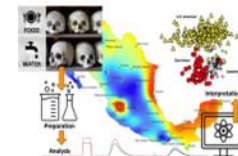
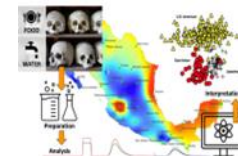


# *Stable Isotope Ratios in Forensics*





[https://www.youtube.com/  
watch?v=R8KdKsydcpQ](https://www.youtube.com/watch?v=R8KdKsydcpQ)



# *Stable Isotope 'Profiling' or Chemical 'DNA': A New Dawn for Forensic Chemistry?*

## Analogies between DNA and Light Stable Isotopes

### Biological DNA versus Chemical "DNA"

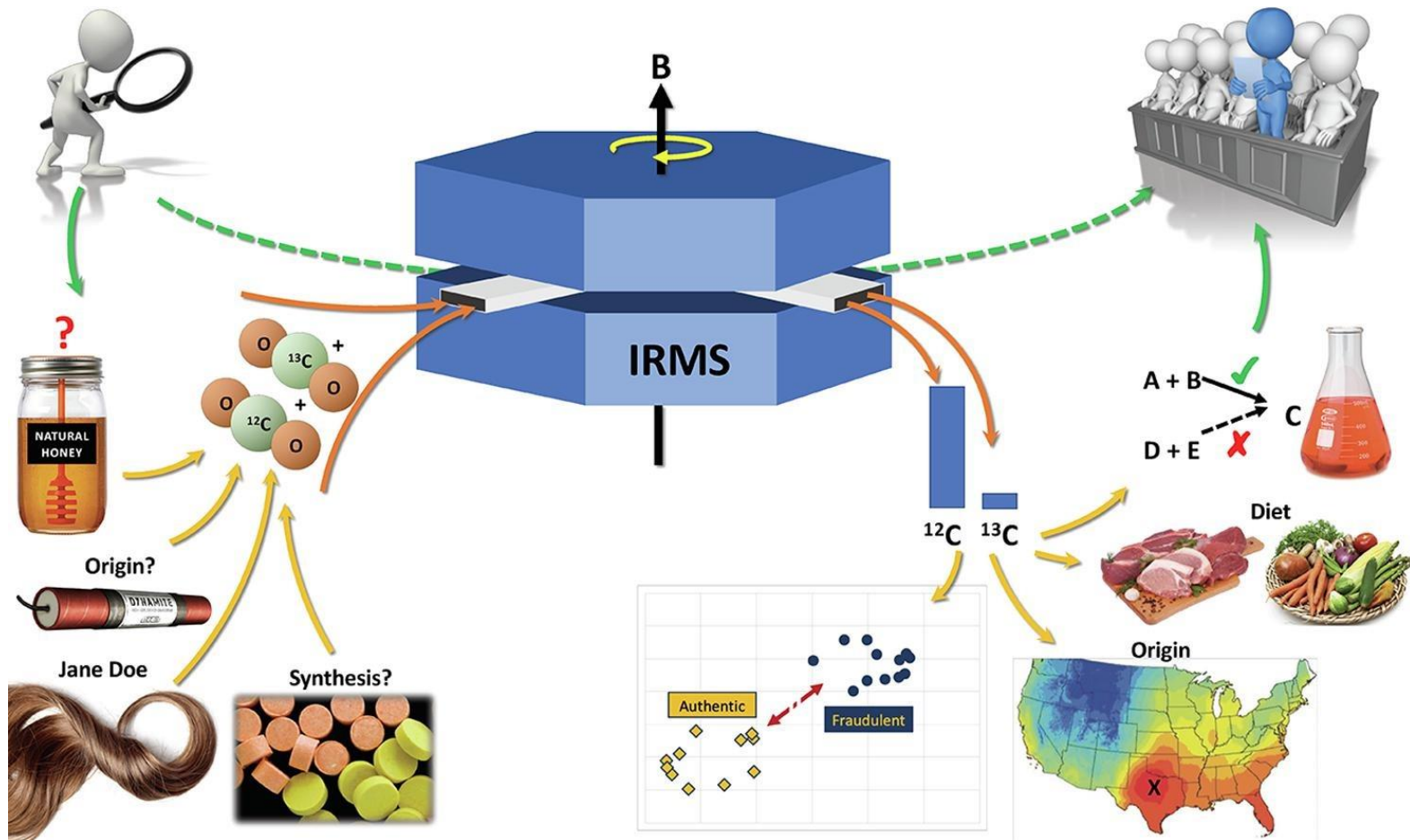
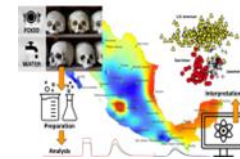
Alphabet of Biological DNA (letters)

A  
C  
G  
T  
[U]

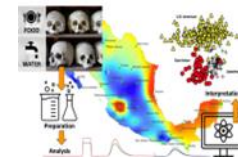
Alphabet of Chemical DNA (letters)

$^2\text{H}$   
 $^{13}\text{C}$   
 $^{15}\text{N}$   
 $^{18}\text{O}$   
[ $^{34}\text{S}$ ]

Note: This is for illustrative purposes only and does not denote any equivalence between DNA bases and chemical elements.

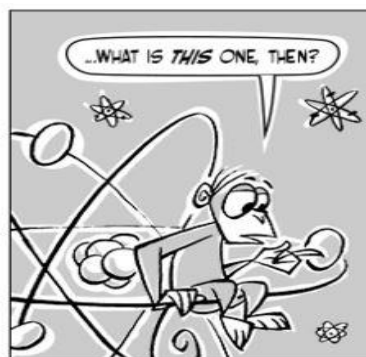
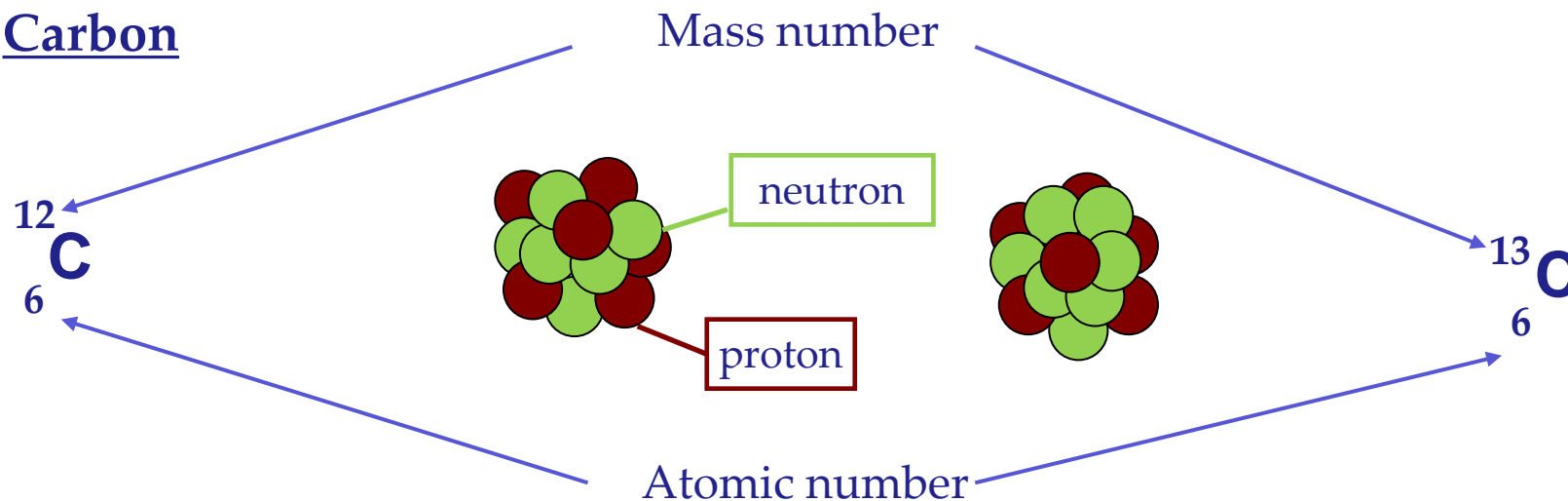


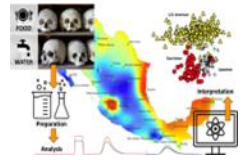
Matos, P. V. Mayara, Forensic Chemistry, 2019



**Isotopes:** forms of the same atom that differ only in their number of neutrons

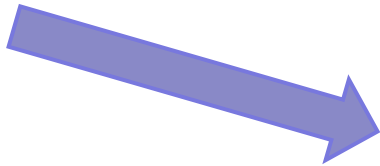
Carbon





# Stable Isotopes

Biological



Isotopic ratio

}	Hydrogen	${}^1_1\text{H}$	${}^2_1\text{H}$	
	Carbon	${}^{12}_6\text{C}$	${}^{13}_6\text{C}$	
	Nitrogen	${}^{14}_7\text{N}$	${}^{15}_7\text{N}$	
	Oxygen	${}^{16}_8\text{O}$	${}^{17}_8\text{O}$	${}^{18}_8\text{O}$
	Sulfur	${}^{32}_{16}\text{S}$	${}^{33}_{16}\text{S}$	${}^{34}_{16}\text{S}$



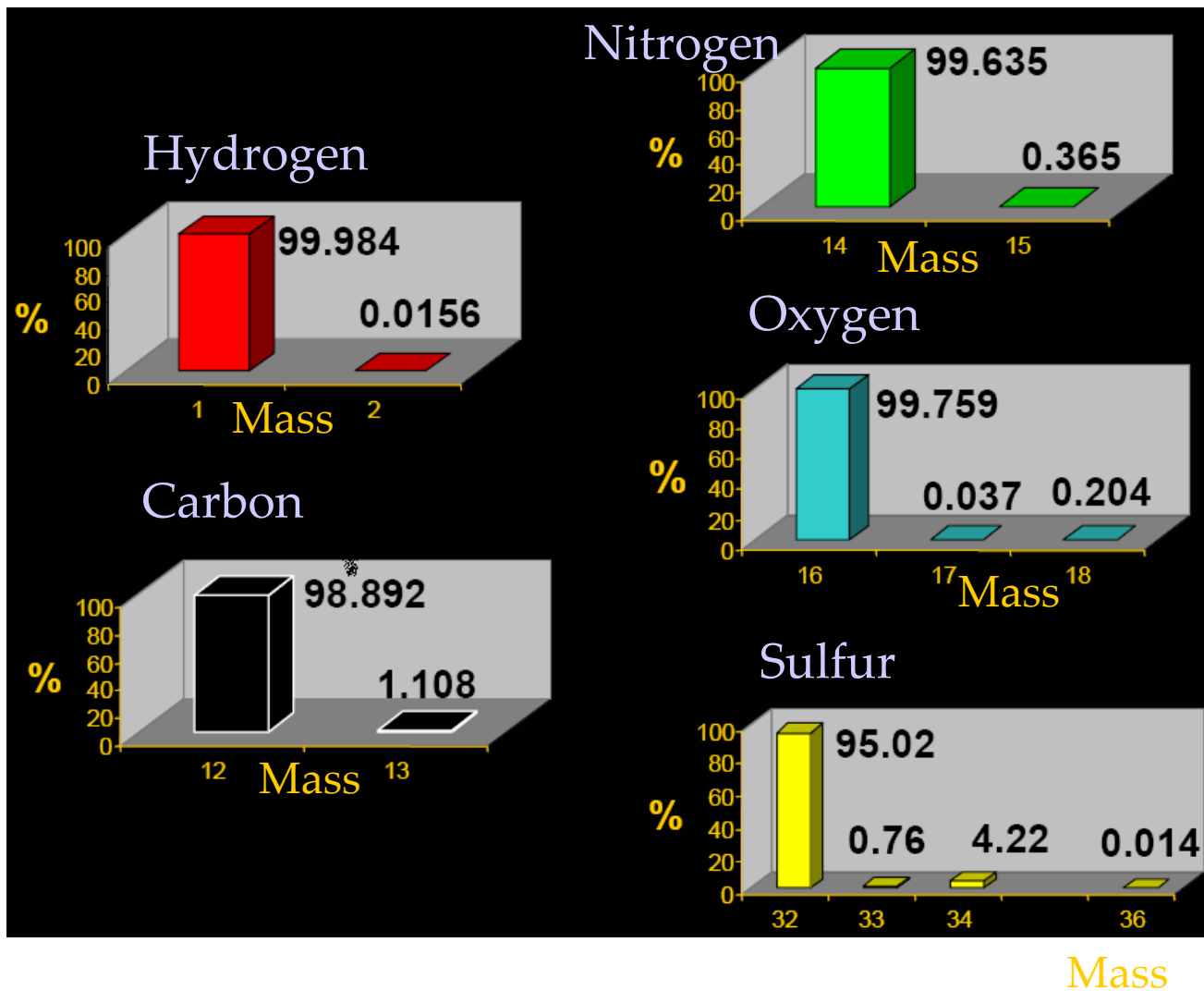
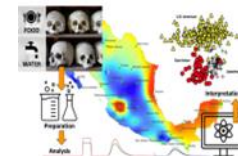
( B, Li, Mg, Ca, K,....)

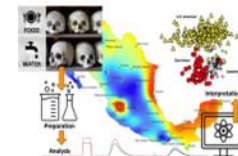
radiogenic heavy isotopes: Sr, Nd, Hf, Os, Pb





# Isotope abundances





**TABLE 1.3:** Chemical and physical properties of three of the nine isotopologues of water.

Property	H <sub>2</sub> <sup>16</sup> O	D <sub>2</sub> <sup>16</sup> O	H <sub>2</sub> <sup>18</sup> O
Boiling Point (°C)	100.00	101.42	
Freezing Point (°C)	0.00	3.82	
Density at 0°C (gm/cm <sup>3</sup> )	0.999841	1.10469	
Vapor Pressure at 20°C (bars × 10 <sup>2</sup> )	2.3379	2.0265	2.3161
Temperature of Maximum Density (°C)	4.0	11.6	
Critical Temperature (°C)	374.1	371.5	
Critical Pressure (bars)	220.6	221.5	
Ionization Product $K_w$ at 25°C	$1 \times 10^{-14}$	$0.3 \times 10^{-14}$	
Dielectric Constant at 20°C	80.36	79.755	
Surface Tension at 19°C (dynes/cm)	73.66	72.83	
Viscosity at 20°C (centipoise)	1.009	1.260	
Refractive Index $n_d$ at 20°C	1.33300	1.32844	
Representative Solubilities at 25°C (g/g of water)			
NaCl	0.359	0.305	
BaCl <sub>2</sub>	0.357	0.289	

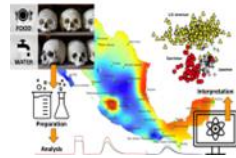
(From Weast, 1970.)

- The difference in mass between isotopes gives rise to different behaviors when involved in physical and chemical processes.
- *E.g.*, the H<sub>2</sub><sup>18</sup>O molecule is much heavier than the H<sub>2</sub><sup>16</sup>O molecule so that during the evaporation process the vapour is more enriched by <sup>16</sup>O.





# *Isotopic fractionation*



**Physical, chemical, biological processes**



*Isotopic fractionation (or isotopic discrimination):*

variation in the isotope abundance ratios resulting from chemical, physical and biological processes, as a consequence of the different physical and chemical properties between the heaviest and the lightest isotope.

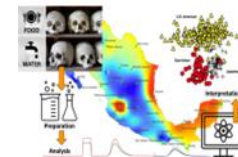


**Vapour enriched in  ${}^{16}\text{O}$**

*“isotope fingerprint”*



# Isotopic fractionation



- **Thermodynamic isotopic effect** - associated with equilibrium processes and favors the accumulation of the heavier isotope (compound more enriched in the heavier isotope) (e.g., rainwater condensation).
- **Kinetic isotopic effect** - results from differences in atomic mass of isotopes in the rate of a reaction. It is associated with non-equilibrium or incomplete processes (e.g., evaporation, diffusion.)

Lighter isotopes



Weaker bonds (easier to break)

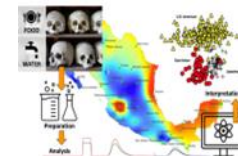
SOMETIMES THE EXTRA NEUTRON MAKES A DIFFERENCE. IT'S HARDER TO PUSH THE HEAVY MOLECULES UP AN ENERGY HILL ...



