\text{H}$  data of eleven 2-cm hair segments record stepwise variations. The Sr data are compatible with the Netherlands, while the Pb ( $\sim 1.13$ ) and H (avg.  $-65\text{‰}$ ) data are more challenging to interpret due to the lack of a comprehensive European reference data. Nevertheless, the data point toward residential mobility in Europe, certainly in a different part of Europe compared to her first years or life. Moreover, the C and N isotope data of the hair sections undoubtedly reflect a period of malnutrition or starvation prior to death. Unfortunately, despite the new insights in possible childhood geolocations, media attention in the Netherlands and Germany, and recently received tips, the girl remains unidentified. In 2016, following the Dutch judiciary, the German judiciary approved a large-scale DNA kinship study with the aim to identify the girl through a family member.

## **Successful identification of a female body found in Burgenland (Austria) in 1993**

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In 1993, a partly skeletonised body of a woman, wrapped in plastic bags, was found beneath bushes in a horse paddock in Burgenland, Austria, close to the Hungary/ Slovakia borders. The police assumed that the deceased woman (age estimated 25-30 years) came from Southeastern Europe, Poland or the former Yugoslavia. Despite extensive investigations, the woman remained unidentified. In 2016, the Austrian Federal Ministry of the Interior commissioned isotope geochemistry investigations on the unknown female. The only remaining tissues were two bones (humerus and femur). C, N, S and H isotope analysis were performed on the femur collagen, and Sr and Pb isotope analysis on the mineral part of the bone.

The integrated C-N-S-H isotope signature of the bone collagen was most comparable to reference hair samples from “Africa” (Namibia or Kenya) or “Central/ South America” (Brazil or Martinique). The results of the stable isotopes of the bio-elements gave no indication for a European origin. Furthermore, the Sr and Pb isotope data suggested that the unidentified woman came from a region where the underlying geology does not contain a large proportion of Pre-Cambrian basement. Comparison of Pb isotope ratios with teeth databases establish that the Pb data were incompatible with most

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parts of Europe and much of Africa, and large parts of North and South America. Importantly the Pb isotope data indicating a component derived from US Pb. The integrated isotope data was therefore used to predict her origin from the Northern Caribbean.

Based on the conclusion of the isotope report, the police concentrated further investigations on the prostitution circles in the region of Austria where the body was found. The police investigated a missing prostitute from the Dominican Republic and through Interpol obtained a DNA-profile from her sister that proved a genetic match. Within a few weeks of receipt of the isotope report, the woman was identified.

## **The stable carbon and nitrogen isotopic composition of fingernails in Brazil: from isolated villages to large urban areas**

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The idea of the use of carbon and nitrogen stable isotopic ratios in food consumption was proposed in the beginning of the 1980s when small differences between the isotopic ratios of food and consumer were observed. Urban populations have access to an immense variety of food products due to market integration, while such access decreases in more isolated areas. Based on that, our main objective in this paper is to explore the possibility of the use of stable carbon and nitrogen isotopic ratios of fingernails as a forensic tool to differentiate between urban and village populations. We analyzed more than 2,000 samples of fingernails from 20 municipalities and more than 20 isolated villages in two different regions of Brazil: Amazon and Cerrado. In these two regions, a clear difference between urban and village populations were observed. In villages, staple foods are rice, beans, and cassava in both areas of Cerrado and Amazon, plus freshwater fish in Amazon and bushmeat in Cerrado, all with a strong C3 signal. On the contrary, in urban areas with larger market integration, a strong C4 signal was detected due to the consumption of industrialized purchased foods such as sweet beverages, sugars, frozen chicken, preserved meats, dairy products and derivatives. We also found that in isolated communities, high consumer buying power tend to have a similar diet

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to the residents of urban areas. Such differences result in a strong variation in fingernails stable isotope ratios according to the degree of market integration of each region. In turn, such variation can be used as a forensic tool, allowing to discriminate people from areas with predominance of C3 or C4-like food.