... SO THAT PRODUCTS HAVE MORE OF THE LIGHT ISOTOPE AND LESS OF THE HEAVY ISOTOPE.

React faster

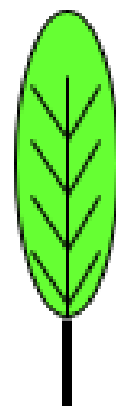
Products enriched in lighter isotopes



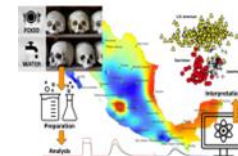
will be relevant?



1,0860 %  $^{13}\text{C}$



1,0805 %  $^{13}\text{C}$



*and now?*

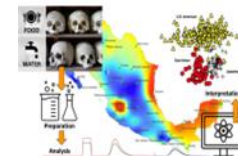
Isotopic values in *delta notation,  $\delta$*



$$1,0860 \text{ } ^{13}\text{C} = -23 \text{ } \text{‰}$$



$$1,0805 \text{ } ^{13}\text{C} = -28 \text{ } \text{‰}$$



# $\delta$ Notation

$\delta$  values are obtained using the following formula:

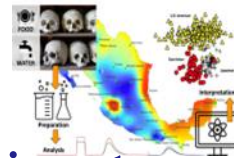
$$\delta_{S/R} = [R_S/R_R - 1] \times 10^3 \text{ ‰}$$

where:

$R_S$  :sample isotopic ratio

$R_R$  :reference isotopic ratio

*Per mil (‰)*



- $\delta > 0$ , sample is **enriched** in the heaviest isotope relative to the standard.
- $\delta < 0$ , sample **depleted** in the heavier isotope compared to the standard

$^{13}\text{C}/^{12}\text{C}_{\text{PDB}} = 0,011237$  Internacional Standard

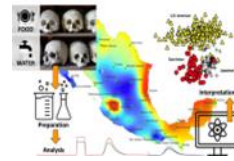
### Examples

$^{13}\text{C}/^{12}\text{C}_{\text{Amostra}} = 0,011248$  + 1 ‰ (variação vs PDB)

$^{13}\text{C}/^{12}\text{C}_{\text{Amostra}} = 0,011226$  - 1 ‰ (variação vs PDB)

$\delta^{13}\text{C}, \delta^{15}\text{N}, \delta^2\text{H}, \delta^{18}\text{O}, \delta^{34}\text{S}$



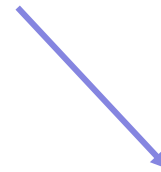


# *Reference Materials*

To correctly compare the results of isotopic ratios between laboratories, it is essential to **normalize** the results to a common international scale:

## **International Reference Materials**

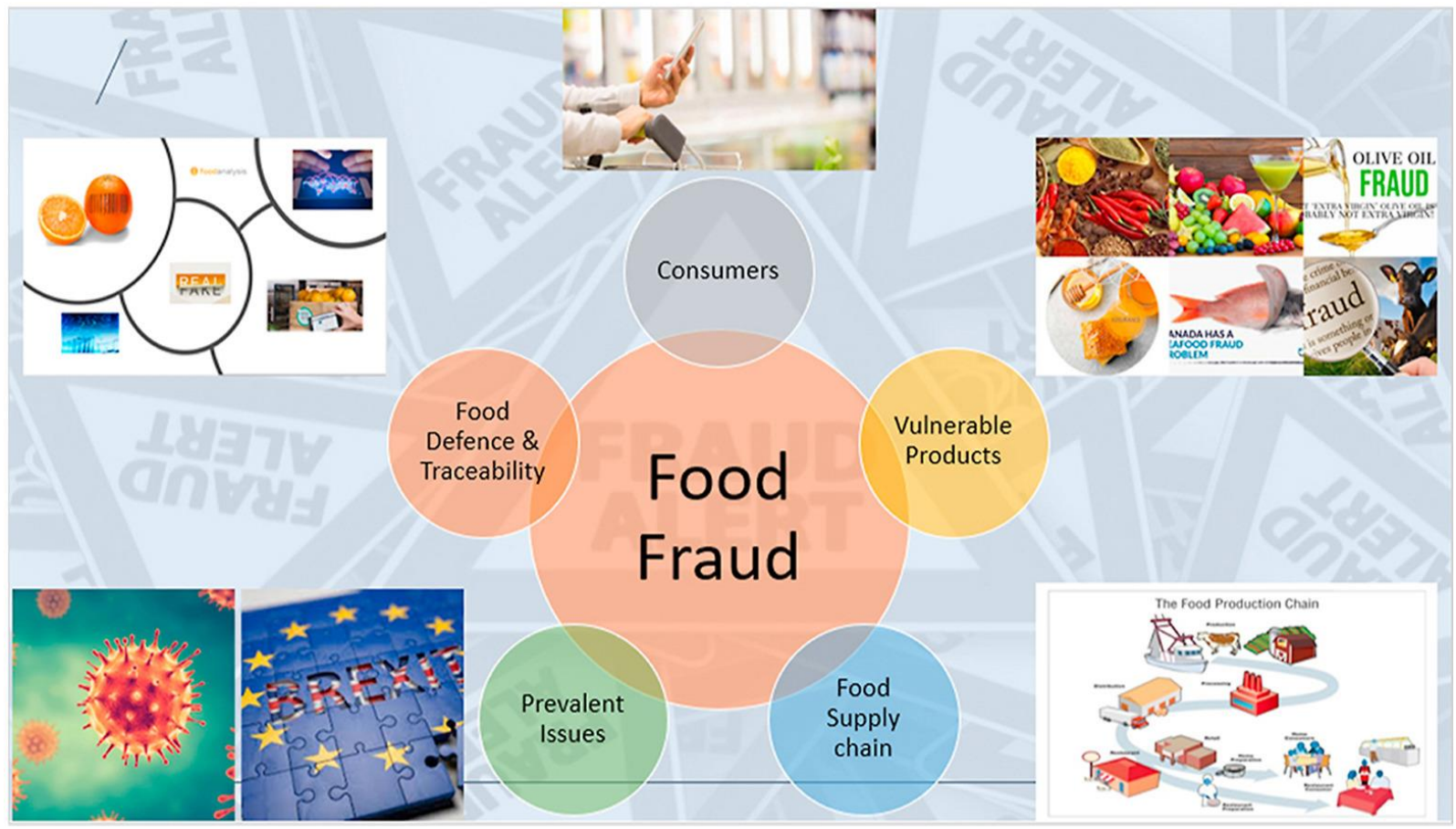
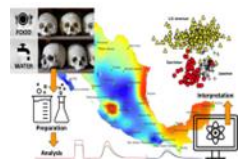
Reliable and traceable analytical results



**(C)RM**

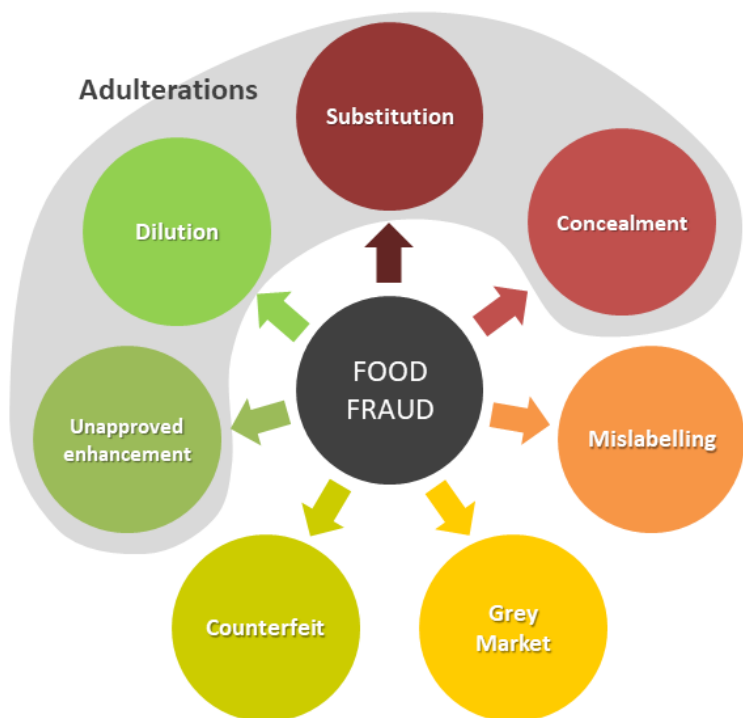
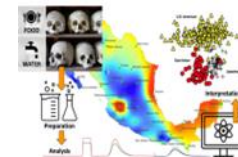


# Economic/crime food fraud





# Economic/crime food fraud



**Grey market:** production, theft, and diversion involving unauthorised sales channels for products. Ex. - sale of excess unreported product when there are production agreements or quotas for the product and the product in question is deliberately produced in excess of these. A fish product originating from illegal, unreported, and unregulated (IUU) fishing is another example. This term also applies when there is a geographical restriction on the sale and distribution of the product, and the product in question is deliberately sold or distributed in other areas

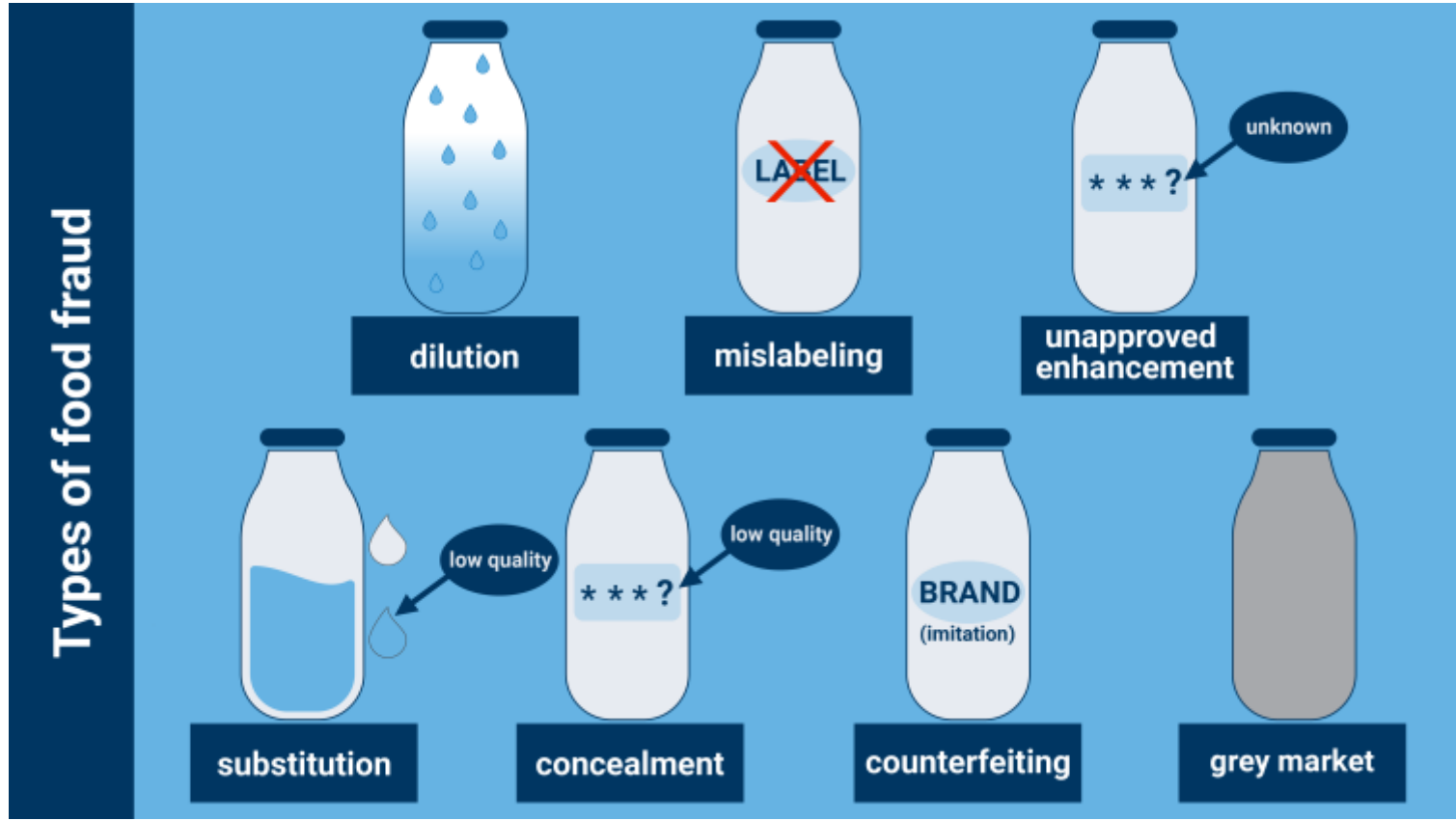
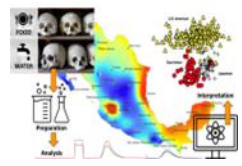
**Unapproved enhancement:** "process of adding unknown and undeclared compounds to food products in order to enhance their quality attributes". Ex. the melamine in milk falls under this category, as adulteration with melamine in milk products aimed at enhancing nitrogen content or use of such as Sudan dyes in spices.

**Counterfeit** infringement Intellectual Property Rights (IPR)

**Concealment** : the "process of hiding the low quality of food ingredients or products". Injecting poultry with hormones to conceal disease is an example of this, as well as meat treated with carbon monoxide.

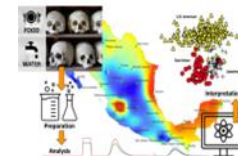


# Economic/crime food fraud





## *Economic/crime food fraud*



# **ECONOMIC FRAUD**

**“EUR 230 million worth of fake food and beverages seized in global OPSON operation targeting food fraud”**

Operation OPSON VI, the joint Europol-INTERPOL operation targeting counterfeit and substandard food and drink, as well as the organised crime networks behind this illicit trade, has resulted in the seizure of **9 800 tonnes**, over **26.4 million litres**, and **13 million units/items** worth an estimated EUR 230 million of potentially harmful food and beverages ranging from every day products such as alcohol, mineral water, seasoning cubes, seafood and olive oil, to luxury goods such as caviar.

**Portugal – More than 300 000 tin cans of fish seized in a factory**

<https://www.europol.europa.eu/media-press/newsroom/news/eur-230-million-worth-of-fake-food-and-beverages-seized-in-global-opson-operation-targeting-food-fraud>

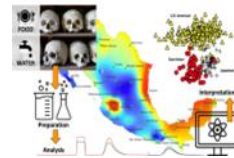






























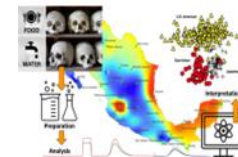
# Economic/crime food fraud



Stable isotope	What can be determined?	What food fraud can be identified?	What products can be affected?
<b>Carbon</b>	Photosynthesis (C3, C4 and CAM pathways)	Adulteration (the substitution of cheaper ingredients)	 Honey  Liquor  Wine  Olive oil  Butter
<b>Hydrogen</b>	Local-regional rainfall and geographical area	Dilution of beverages; origin of product	 Coffee  Liquor  Wine  Water  Sugar  Meat
<b>Nitrogen</b>	Fertilizer assimilation by plants	Mislabelling (Organic and non-organic)	 Vegetables  Meat
<b>Oxygen</b>	Local-regional rainfall and geographical area	Dilution of beverages; origin of product	 Coffee  Liquor  Wine  Water  Sugar  Meat
<b>Sulfur</b>	Local soil conditions; proximity to shoreline	Origin of product	 Vegetables  Meat  Honey



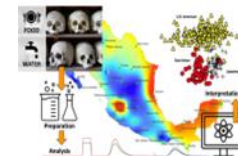
# Authenticity and Provenance of Beverages



- Counterfeit brand products ranges from sparkling water to fruit juices and from beer and wine to white spirits and whisky.
- Control of flavours, fragrances and essential oils. Substituting synthetic or 'nature identical' for natural flavours or fragrances is an all too easy way to defraud consumers by, for example, selling a product containing synthetic flavour or fragrance as the pure natural product or a product based on priced natural ingredients only. Natural lavender oil, for example, contains 30–60% linalyl acetate and retails at about £450/l. Synthetic linalyl acetate (97%) costs £108/l while natural linalyl acetate (around 80%) costs £180/l. Similarly, an extract of natural -decalactone, a flavor compound contained in peach and apricots, costs approximately £480/kg while the synthetic compound only costs £60/kg.

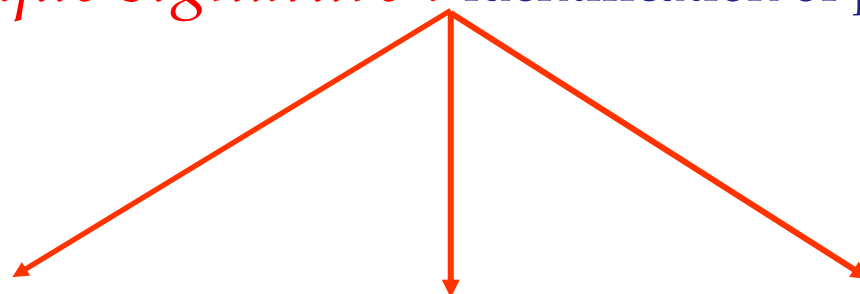


Foodstuffs, foods and drinks, has an



*isotope fingerprint,*

a *unique signature* : identification of products



**Botanical origin**

Baunilha, azeite,  
açúcar, carne, mel  
(tipo de  
alimentação)

**Geographical origin**

Vinho, bebidas  
espirituosas, queijo,  
café, açúcar, carne  
(tipo de alimentação).

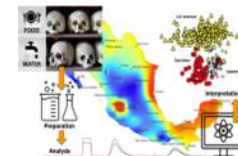
**Fraudulent  
practices \***

Mel, vinho, bebidas  
espirituosas,  
sumos)

\*These processes can be trace back by using **C, N, H, O and S isotopes**

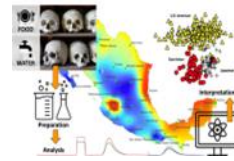


...*“isotope fingerprint”*...



distinguish “cat from hare”





# One of the most common frauds is *adulteration*

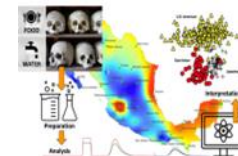
According to the CWA:

"A type of food fraud which includes the intentional addition of a foreign or inferior substance or element; especially to prepare for sale by replacing more valuable with less valuable or inert ingredients."





# *Adulteration/counterfeiting/authenticity*



*why cheat?*

**Motivation :**

**ECONOMIC**

Where there is money there will be criminals trying to take their cut

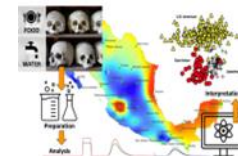
**Public Health Safety Problem?**

**Who loses? The consumer, honest entrepreneurs**





# OCDE

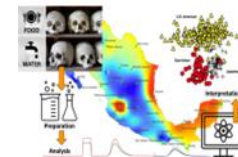


In a report on *The Economic Impact of Counterfeiting and Piracy*, published in 2007 (<http://www.oecd.org/dataoecd/13/12/38707619.pdf>; last accessed 24 July 2009), the Organization for Economic Cooperation and Development (OECD) stated that in 2005:

*. . . the volume of tangible counterfeit and pirated products in international trade could be up to US\$200 billion. This figure does not, however, include counterfeit and pirated products that are produced and consumed domestically, nor does it include the significant volume of pirated digital products that are being distributed via the Internet. If these items were added, the total magnitude of counterfeiting and piracy worldwide could well be several hundred billion dollars more.*



# *E.U. Wine Databank*



- ❖ Portuguese Wine Databank (*E.U. Wine Databank*)  
(isotopic parameters  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ )

- ❖ Geographical origin of Portuguese Wines

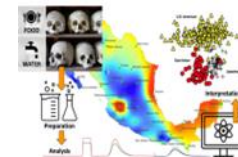
- ❖ Adulteration/authenticity detection (water /sugar addition).

$\delta^{13}\text{C}$  wine ethanol = -20 a -26 ‰  
 $\delta^{13}\text{C}$  ethanol, cane sugar = -10 a -14 ‰

$\delta^{18}\text{O}$  water from grapes = +1 a +10 ‰  
 $\delta^{18}\text{O}$  mineral water = -3 a -8 ‰

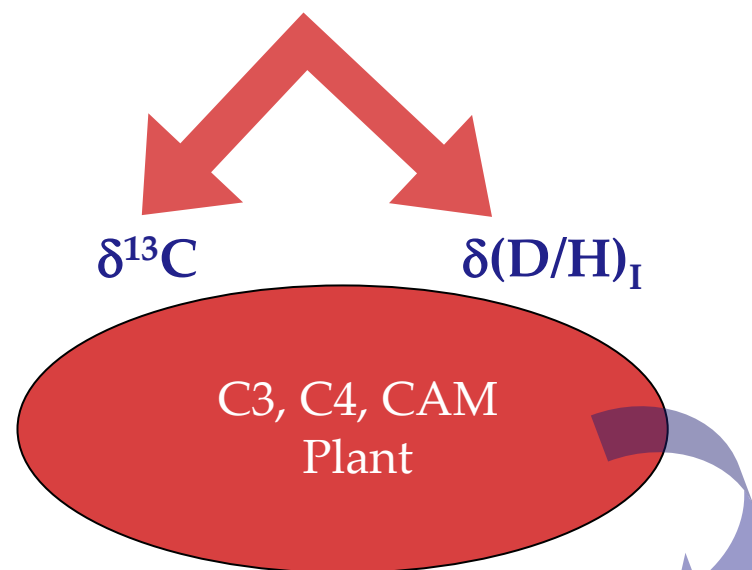
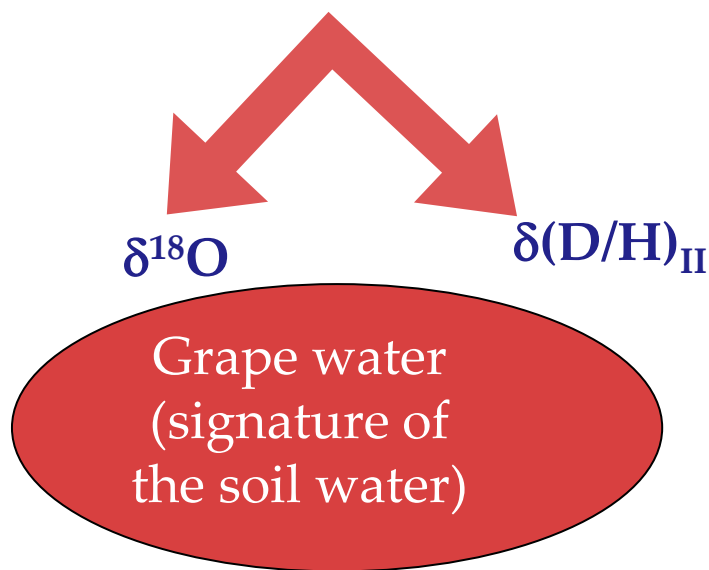


# Food Authenticity



## Geographical Origin

## Botanical Origin



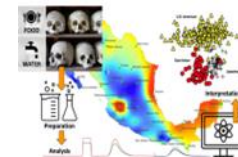
C3	C4	CAM
( $\delta^{13}\text{C}$ : -35 a -22 ‰)	( $\delta^{13}\text{C}$ : -17 a -9 ‰)	( $\delta^{13}\text{C}$ : -35 a -9 ‰)
<b>Vine</b> , beet, rice, wheat, soya	Cane sugar, maize	Pineapple, vanilla

*Authenticity/Adulteration*

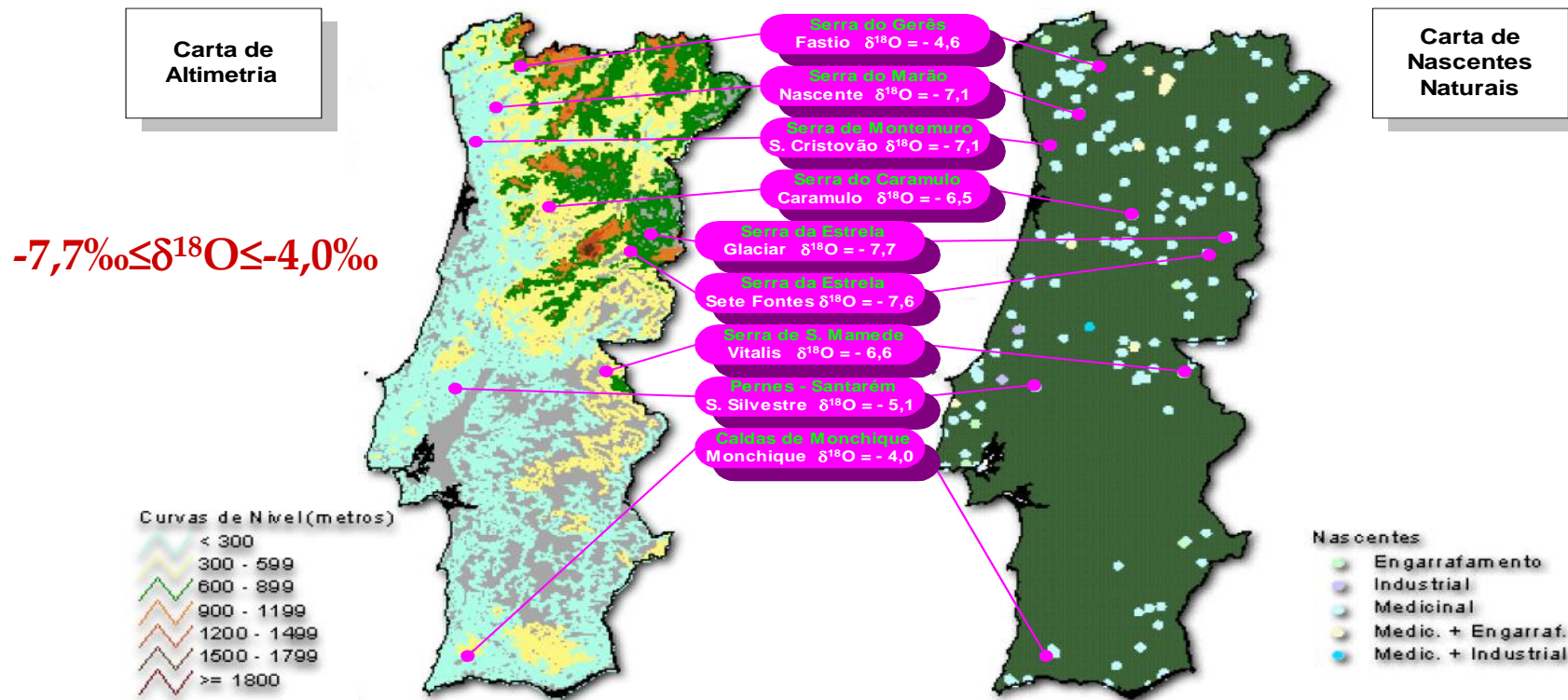
**adulteration:** addition of cane or beet sugar, or addition of water



# Wine



$\delta^{18}\text{O}$  values for several portuguese commercial waters (internal standards).

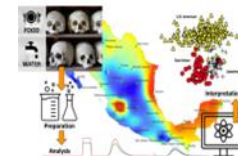


Portugal map (left: Altimetry map and right: Spring waters map) with the  $\delta^{18}\text{O}$  values assigned.

$\delta^{18}\text{O}/^{16}\text{O}$  values range from -4.0 to -7.7 ‰ depending on the altitude



# Wine



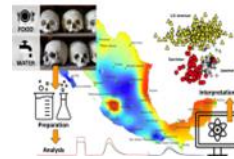
- ❖ Portuguese Wine Databank (*E.U. Wine Databank*) (isotope parameters  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ,  $\delta(\text{D}/\text{H})_{\text{I}}$  and  $\delta(\text{D}/\text{H})_{\text{II}}$ ) (since 2000)
- ❖ Geographic origin of portuguese wines.
- ❖ Adulteration/authenticity detection (water /sugar addition).

$$\left\{ \begin{array}{l} \delta^{13}\text{C ethanol wine} = -20 \text{ a } -26 \text{ ‰} \\ \delta^{13}\text{C ethanol cane sugar} = -10 \text{ a } -14 \text{ ‰} \end{array} \right.$$

$$\left\{ \begin{array}{l} \delta^{18}\text{O water wine} = +1 \text{ a } +10 \text{ ‰} \\ \delta^{18}\text{O mineral water} = -3 \text{ a } -8 \text{ ‰} \end{array} \right.$$



# Wine

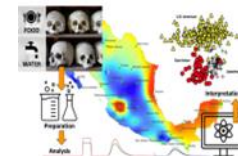


❖  $\delta^{18}\text{O}$  values for mineral waters (majority, has negative  $\delta^{18}\text{O}$  value) are very different from wine water (positive  $\delta^{18}\text{O}$  value).

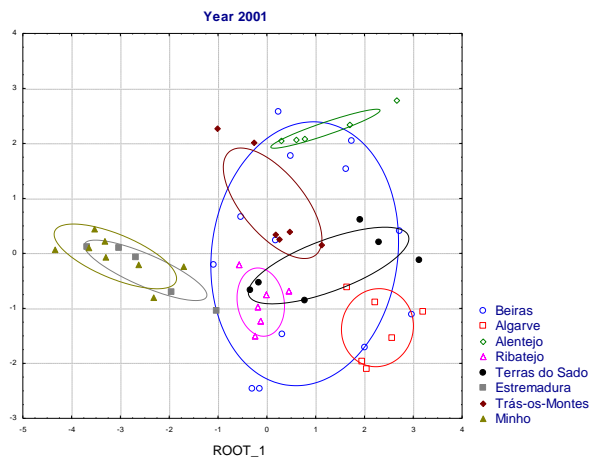




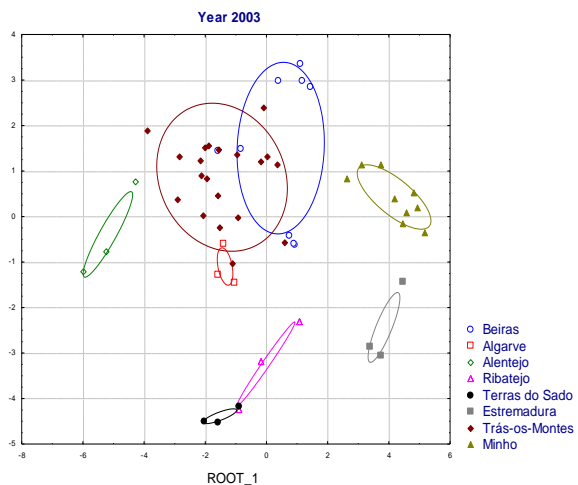
# Wine



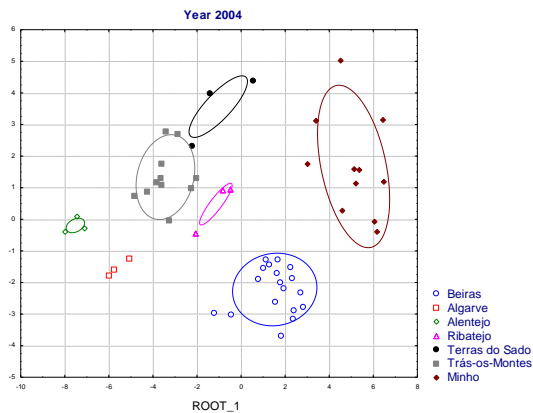
Grouped by Wine growing zones (vintages) 2001, 2002, 2003, 2004 and 2005)