## **Stable isotopes of H and O used as a forensic tool to investigate environmental issues related to groundwater**

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What is recharging the shallow aquifer reservoir in São Paulo City, Brazil, is the main question of this work. São Paulo is the biggest urban agglomerate in Brazil and depends on both surface and groundwater reservoirs. Understanding the sources of them is important to achieve sustainability. Isotopic data from groundwater, rainfall, public water supply system and sewage were obtained to investigate and quantify different contribution from distinct sources to groundwater. After a hydrogeochemical study, wells were separated in three major groups. The source of water for each group were calculated using ISOSOURCE (EPA), considering 1% for increment and 0,01 for tolerance values. There were analyzed water from monitoring wells in three different depths, 4.5, 7.5 and 13.5 mbgl. For the shallowest groundwater (4.5 mbgl), results show that main source for recharge is leaking from public water supply system (42.5%) and sewage pipes (24,5%). This means that anthropogenic sources contribute with 67% of the recharge, whereas natural sources correspond to 33,1% (rainwater = 18,7%; ancient groundwater = 14,4%). At 7.5 mbgl, water from supply system is contributing with 47,7%, and sewage with 28,1%, being 75,8% of anthropogenic recharge. Natural recharge

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is 24,2% (16,1% and 8,1% for rain and deep groundwater respectively). As the pipes are more or less at 4 meters depth, is comprehensible that the middle depth has an anthropogenic contribution higher than the upper level. At 13.5 mbgl, the natural contribution is higher (60,6%) than the anthropogenic (39,3% - 19,6% = water supply system; 19,7% = sewage). These results indicate that this aquifer has an important contribution from anthropogenic sources and contaminated water from sewage may contribute with almost 30% of the total recharge. Concerning climate change and the importance of this resource for cities, public policies should be developed to avoid a water collapse. (Financial Support from FAPESP 04/05360-6, 2010/20876-0)

## **Isotopic analysis of fertilizers and soil correctors used in intensive organic farming**

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In the last decades there has been an increasing demand of organic foodstuffs. The higher market price of these products, due to a yield gap in comparison to the conventionally produced foodstuffs and the certification costs, make them attractive for fraudulent techniques along the different levels of the supply food chain. As it was stated: "food fraud generally occurs where the potential for is high and the risk of getting caught are low".

Inputs allowed to be used in intensive organic production are fixed in the legislation guidelines EC 834/2007 and EC 889/2008. In this sense, protein hydrolysed fertilizers (PHFs) are new fertilizers that are starting to be used in intensive organic farming. PHFs of plant origin are classified in the Annex I of 889 as: 'products and by-products of plant origin for fertilizers'. The organic farming regulation (EC 889/2008) also allows the use of synthetic chelating or complexing agents (soil conditioners) with unknown quantities of nitrogen. Thus, the amount added to the field can affect to the isotopic data from organic farming foodstuffs. Therefore, it is important to identify the bio stimulants and guarantee the organic origin of the nitrogen to avoid cases of misleading techniques in organic inputs. However, it is a challenge to assure the origin of these agricultural inputs. Several analytical attempts have been approached and recent advances using the ratio of the stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) by

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S-IRMS have been reported. In this study, we analysed the  $\delta^{15}\text{N}$  and nitrogen percentage of different PHFs and organic correctors. This methodology enabled us to establish differences among both organic inputs. It was remarkably the high percentage of nitrogen found in several organic deficiency correctors and their low  $\delta^{15}\text{N}$  values, in the range of the conventional fertilizers. These results may open a new regulatory framework to determine the use of these fertilizers in intensive organic agriculture.

## **Discrimination between commercial and Italian natural saffron using stable isotope analysis**

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Saffron is one of the most ancient and valuable spices in human history. It derives from the dried stigmas of *Crocus sativus* L. The cultivation, harvest and manufacturing of this spice is entirely manual. To produce a kilo of saffron it is necessary to pick up about 150.000 flowers, which entail around 500 hours of work. Because of its high cost, its beneficial effects and the great demanding production, saffron has often been subject of adulterations. The frauds consist both in adding extraneous substances to enhance organoleptic properties of saffron and in declaring a different geographical origin than the real one, as saffron quality is closely related to the terroir of production. In the present work,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ ,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of 55 samples of bulk saffron have been analysed. The set of samples is composed by 9 commercial samples and 46 natural Italian samples, coming from 10 different regions. The aim of this work is to check the possibility to discriminate between commercial and natural saffron. Moreover, a geographical characterization was carried out to study the changes in the isotopic values depending on the origin of the samples.