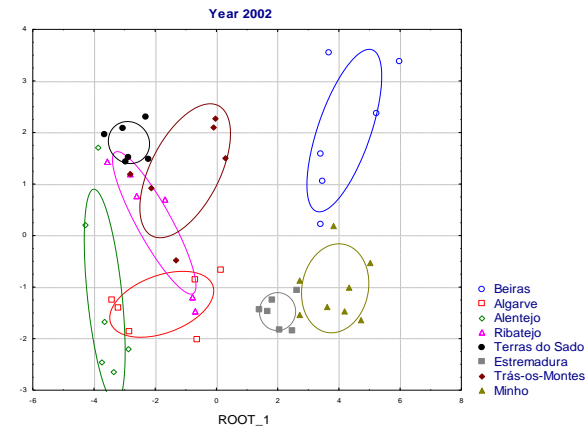
Vintage 2001



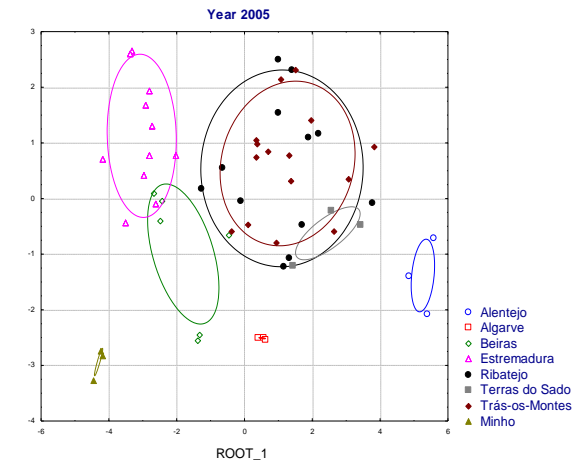
Vintage 2003



Vintage 2004



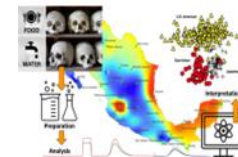
Vintage 2002



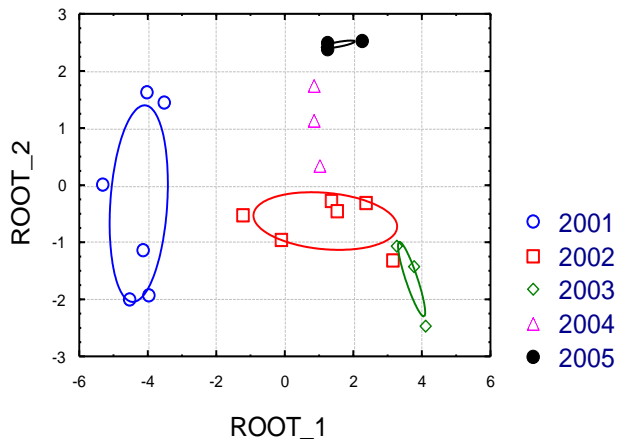
Vintage 2005



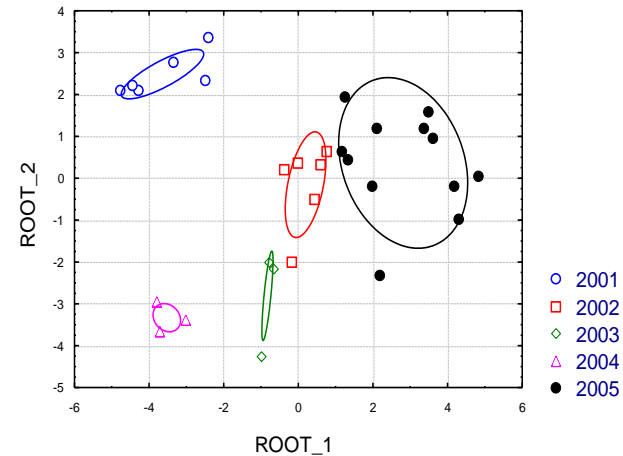
# Wine



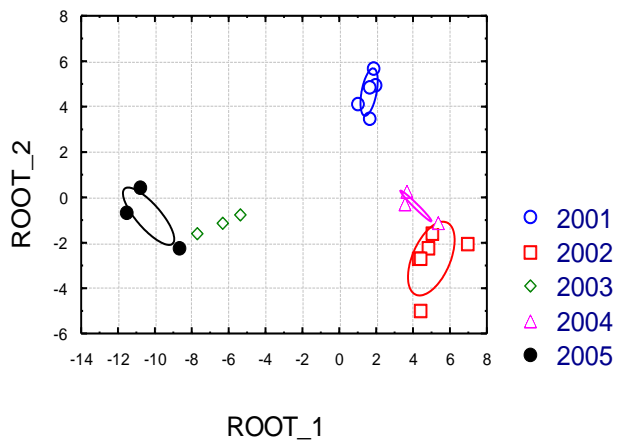
### ALGARVE (2001-2005)



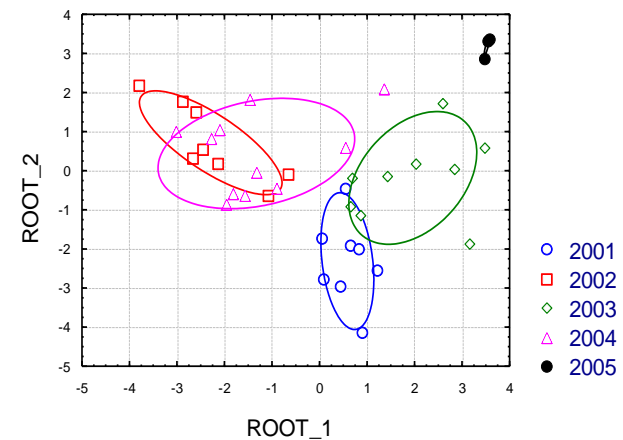
### Ribatejo 2001-2005



### Alentejo 2001-2005



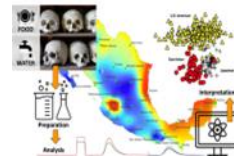
### Minho 2001-2005



Algarve,  
Alentejo,  
Ribatejo, Minho  
(Grouped by  
vintages)



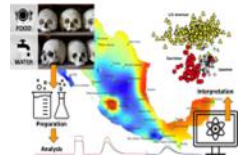
# Databases



- Food authentication and traceability: reference dataset: Databank
- Official databanks based on regulations (EU Wine Databank)
- Databank created by product-specific association or Consortium (AIJN for fruit juices, Grana Padano PDO cheese).



# *The murky world of herb and spice fraud*

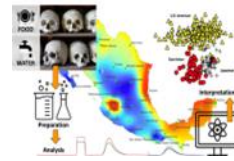


Global herb and spice industry is valued at approximately **US\$4 billion** and continues to grow. **Vanilla** and **saffron** as examples, the former is worth more by weight than silver, while the **latter is more valuable than gold!**





# Yellow Dye in Turmeric Linked with Lead Poisoning in Bangladesh



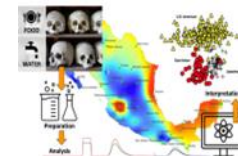
## *Cromato de chumbo*



There are 800 million children in the world with lead blood levels greater than recommended standards, and many will go on to suffer permanent brain damage. One million adults die every year because of exposure to lead

*Jenna Forsyth, PhD, a research scientist with the Stanford School of Medicine*

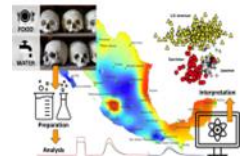




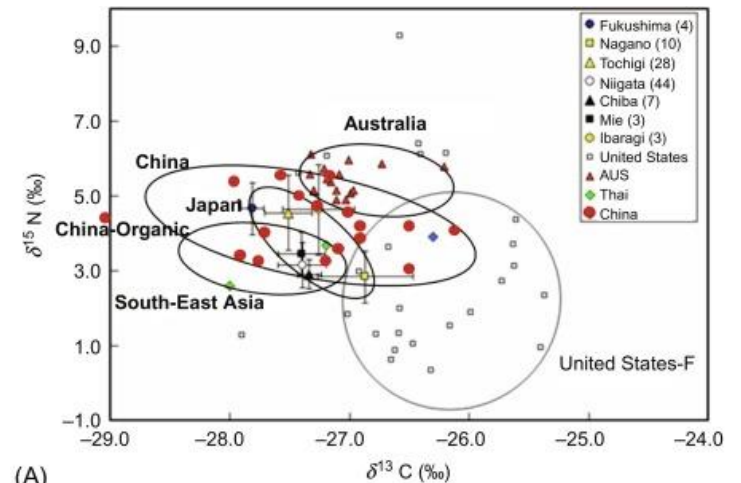
*“It was law enforcement theater,”* Forsyth said

After the intervention : at the markets, incidence of adulterated spice plunged from 47 % in Sep 2019 to 5 % in the first quarter of 2020, to no detectable lead in 2021.

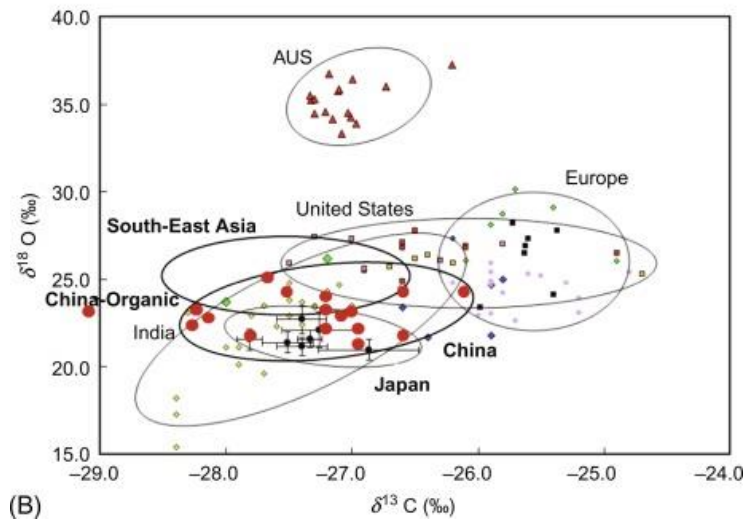
16 months after the intervention lead levels in the blood of sample test subjects dropped by a median of 30 %.



Relationships (A) between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and (B) the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of Koshihikari cultivated in Japan and other countries.

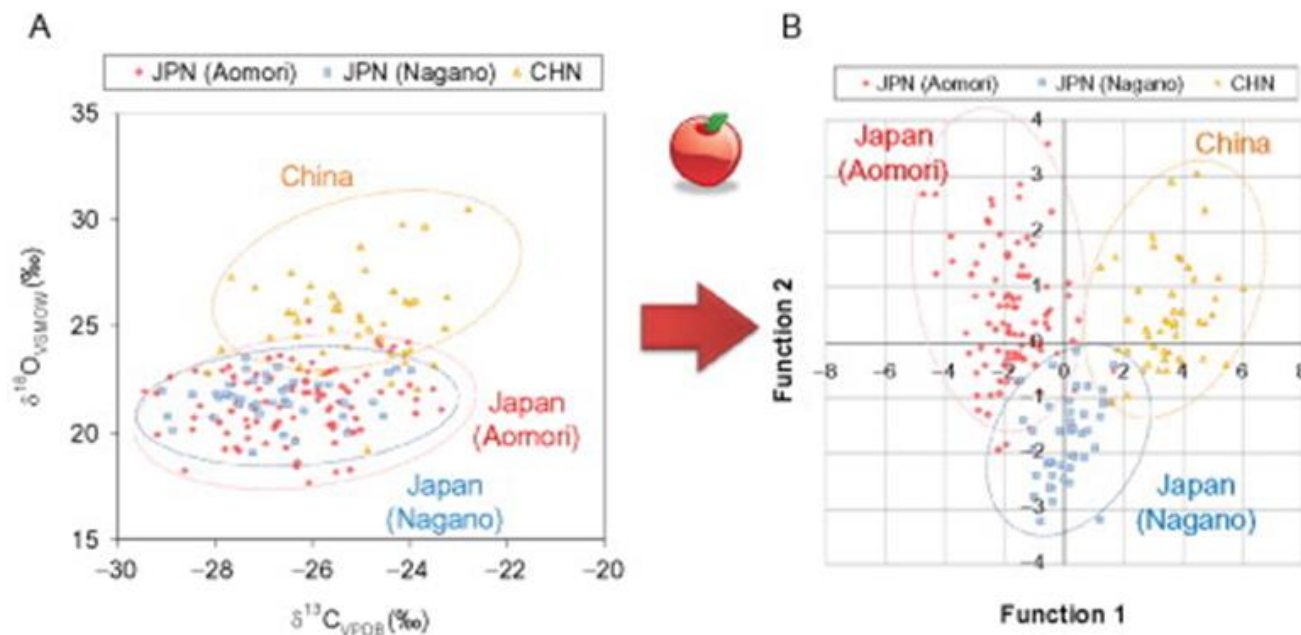
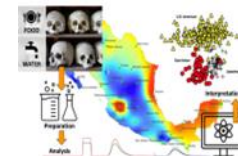


(A)



(B)

(BIOCHEMICAL STABLE ISOTOPE ANALYSIS IN FOOD AUTHENTICITY, Takashi Korenaga, Yaeko Suzuki, Yoshito Chikaraishi  
Engineering Tools in the Beverage Industry, Volume 3: The Science of Beverages, 2019, Pages 209-227)



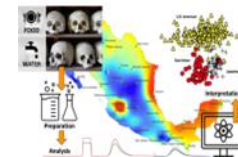
Distributions of the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of Chinese, Japanese (Aomori and Nagano) apples (A) and the corresponding dendrogram using the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values and nine elements (Mg, Ca, Mn, Fe, Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Cd, Cs, Ba, Tl, and Pb) of them (B)

(Suzuki, Y., & Nakashita, R. (2013). *Authentication and Traceability of Fruits and Vegetables*. *Comprehensive Analytical Chemistry*, 461–477. doi:10.1016/b978-0-444-59562-1.00018-9)





# Others...



Baunilha (vanilina) aroma mais usado em todo o mundo. Facto: A produção da baunilha natural apenas cobre 1 % da necessidade global.

99 % deste aroma é produzido sinteticamente (origem petróleo) ou com biotecnologia (ex. do ácido ferúlico e do eugenol).

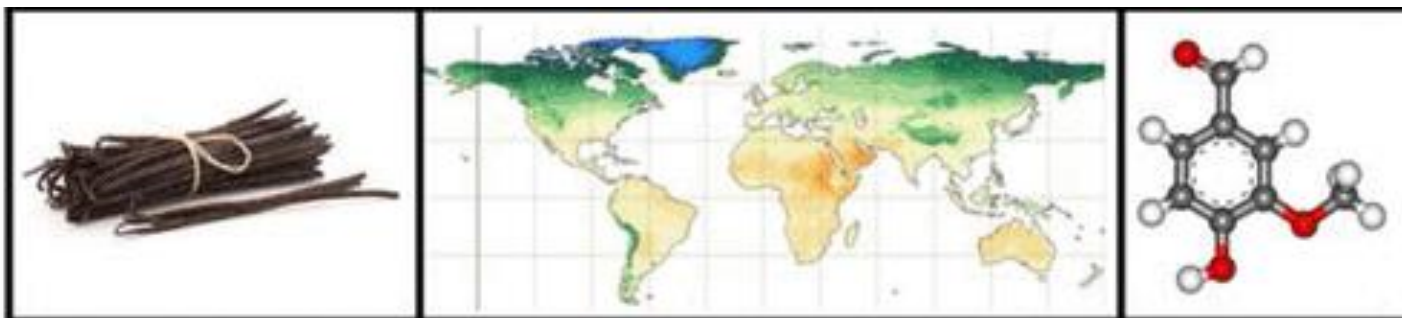
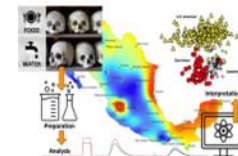
**Preço: natural vs sintética**

**100 : 1**



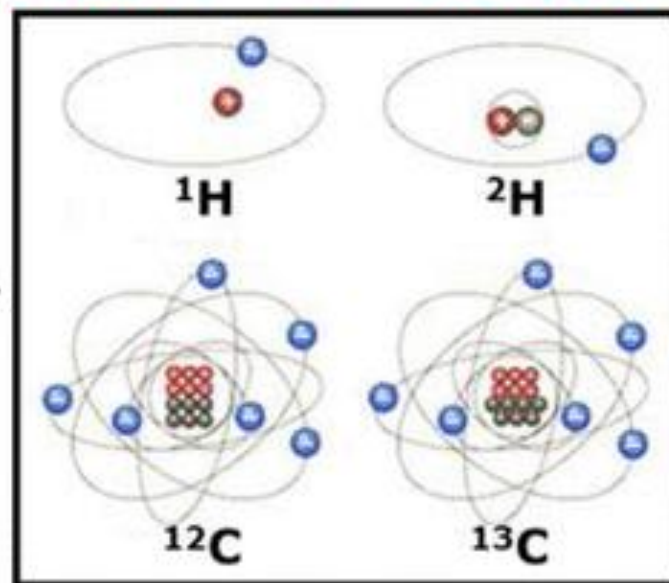
Vanilina natural e sintética são quimicamente semelhantes. Mas a “assinatura isotópica pode ser usada para as diferenciar.

**How can consumers be protected from false declaration and fraud?**



## AUTHENTICITY and TRACEABILITY of VANILLA

USING STABLE ISOTOPES





# Official Methods

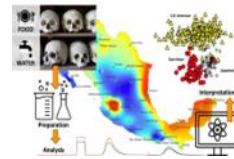
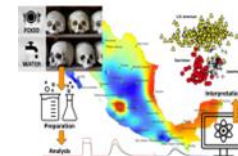


Table 1. Official methods for isotope analysis using isotope ratio mass spectrometry.

Product	Official method	Isotope fingerprint	Sample	What does it address?	Analytical solution
<b>Wine</b>					
	OIV-MA-AS2-12	$\delta^{18}\text{O}$	Water	Adulteration, Geographical origin, Year of vintage	Thermo Scientific™ GasBench II System, Thermo Scientific™ Dual Inlet
	OIV-MA-AS312-06	$\delta^{13}\text{C}$	Ethanol, Wine must, Grape sugar	Adulteration, origin	Thermo Scientific™ EA IsoLink™ IRMS System, Thermo Scientific™ GC IsoLink II™ Interface for GC-IRMS
	OIV-AS312-07	$\delta^{13}\text{C}$	Glycerol in wines	Adulteration by addition of glycerol from C4 maize or fossil sources	GC IsoLink II Interface for GC-IRMS, Thermo Scientific™ LC IsoLink™ Interface for IRM-LC/MS
	OIV-OENO 510-2013	$\delta^{13}\text{C}$	Acetic acid in wine, vinegar		GC IsoLink II Interface for GC-IRMS, EA IsoLink IRMS System
OIV-OENO 510-2013	$\delta^{18}\text{O}$	Water in wine, vinegar	Adulteration, Geographical Origin, Year of Vintage	Thermo Scientific™ GasBench II System, Dual Inlet	
<b>Sparkling wine</b>					
	OIV-MA-AS314-03	$\delta^{13}\text{C}$	$\text{CO}_2$ in sparkling wine	Origin and authenticity of sparkling wine	GasBench II System, EA IsoLink IRMS System, GC IsoLink, Dual Inlet
<b>Spirits</b>					
	OIV-AS312-07	$\delta^{13}\text{C}$	Glycerol in spirits	Adulteration by addition of glycerol from C4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, LC IsoLink Interface for IRM-LC/MS
<b>Fruit Juice</b>					
	EU - CEN				GasBench II System, LC IsoLink Interface

<b>Spirits</b>					
	OIV-AS312-07	$\delta^{13}\text{C}$	Glycerol in spirits	Adulteration by addition of glycerol from C4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, LC IsoLink Interface for IRM-LC/MS
<b>Fruit Juice</b>					
	EU - CEN 1995	$\delta^{13}\text{C}$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	LSA - AOAC 1981	$\delta^{13}\text{C}$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU - CEN 1998	$\delta^{13}\text{C}$	Sugars and pulp	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU - CEN 1995	$\delta^2\text{H}$ and $\delta^{18}\text{O}$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	AOAC method 2004.01	$\delta^{13}\text{C}$	Ethanol (From Fermentation)	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
<b>Fruit Juice (Concentrate)</b>					
	AOAC 1992	$\delta^{18}\text{O}$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, EA IsoLink IRMS System
<b>Honey</b>					
	AOAC method 991.41	$\delta^{13}\text{C}$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
	AOAC method 998.12	$\delta^{13}\text{C}$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
<b>Cheese</b>					
	EU Reg 548/2011	$\delta^{13}\text{C}$	PDO	PDO Grana Padano	EA IsoLink IRMS System



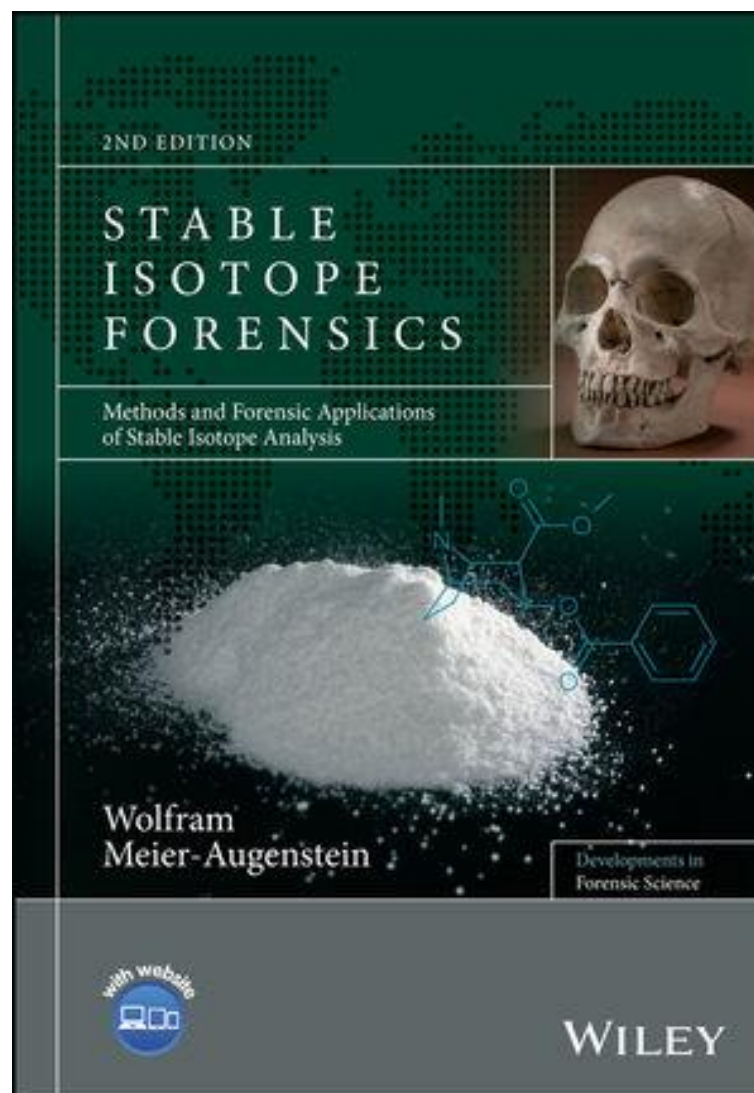
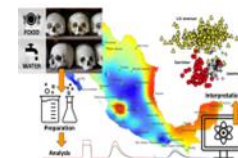
**IRMS can contribute towards greater transparency and security for food.**

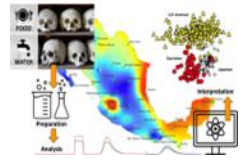






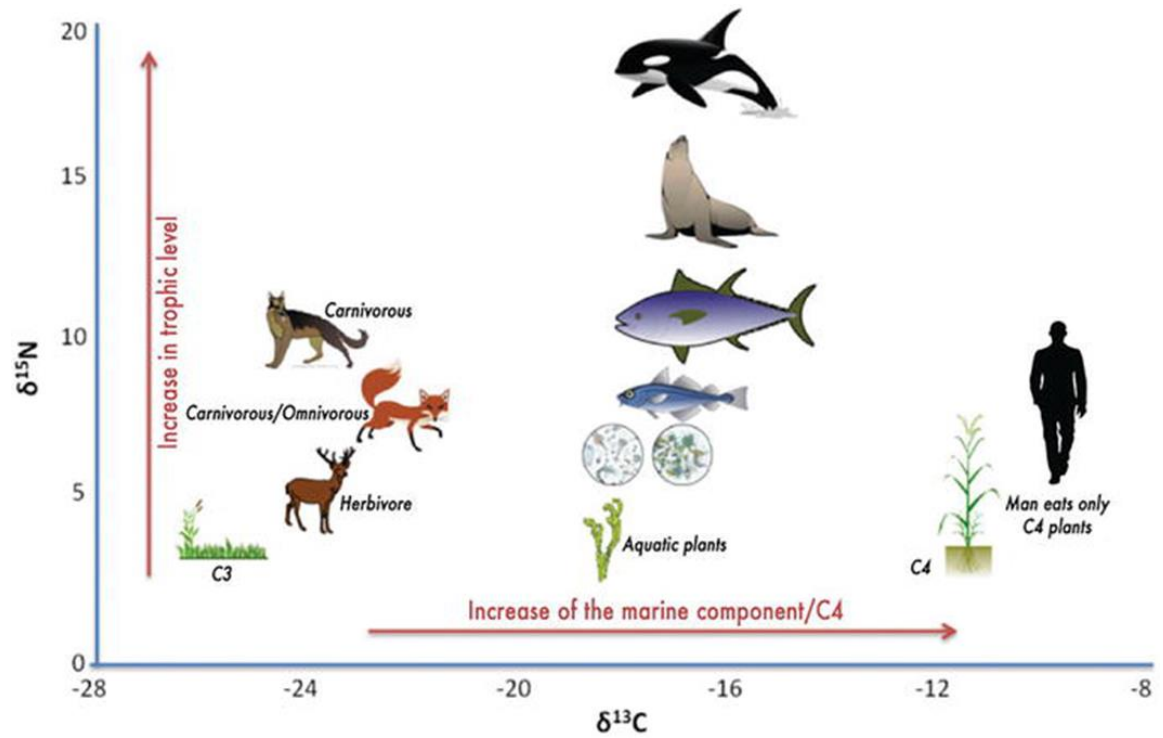
# Stable isotopes forensics





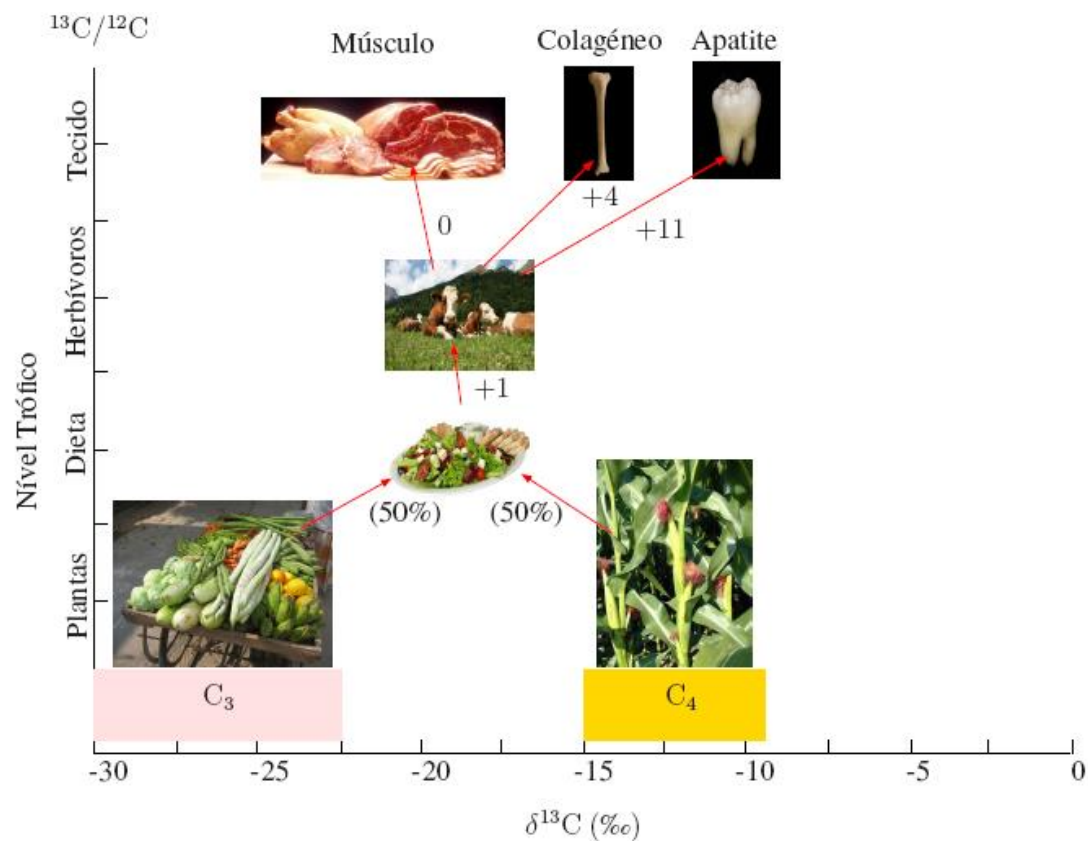
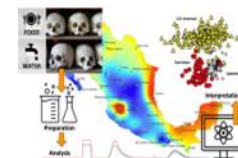
# STABLE ISOTOPES IN HUMANS

*“You are what you eat”*





# Isotope fractionation in diet

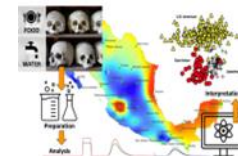


e.g., in mammals and birds whose feed is known, the difference of  $\delta^{13}\text{C}$  (‰) between collagen and feed is about +5 ‰.



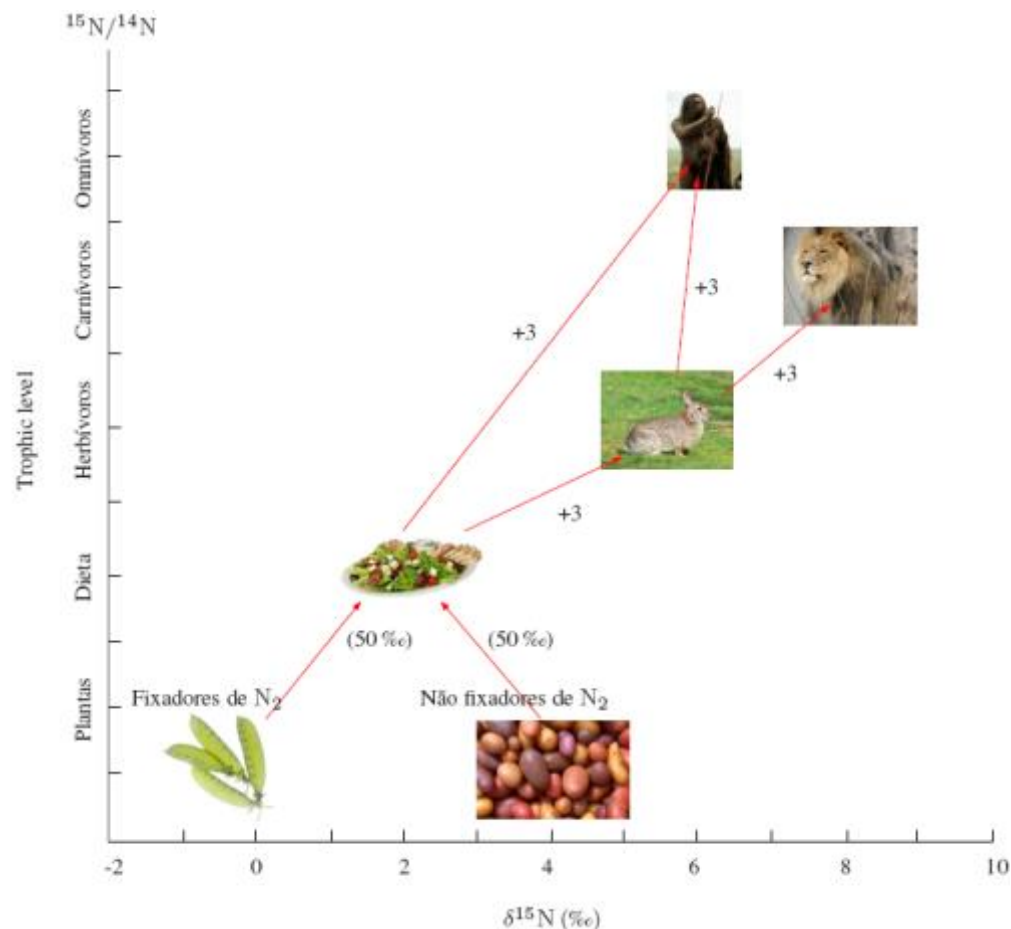


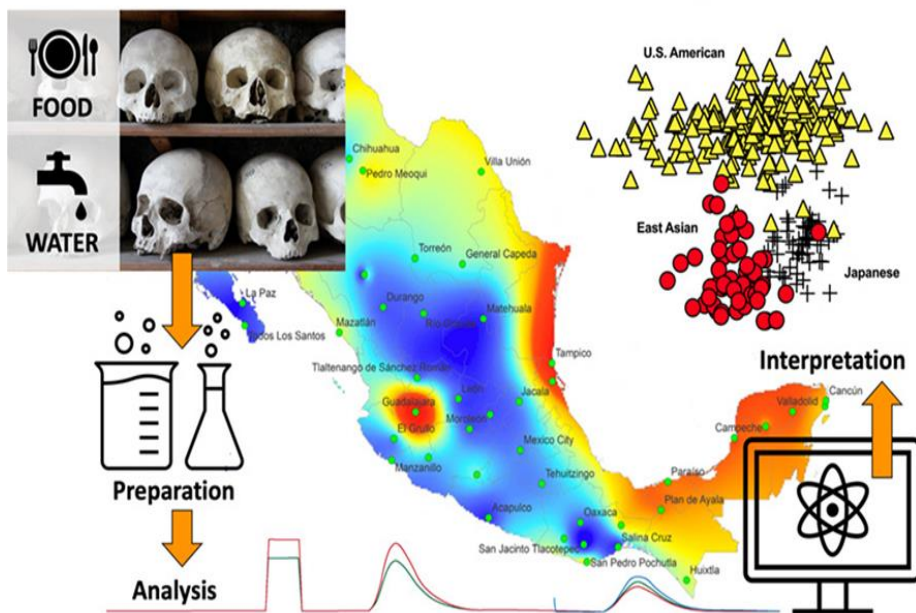
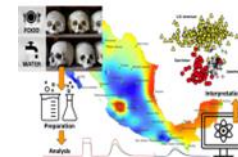
# Isotope fractionation in diet



Fractionation of  $\delta^{15}\text{N}$  (‰) (present in collagen) in the food web - herbivores, carnivores and omnivores..