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## **$^{13}\text{C}/^{12}\text{C}$ isotope ratios of citric acid determined by HPLC-co-IRMS for juices authenticity**

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Citric acid is one of the most widely used additives in beverages. Stable isotope ratio technique is a powerful tool for food adulteration detection, and the carbon isotope ratio characteristic of citric acid has therefore been proposed as a means of detecting the addition of citric acid, however, the pioneering method is rather cumbersome, due to the pure citric acid required for the analysis and the complicated preparation procedure of isolation comprised three chromatographic clean-up steps and a subsequent preparative liquid chromatographic separation, meanwhile, the isotope fraction risk was increased. Finally, these purification steps and one by one sample preparation limited the use of this characteristic in authenticity control routine applications.

HPLC-co-IRMS via an interface allowing the chemical oxidation of organic matter was used to online determine carbon 13 isotope ratio ( $\delta^{13}\text{C}$ ) of organic acids, glucose, fructose, ethanol and glycerol while equipped a  $\text{H}^+$  ion exchange column, but limit for citric acid direct analysis due to the existence of sucrose molecule in the sample. As sucrose can be degraded into glucose and fructose, the pretreatment conditions of amount of sulfuric acid solution addition and incubation time were optimized to remove sucrose molecules, therefore

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the samples can be injected into HPLC-co-IRMS system directly. Upon the experiences obtained, the elimination of sucrose on  $\delta^{13}\text{C}$  analysis of citric acid were discussed with regard to mix citric acid with different sucrose reagent (with  $\delta^{13}\text{C}$  values from -10‰ to -28‰) . The measured  $\delta^{13}\text{C}$  value of citric acid from different samples after pretreatment kept consistent with pure citric acid reagent, which allows a set of juice samples can be pretreated at the same time. Then, analysis were performed on 19 different orange fruits and squeezed in the laboratory with the standard deviation better than 0.25‰, and a variation of  $\delta^{13}\text{C}$  values of -28.35‰ to -25.04 was found for citric acid.

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## Isotope Ratio Preservation in Burned Pig and Human Bones

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Stable and radiogenic isotope signatures in bone may alter due to post mortem events, causing inaccurate inferences of dietary and geographical life history information. This study examines fidelity of perimortem isotope composition during burning by simulating body disposal attempts and analyzing common geoprofiling isotope systems ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  values) and lesser studied systems ( $\delta^{34}\text{S}$  and  $\delta^{88}/^{86}\text{Sr}$  values).

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Racks of fleshed pig ribs from the same farm, ensuring consistent geolocation and food source for the animals, provided both control ( $n = 15$ ) and experimental samples ( $n = 48$ ). One bone from each rib rack functioned as an unburned control while remaining ribs were sectioned and burned outdoors in an open flame for a preassigned length of time and accelerant treatment (max. temp. =  $566^{\circ}\text{C}$ ). Additionally, for dry human skeleton elements, one rib and femur pair from four individuals was kept as a control, and another pair was heated in a muffle furnace ( $n = 8$ , max. temp. =  $800^{\circ}\text{C}$ ). Bone collagen, carbonate, and strontium were analyzed by EA-IRMS, Gas Bench-IRMS, and MC-ICP-MS, respectively.

Isotope ratios for burned samples were normalized to the unburned control from the same individual to determine burn induced shifts in isotope ratios. Standard deviations for the normalized values of each individual were compared to the range of the unburned controls. The variation for each isotope system between burned and control samples was well within the range seen in the control samples, except for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of carbonate in human samples, which showed a depletion at  $400^{\circ}\text{C}$  and  $800^{\circ}\text{C}$ , respectively. Collagen was not extracted from human samples burned at  $\geq 400^{\circ}\text{C}$  but was extracted from pig samples above this temperature.

This research suggests that, within the parameters of this study, the isotope signatures of burned bone retain the geoprofiling inference of unburned bone, except for carbon and oxygen isotopes of carbonate from dry bone.



## **Sampling potato tubers to determine their geographical origin**

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Analyzing stable isotope composition of food is one of the most promising methods to investigate its place of origin, perceived as indicator of quality. The aim of this work was to evaluate the local variability of potato tubers and to create an adequate sampling method to study the geographic origin of potatoes.