As you move up the nutrition chain, higher the enrichment is  $^{15}\text{N}$  - ca. +3 (‰) in each stage.





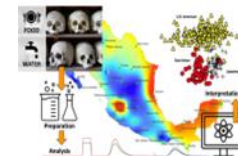
*Forensic application of stable isotope ratio analysis capitalizes on the fact that certain isotopes: “nature’s recorders” and the isotopic composition of human tissues reflects the isotopic composition of what the individual ingested.*

*How food and water vary isotopically across landscapes :*

*interpretation of the isotope records in human tissues can help to reconstruct life history, including diet, geographic provenance, and travel movements before death.*



## *Answer to...*



**How many people disappear every year around the world and cannot be identified?  
(human trafficking, violent crimes, involuntary disappearances...)**

## **Forensic identification in post-conflict?**

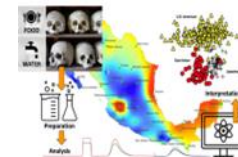
**Colombia: ~80 000 (political-social conflicts)**

**Ex-Yugoslavia:: ~40 000 (armed conflict)**

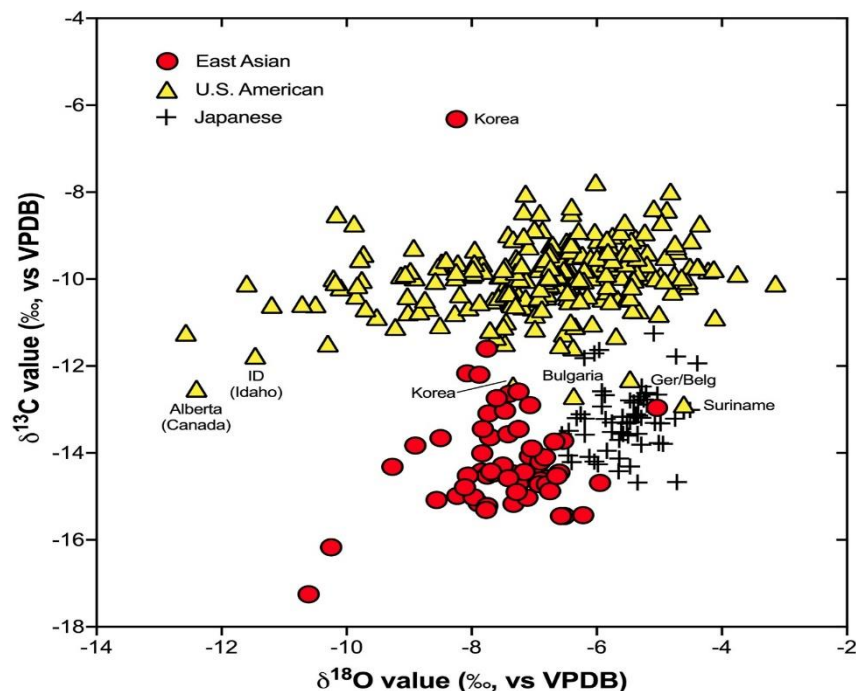
**Iraque, etc, etc, etc,...**



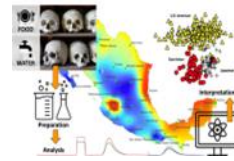
# Military conflicts



To investigate the isotopic discrimination of Japanese (n = 62) from Americans (n = 202) for the purpose of potentially segregating World War II soldiers. The authors stated that discrimination was “100% accurate”.



Distribution of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of tooth enamel carbonate for **East Asian** (red circles), **U.S. American** (yellow triangles), and Japanese (plus signs) individuals, demonstrating that populations can isotopically differ.

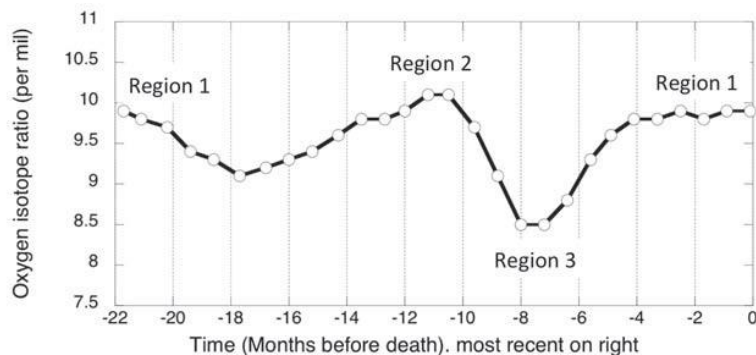
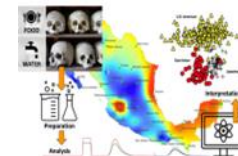


# *Humanitarian crises*

Similar to armed conflicts, humanitarian crises can also lead to large numbers of missing and unidentified persons. For instance, at least 15,000 individuals are known to have died at European Union borders in the past decade as a result of the migrant or refugee crisis faced by Mediterranean countries (Olivieri et al., 2018; Robins, 2019).



# “Saltair Sally”



(B) A plot of the changes in the oxygen isotope ratio of Saltair Sally’s hair over the last 22 months of her life. (C) The isoscapes of three geographical regions where Saltair Sally had traveled prior to her death are based on the tap water isoscape shown and the relationship between the oxygen isotope ratios of tap water and hair. The blue spot on isoscape Region 1 is Salt Lake City (Utah) from where Saltair Sally’s body was recovered. The yellow spot on isoscape Region 2 is Seattle (Washington) where she was originally from.



Region 1

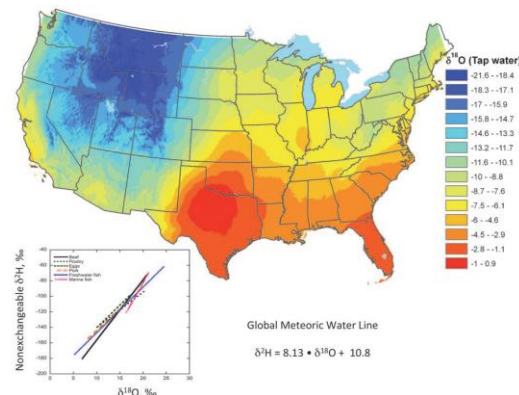


Region 2



Region 3

## 2012: Nicole Bakoles

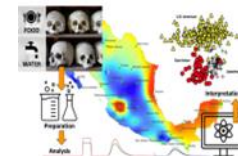


Ehleringer, J. R. , Elements, 2015

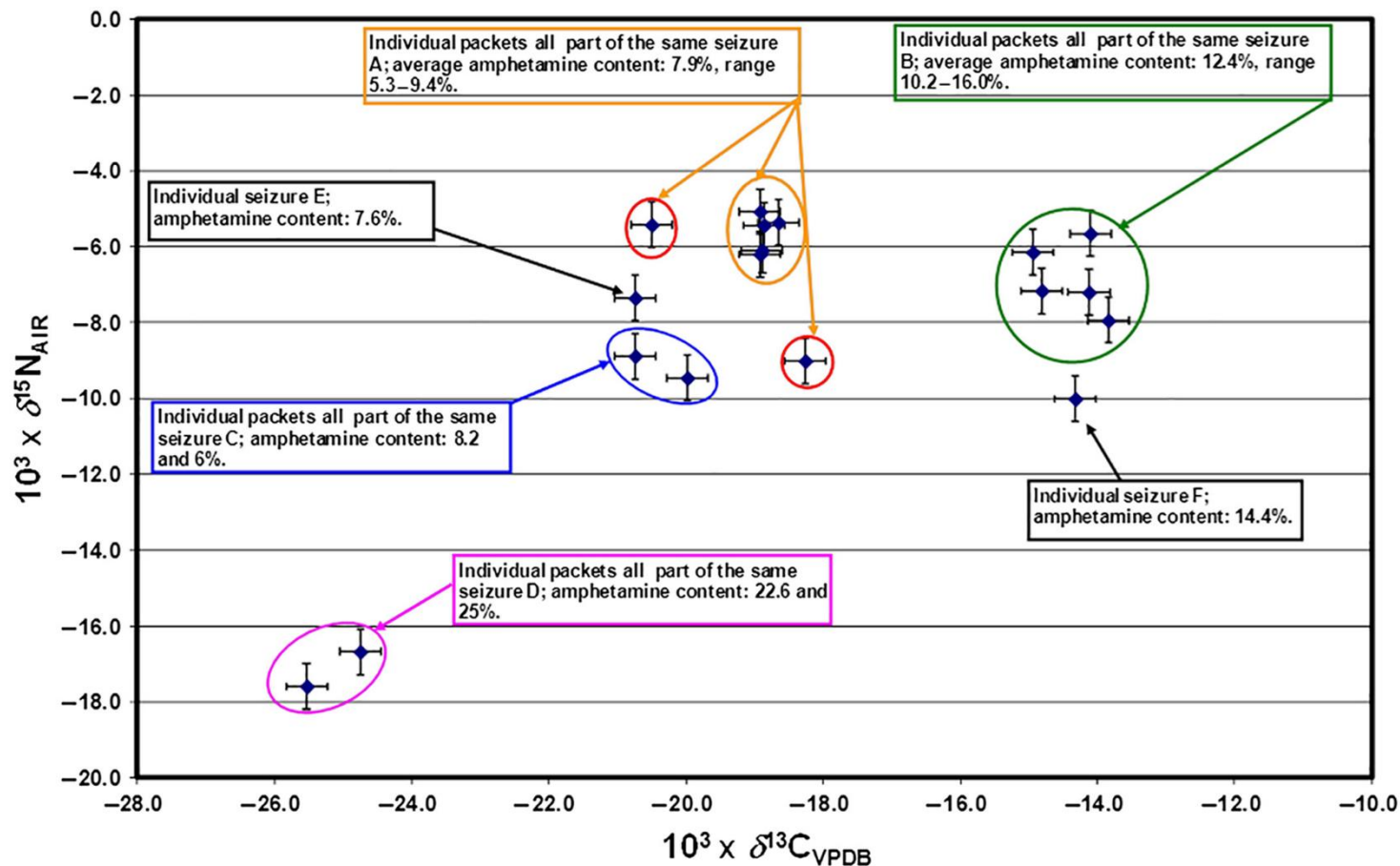




# Illicit drugs



## $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ signatures of 18 amphetamine samples from six seizures



Meier-Augenstein, W. (2019). Forensic stable isotope signatures: Comparing, geo-locating, detecting linkage. Wiley Interdisciplinary Reviews: Forensic Science, 1(5). doi:10.1002/wfs2.1339



# Illicit drugs

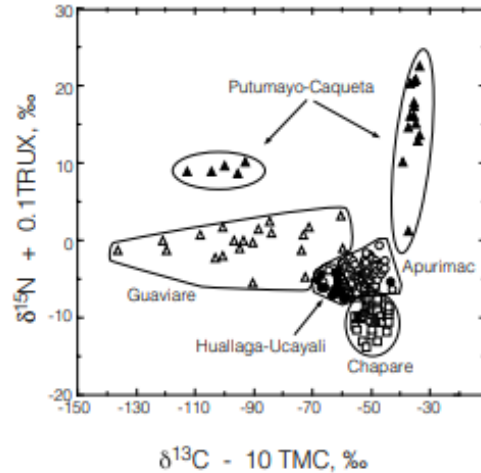
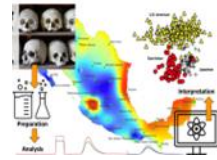


Figure 1. Identification of the regions where cocaine is grown based on a combined model which includes carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios as well as abundances of minor alkaloid components (TMC = trimethoxycocaine, TRUX = truxilline). Symbols are Bolivia ( $\square$ ), Colombia ( $\Delta$ ), and Peru ( $\circ$ ), with regions within a country distinguished by closed and open symbols. This model correctly identifies the region-of-origin with a precision of 96%. Data are adapted from Ehleringer et al. [33].

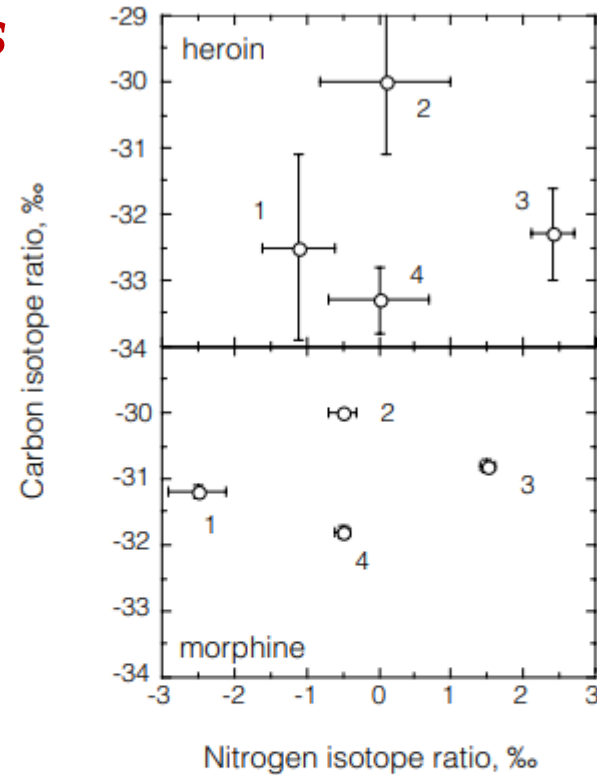
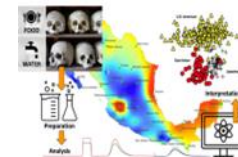


Figure 2. Carbon and nitrogen isotope ratios of bulk heroin samples (top) and the associated extracted morphine (bottom) derived from authentic samples from Mexico (1), Southwest Asia (2), Southeast Asia (3), and South America (4). The data are means and 95% confidence intervals. Data are adapted from Ehleringer et al. [34].



# EXPLOSIVE



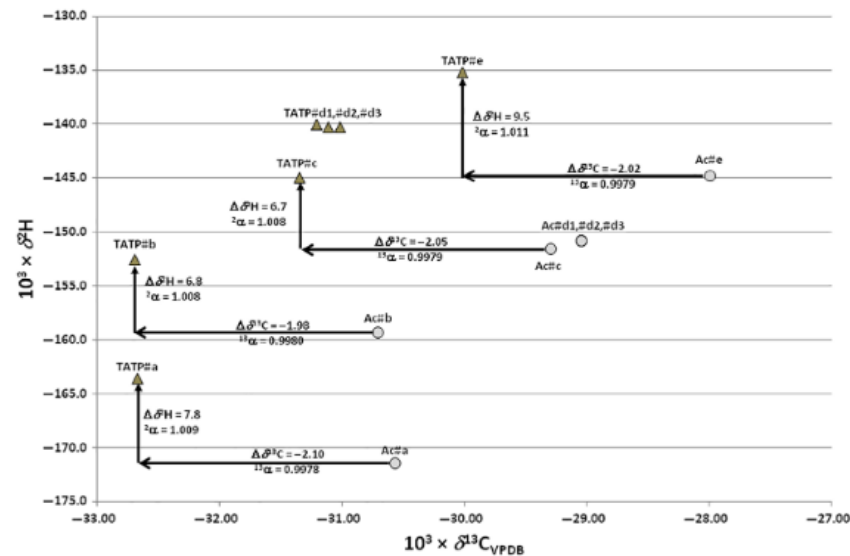
TATP also known as “Mother of Satan” triacetone triperoxide.

High susceptibility of TATP to accidental detonation, causing injuries and deaths among illegal bomb-makers

## *Liverpool Women’s Hospital, 2021*



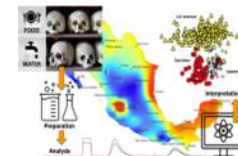
**FIGURE 7** Bivariate isotope profile plot of  $\delta^2\text{H}$  and corresponding  $\delta^{13}\text{C}$  values of TATP made from acetone from different sources showing direction and magnitude of isotopic fractionation. Reproduced with permission of John Wiley & Sons Ltd, from “Stable Isotope Forensics—Methods and Forensic Applications of Stable Isotope Analysis 2ed.” Meier-Augenstein (2018)



**Also: 2015 Paris attacks, the Manchester Arena bombing in 2017**



# Counterfeiting of drugs



## WHO

Counterfeiting of  
drugs



10 % global market

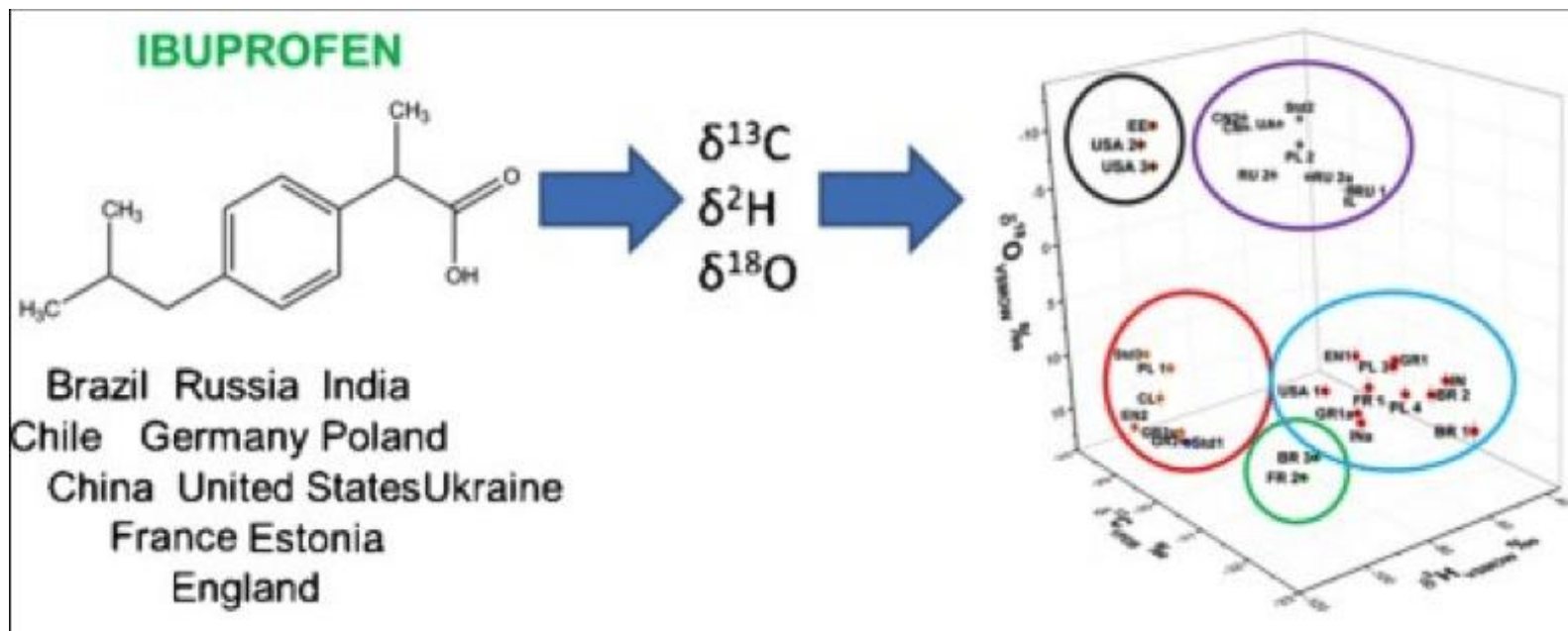
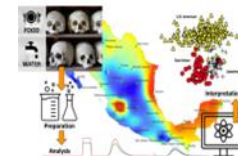
pharmaceutical industry of US\$150 billion per year.

**dangerous, posing health and safety  
risks to consumers**

(<http://www.who.int/medicines/services/counterfeit/impact/ImpactFS/en/>)



# Counterfeit Pharmaceuticals

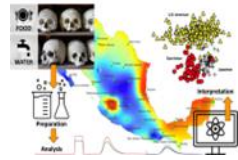


Gilevska, T., Gehre, M., & Richnow, H. H. (2015). Multidimensional isotope analysis of carbon, hydrogen and oxygen as tool for identification of the origin of ibuprofen. *Journal of Pharmaceutical and Biomedical Analysis*, 115, 410–417. doi:10.1016/j.jpba.2015.07.030





# *"the Miracle on the Hudson"*



$\delta^2\text{H}$  geese  
feathers

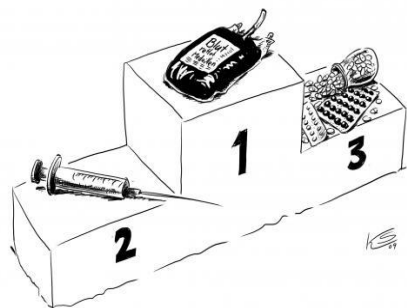
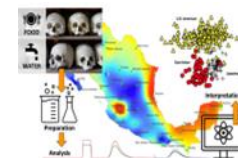


US Airways Flight 1549 was a regularly scheduled US Airways flight from New York City (LaGuardia Airport), to Charlotte and Seattle, in the United States. On January 15, 2009, the Airbus A320 serving the flight struck a flock of birds shortly after takeoff from LaGuardia, losing all engine power.





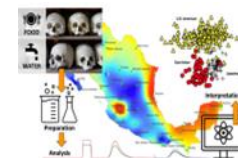
# Sports Doping: How Isotopes Help to Fight the Crime



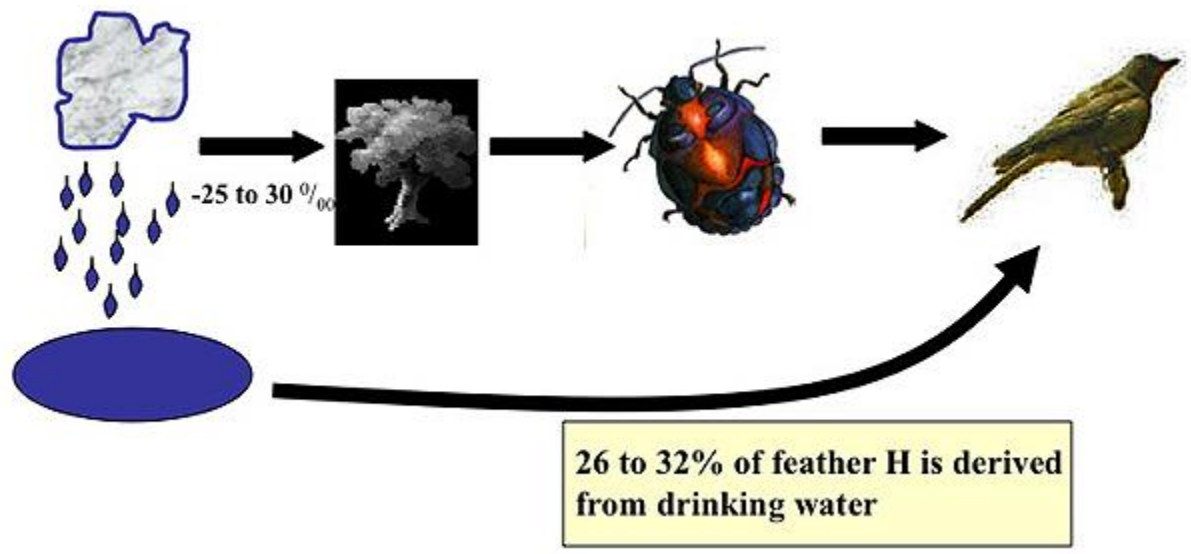
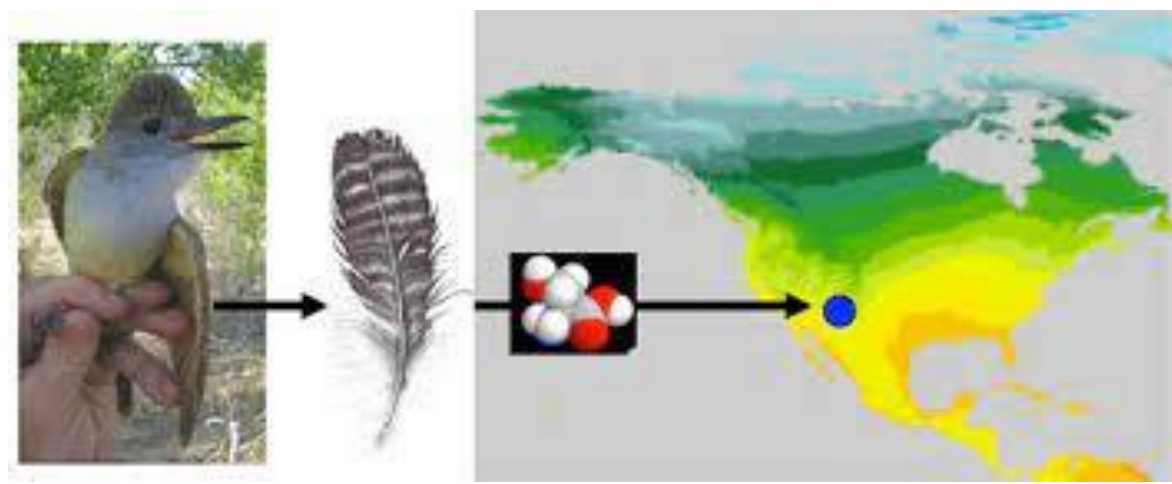
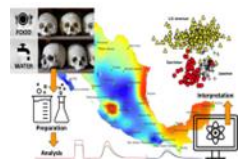
Isotope Fingerprint	What is the interpretation?	Example forensic interpretation	What sample types can be analyzed?
Carbon	Pharmaceutically produced anabolic-androgenic steroids are derived from C3-plant material which means botanical processes (C3 Photosynthesis) define their carbon isotope ratio value, which finally differs from endogenous steroids.	Distinguishing endogenous anabolic steroids from their synthetic analogs.	Urine
Hydrogen	In anti-doping control it is necessary to know metabolic fate of drugs because knowing metabolites accelerates the drug discovery and method development process.	Deuterium labeling of compounds can be used to identify metabolites in complex matrices.	Urine



# *Sports Doping: How Isotopes Help to Fight the Crime*

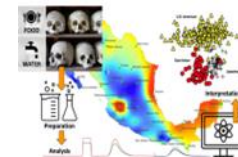


[https://www.thermofisher.com/blog/analyte\\_guru/sports-doping-how-isotopes-help-to-fight-the-crime-part-2/](https://www.thermofisher.com/blog/analyte_guru/sports-doping-how-isotopes-help-to-fight-the-crime-part-2/)

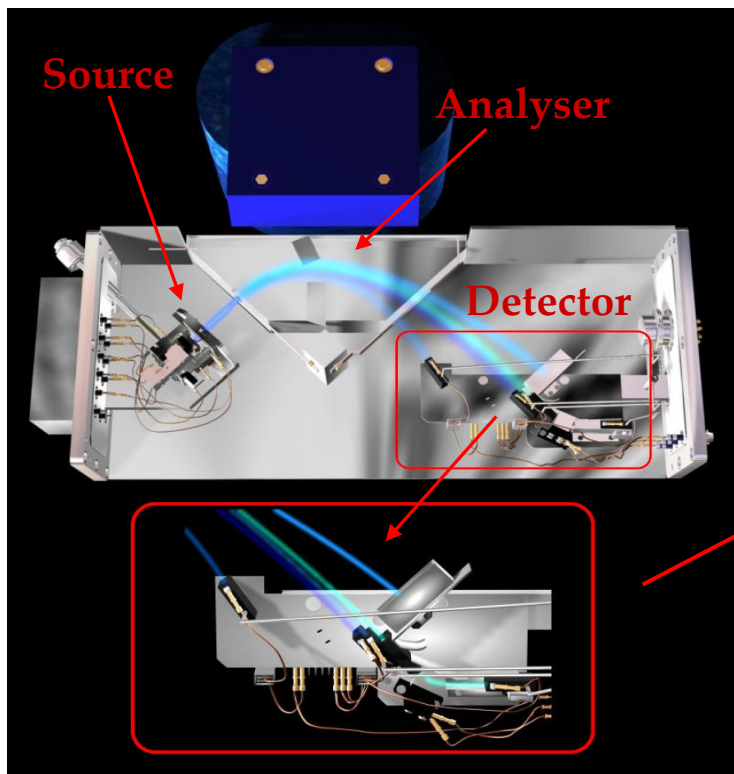




# IRMS – Hyphenated systems



**IRMS** – Measures the ratio between C, H, O, N e S ( $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ ...) stable isotopes gases (e.g.  $\text{CO}_2$ ,  $\text{N}_2$ ,  $\text{CO}$ ...) with high precision.



	m/z					
$(^{12}\text{C}^{18}\text{O}^{16}\text{O})$	46	3			30	66
$(^{13}\text{C}^{16}\text{O}^{16}\text{O})$	45			29		
$(^{12}\text{C}^{16}\text{O}^{16}\text{O})$	44		52	28	28	
			50			
		2				64
	$\text{CO}_2$	$\text{H}_2$	$\text{CH}_3\text{Cl}$	$\text{N}_2$	$\text{CO}$	$\text{SO}_2$
	Gases					

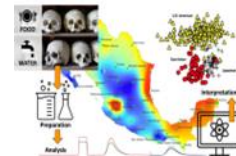
**45/44 ( $^{13}\text{C}/^{12}\text{C}$ ) 46/44 ( $^{18}\text{O}/^{16}\text{O}$ )**

Feixes de iões em direcção aos colectores (“Faraday cups”), Isoprime™.