Six potato tubers per plant were collected from three plants in the central zone of a 2.6-ha-field in northern Italy. Washed and peeled tubers were split in the apical portion attached to the plant (a), the lateral portion in contact with the peel (l), and central part (c). All parts were freeze-dried, powdered and stored in sealed containers in a desiccator before the analysis of carbon ( $\delta^{13}\text{C}$ ), hydrogen ( $\delta^2\text{H}$ ), and oxygen ( $\delta^{18}\text{O}$ ) isotopic composition. To study the effect of conservation, one tuber was kept fresh at 21°C in the dark and freeze-dried just before the analysis while another tuber was stored freeze dried in a desiccator. These samples were analyzed several times in the course of six months. The analytical accuracy and the instrumental error were checked by periodically analyze the same potato sample for two years.

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The results showed that the within-field isotope variability of potatoes is similar between plants and among tubers of the same plants. In particular, the standard deviation of  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  (in ‰, respectively) were 0.61, 9.09 and 0.75 within-field, while they were 0.38, 4.90 and 0.32 among tubers of plant A; 0.55, 11.96 and 0.91 among tubers of plant B; and 0.56, 6.44 and 0.33 among tubers of plant C. The analytical results of the same sample stored fresh and freeze-dried were stable for six months although the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  was larger for the freeze-dried sample. The potato control sample showed a good accuracy of the analysis and instrumental condition. The results of this work offer the statistical support to appropriately sample potato tubers to determine their geographical origin through the analysis of stable isotopes.

## **Implementation of ISO/IEC 17025 standard in isotopic laboratories: Case study for radiogenic and stable isotopic methodologies validation in a Brazilian Research Center**

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The isotopic data have been used in many research fields as an important information to regulatory and make decisions in strategic areas, such as forensic, health and environmental investigations. Considering these valuable application, analytical quality of measurement is the first order of relevance. Despite some good practices adopted to method validation and quality control, there are few isotopic laboratories with Quality Management System (QMS) worldwide based on ISO/IEC 17025 standard. The Research Center of Geochronological and Isotopic Geochemistry at University of São Paulo is one of the first isotopic laboratory on South America and has implemented different methods to analyze radiogenic and stable isotopes of traditional (Pb, Sr, Nd, C

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and O) and non-traditional (Cu, Zn) elements in diverse matrices. In the last year, implementation of QMS in the Research Center has been performed based on ISO/IEC 17025 standard in order to obtain accreditation of those methodologies. Several challenges were found to develop validation reports of those methodologies considering specific characteristics of each isotopic method and few references available for this purpose. Different strategies were employed for report method perform characteristics. Selectivity of traditional (Pb, Sr and Nd) and non-traditional (Cu and Zn) isotopes were evidenced by elution curves. Quantification limits were determined by minimum acceptable standard deviation (SD) based on SD vs signal distribution, which was used to found linearity (working range) also. Accuracy was determined by measurements of certified isotopic standards, whereas precision was quantified by measurements of reference materials in stablished conditions. Expanded uncertainties were estimated by assessment and combination of many sources. An internal guide was developed with those exercises for method validation, which must be shared with other isotopic laboratories.

## **Geographic origin of marine food products: stable isotope approaches at global, ocean basin and local scales.**

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The seafood sector faces many challenges associated with authenticity and traceability. Genetic test of species identity have demonstrated high levels of fraud and species substitution throughout global seafood markets, and have led to improvements in labelling and chain of custody processes.

The spatial origin of seafood is much more difficult to establish however. As wild capture fisheries are mostly managed in terms of spatial fishery units, there is incentive and opportunity to gain from mis-reporting catch location. Stable isotope based tests of origin of seafood could therefore assist global fishery management and seafood traceability.

The isotopic compositions of O H and Sr are essentially homogenous in marine systems, and geographic isotopic gradients are largely expressed in biologically-mediated isotope systems C, N and S. Establishing isotope-based traceability in marine systems is therefore complicated by the relative inaccessibility of the areas under study and additional complications associated with isotopic ecology and physiology.

Here we report on 10 years of development of isotope-based traceability methods suitable for estimating origins of marine seafoods. We report on efforts to predict likely isotopic distinctions between fishery regions on global scales based on biogeochemical modelling: on the degree of isotopic

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discrimination found among major cod fishery units and on efforts to map isotopic variation within shelf seas.

Ultimately the use of stable isotopes as a forensic tool for geographic traceability of marine foodstuffs is promising, but it is still a relatively new approach, and the limitations of the method both in spatial and ecological terms are still being assessed.

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## **Stable carbon isotope ratios for carbohydrates and organic acids in commercial honey samples**

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In the 2000s, liquid chromatography combined with isotope ratio mass spectrometry (LC/IRMS) has been developed, which allows the measurement of stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) composition of highly polar and non-volatile analyses. In recently, LC/IRMS has been used to authenticate and trace products such as honey, wine, and lemon juice.

Honey is a highly nutritious functional food composed mainly of glucose, fructose, minor sugars, organic acids. However, honey is often adulterated with inexpensive ingredients such as corn syrup, glucose syrup, and sucrose syrup, and therefore methods for distinguishing pure honey from adulterated honey are necessary. In this study, determined the  $\delta^{13}\text{C}$  values for glucose, fructose, disaccharides, trisaccharides, and organic acids in 116 honey samples. In addition, gluconic acid which the main component of the organic acid was analyzed by heart-cutting two-dimensional (2D) LC/IRMS. We investigated distinguish pure honey and adulterated honey and pairwise correlations between  $\delta^{13}\text{C}$  values.

On the basis authenticity criteria by  $\delta^{13}\text{C}$ , 77 of the samples were judged to have been pure for 116 honey samples. The carbohydrates and organic acids  $\delta^{13}\text{C}$  values for the 77 pure honey samples ranged from  $-30.2$  to  $-22.8\text{‰}$  and from  $-33.6$  to  $-26.5\text{‰}$ , respectively. The  $\delta^{13}\text{C}$  values for the organic acid of

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honey were reported for the first time. The organic acid is gluconic acid as main component as well as various other acids. Next, we try to analyze the individual organic acids of  $\delta^{13}\text{C}$  by developing 2D-LC/IRMS. As the results, the average of gluconic acid  $\delta^{13}\text{C}$  values was  $-30.0 \pm 0.7\%$ . The correlation coefficient for the relationship between the glucose and gluconic acid  $\delta^{13}\text{C}$  values ( $R = 0.61$ ), which was higher than between glucose and organic acid  $\delta^{13}\text{C}$  values ( $R = 0.45$ ). Gluconic acid is produced via decomposition of glucose by bee glucose-oxidase. The mechanism that gluconic acid produced from glucose was also confirmed by the relationship.



## Determination of carbon stable isotope ratios of ethanol and glucose in sake

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Sake is Japanese traditional beverage made by rice, and composed mainly of water, ethanol, carbohydrate, organic acids, and amino acid, etc. Sake can be classified into two major categories that “junmai-shu” was made only from water, rice, and koji, and “futsu-shu” was added other alcohols and organic acids during made. Recently, there has been some cases where junmai-shu is sold mixed with other alcohols. The other alcohol mainly produced from C4 plants such as sugar cane. On the other hand, the rice which is main materials of sake is C3 plants. Therefore, junmai-shu and fustu-shu can be identified by using the stable carbon isotope ( $\delta^{13}\text{C}$ ). For previous method, it was analyzed by elemental analyzer/isotope ratio mass spectrometry, but it is time-consuming pretreatment and a risk of isotope fractionation. In the 2000s, liquid chromatography combined with isotope ratio mass spectrometry (LC/IRMS) has been developed. In recently, LC/IRMS has been used to authenticate and trace products for various food. In this study, we collected 40 kind of sake (15 junmai-shu without other alcohol, 25 futsu-shu with other alcohol) and determined the ethanol and glucose  $\delta^{13}\text{C}$  values by LC/IRMS.

For 40 kind of sake, the ethanol and the glucose average  $\delta^{13}\text{C}$  values were  $-25.8 \pm 2.3\text{‰}$  and  $-26.9 \pm 2.5\text{‰}$ , respectively. The precision of all samples were  $<0.7\text{‰}$ . For the ethanol  $\delta^{13}\text{C}$  values, 15 junmai-shu were  $-28.3 \pm 0.3\text{‰}$ ,

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25 fustu-shu were  $-24.4 \pm 1.7\text{‰}$ . The ethanol  $\delta^{13}\text{C}$  values of Junmai-shu was 3.9‰ lighter than fustu-shu. For the glucose  $\delta^{13}\text{C}$  values, 15 junmai-shu was 7.6‰ lighter than 4 fustu-shu. Therefore, it is considered that junmai-shu and fustu-shu can classify by using the ethanol and glucose  $\delta^{13}\text{C}$  values. In addition, this analytical method is considered to be very effective for analysis of  $\delta^{13}\text{C}$  values in sake. We expect to expand the scope of application to beverages including alcohol such as wine and beer.

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## **Stable carbon isotope ratio measurement of water-soluble organic aerosol using online wet oxidation/isotope ratio mass spectrometry**

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Liquid chromatography/isotope ratio mass spectrometry (LC/IRMS) is widely used to measure stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of highly polar and nonvolatile samples especially in food science. However, to our best knowledge, few studies are available on field-deployment of the LC/IRMS to investigate  $\delta^{13}\text{C}$  of carbonaceous aerosols. The  $\delta^{13}\text{C}$  analysis can provide information about the sources and atmospheric processes of carbonaceous aerosols and their constituents. The  $\delta^{13}\text{C}$  for carbonaceous aerosols (total carbon, elemental carbon, and organic carbon) have been measured using EA/IRMS, but determining the  $\delta^{13}\text{C}$  of water-soluble organic carbon (WSOC) is complicated and requires a time-consuming pretreatment process. In this study, we developed an online wet oxidation/IRMS method for determining the  $\delta^{13}\text{C}$  of WSOC in aerosols. This simple method is quick because the aerosol samples were collected with a quartz fiber filter, extracted with water, and then analyzed without pretreatment. We evaluated the accuracy, precision, and detection limit of the method, and demonstrated to measure the  $\delta^{13}\text{C}$  of WSOC in ambient aerosols. The aerosol samples were collected at two sites in Japan using high-volume samplers from July 2017 to February 2019. The  $\delta^{13}\text{C}$  of

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WSOC were measured by means of wet oxidation/IRMS. The run required only approximately 6 min because this method conducted were done without a LC column. This method showed high accuracy (0.1‰) and precision (0.1‰), and the limit of detection was sufficiently low for WSOC analysis. The preparation and analysis time was a third to a half of that required for analysis by previously reported EA/IRMS methods. The  $\delta^{13}\text{C}$  of WSOC ranged from  $-28\text{‰}$  to  $-19\text{‰}$ , showing seasonal trend. This range was similar to values obtained in previous studies, suggests that the sources of the WSOC were terrestrial C3 plants and fossil fuels. This wet oxidation/IRMS method could be used for various water - soluble components, and it expands the applications.

## **Challenges in food provenancing and traceability with processed food and beverages**

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**Stewart G Walker**

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The use of analytical techniques to determine and compare the chemical and isotopic content of food and beverages, and determine the most likely place of origin, is well established. Ideally, genuine samples of products, soils and water from places of origin can be collected and compared to samples known to come from these origins. However, processes – including ageing and transportation – have the potential to alter the chemical and isotopic make-up of the final product.

A multi-national program of the International Atomic Energy Agency/Regional Cooperative Agreement (RAS5081) includes developing analytical and provenancing capacity to enhance food safety and authentication in countries as far apart as Jordan, Fiji, Mongolia and New Zealand. This presentation will present results from investigations into changes when two important dietary staples - rice and coffee - are processed. Rice is a staple for more than half the worlds population with more than 3.5 billion relying on rice each day and coffee is the second most traded material (after oil).

Rice was boiled in Australian, Norwegian and Italian water. Raw/green coffee beans were sourced from 17 known and one unknown origin from 16 different countries. If the chemical and isotopic content of coffee beans changes during roasting it will not be possible to prove that the coffee the customer is drinking came from where it is claimed.

Carbon and nitrogen isotope ratios and multi-elemental analysis (ICPMS) were determined for green, light, medium and dark roasted beans. Although carbon

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is known to be lost in the Maillard reaction, the large carbon content of the beans (> 50%) meant that the overall  $\delta^{13}\text{C}$  did not change significantly.  $\delta^{15}\text{N}$  however did show a regular fractionation. So when coupled with trace elements that are 'conserved' during the process isotope ratios and trace elements can be used to eliminate some potential sources as a roasted bean can not have come from a raw bean that is more depleted in  $^{15}\text{N}$ .



## ORGANISING COMMITTEE:

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