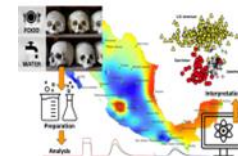


# Stable Isotopes Lab





# *Stable Isotopes Lab*



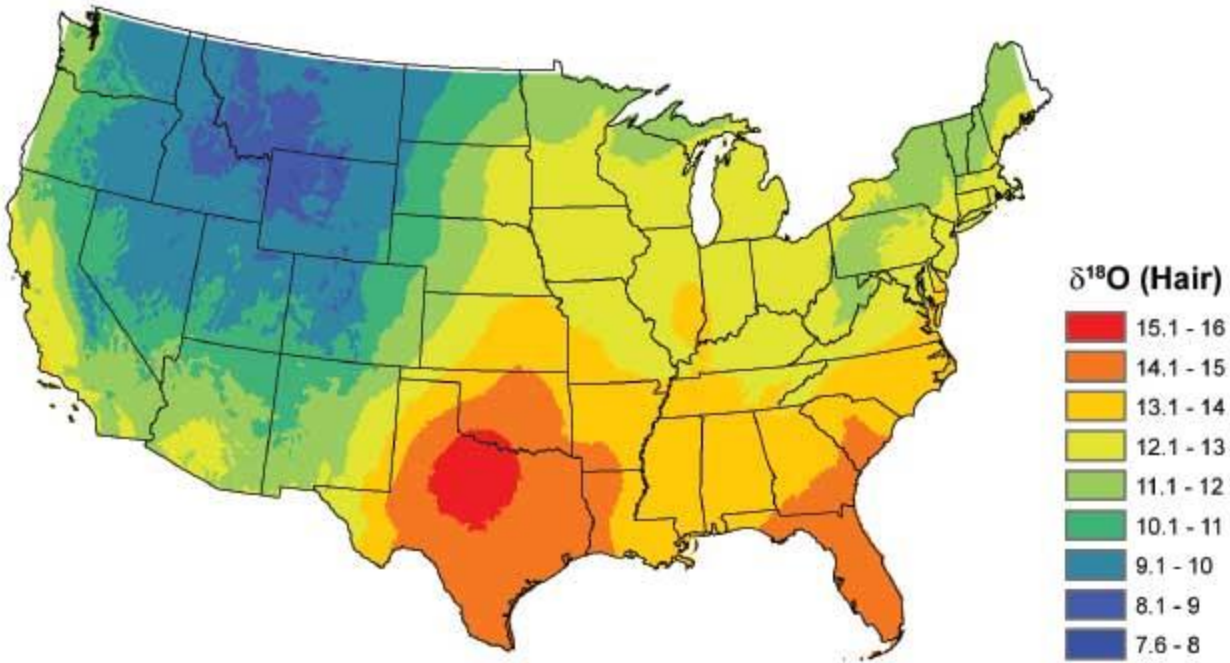
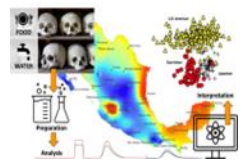
IRMS, ISOCARB, Dual-Inlet





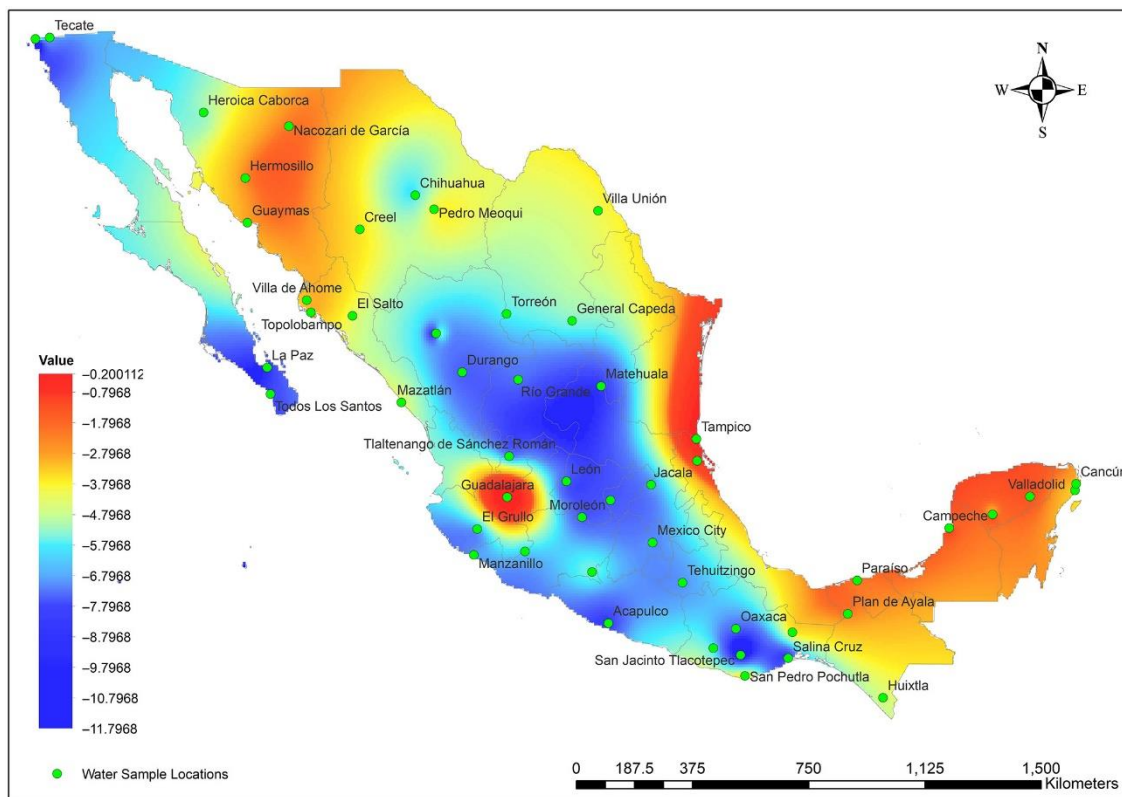
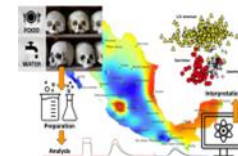


# Isoscapes



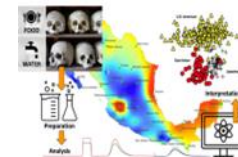


# Isoscapes

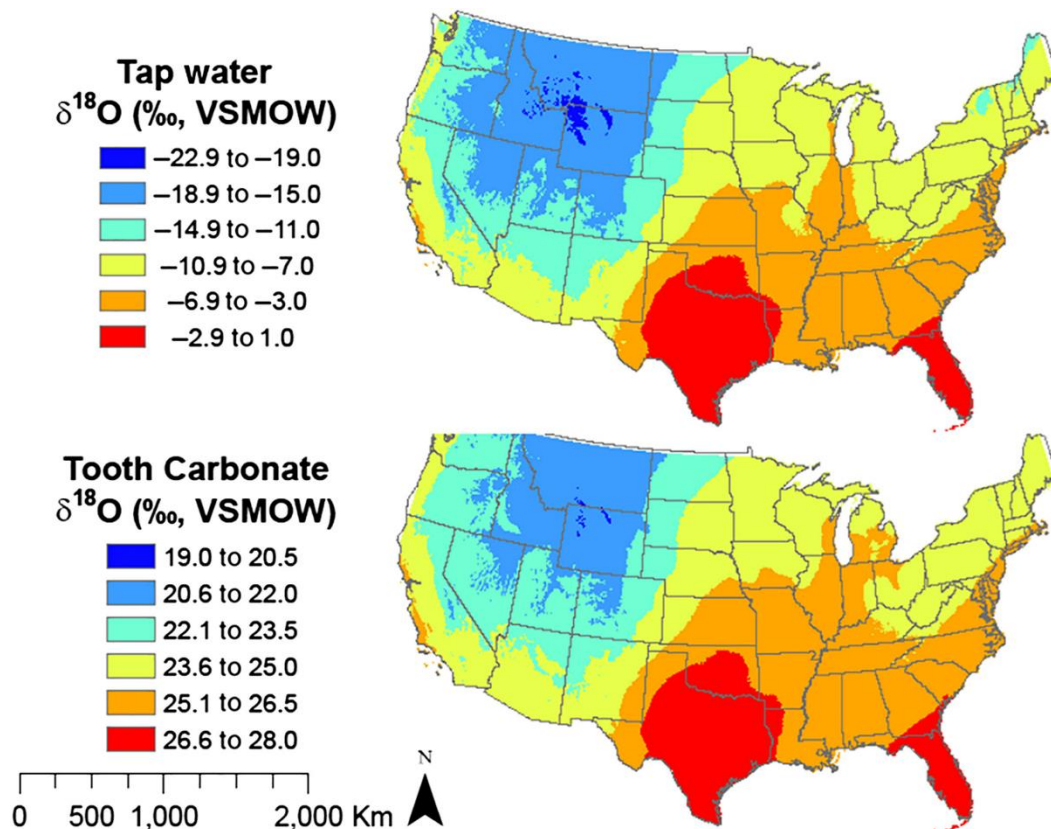




# Isoscapes



Isoscape of  $\delta^{18}\text{O}$  values in tap water across USA (top) and isoscape of  $\delta^{18}\text{O}$  values in the carbonate fraction of tooth enamel bio-apatite throughout the USA (bottom)

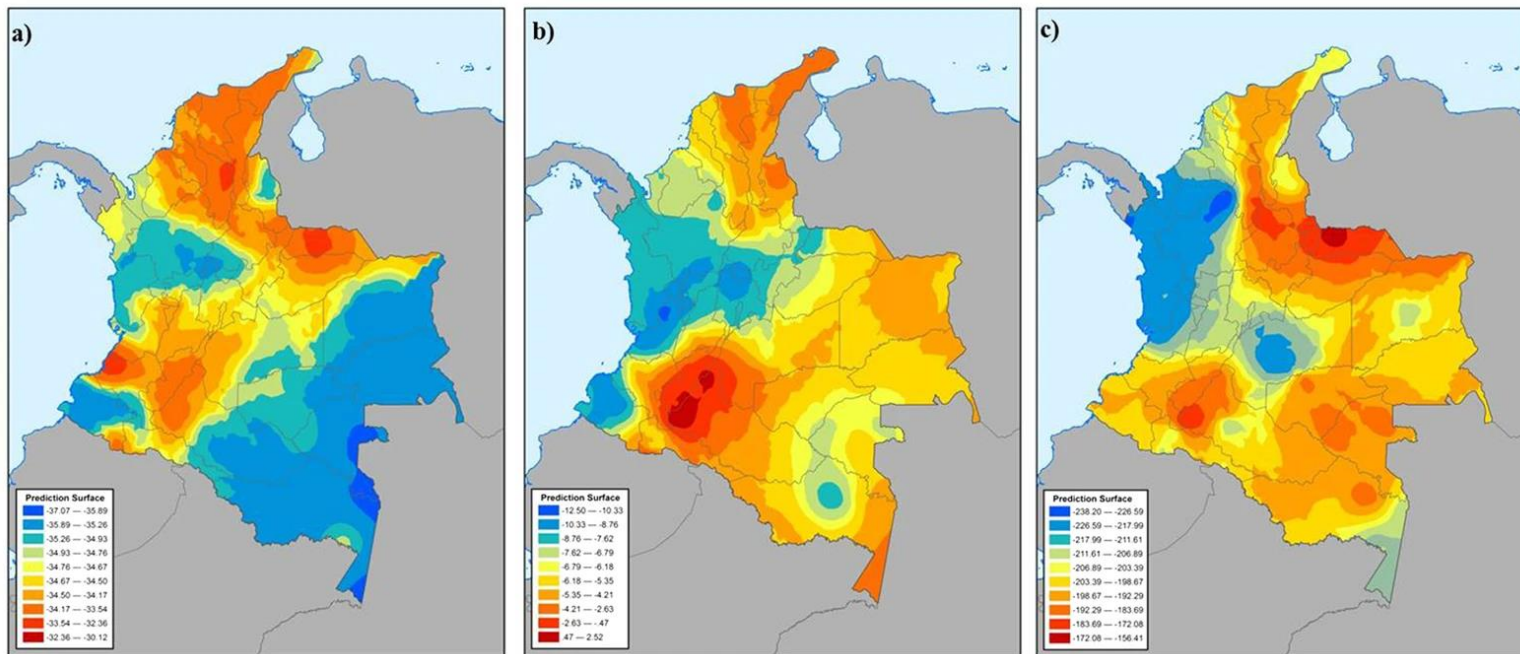
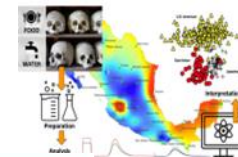


Wolfram Meier-Augenstein, Forensic stable isotope signatures: Comparing, geo-locating, detecting linkage , WIREs Forensic Sci. 2019;

1: e1339.



# Isoscapes



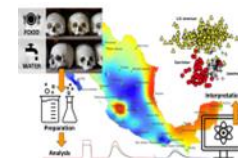
*Cocaine isoscapes of (a)  $\delta^{13}\text{C}$ , (b)  $\delta^{15}\text{N}$  and (c)  $\delta^2\text{H}$  in Colombia interpolated from 336 authentic coca leaf samples.*

*The isoscapes were created with ArcGIS Advanced software (Environmental Systems Research Institute).*

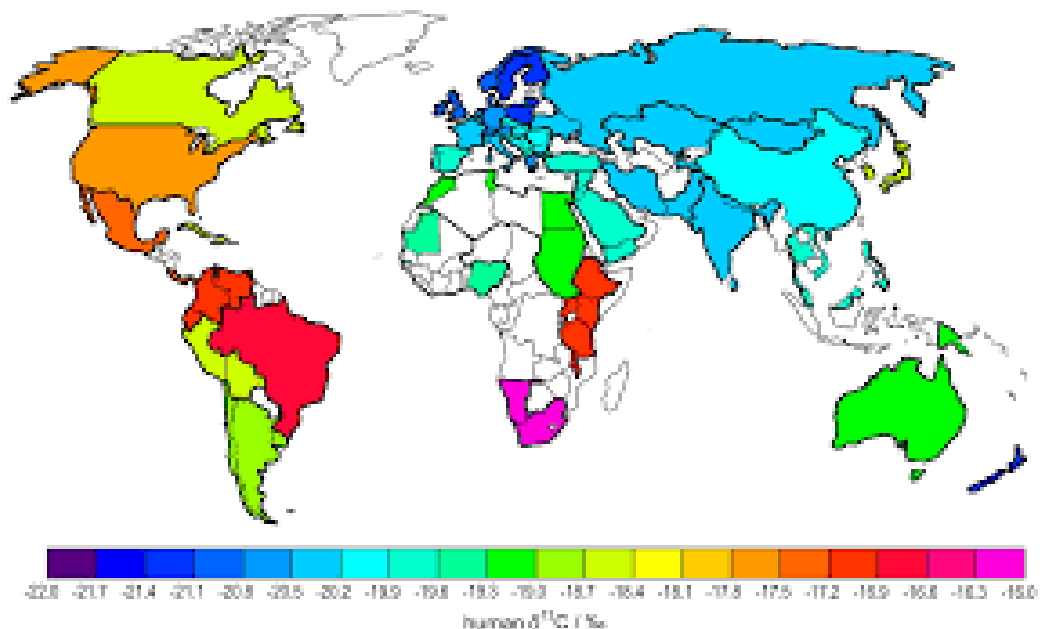
Mallette, J., Casale, J., Jordan, J. *et al.* Geographically Sourcing Cocaine's Origin – Delineation of the Nineteen Major Coca Growing Regions in South America. *Sci Rep* 6, 23520 (2016). <https://doi.org/10.1038/srep23520>



# Isoscapes



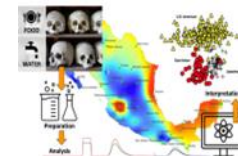
$\delta^{15}\text{N}$  data of more than 400 hair and nail samples of contemporary humans living all over the world



Global spatial distribution of natural stable carbon isotope ratios of contemporary human hair and nails. For countries marked white no data are available. Solid black country borders indicate individual countries and country cluster for which isotope data were summarised.

Frank Hülsemann, Christine Lehn, Sabine Schneiders, Glen Jackson, Sarah Hill, Andreas Rossmann, Nicole Scheid, Philip J. H. Dunn, Ulrich Flenker and Wilhelm Schänzer, *Rapid Commun. Mass Spectrom.*, 2015, 29, 2111–2121.





Forensic Chemistry  
Volume 33, May 2023, 100486



## Assessment of the stable isotope ratio variability of cling films purchased in Australia

Joe Meikle<sup>a, b</sup>, Kylie Jones<sup>a</sup>, Sarah L. Cresswell<sup>b</sup>, Carney Matheson<sup>b</sup>, James F. Carter<sup>c</sup>

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<https://doi.org/10.1016/j.forc.2023.100486>

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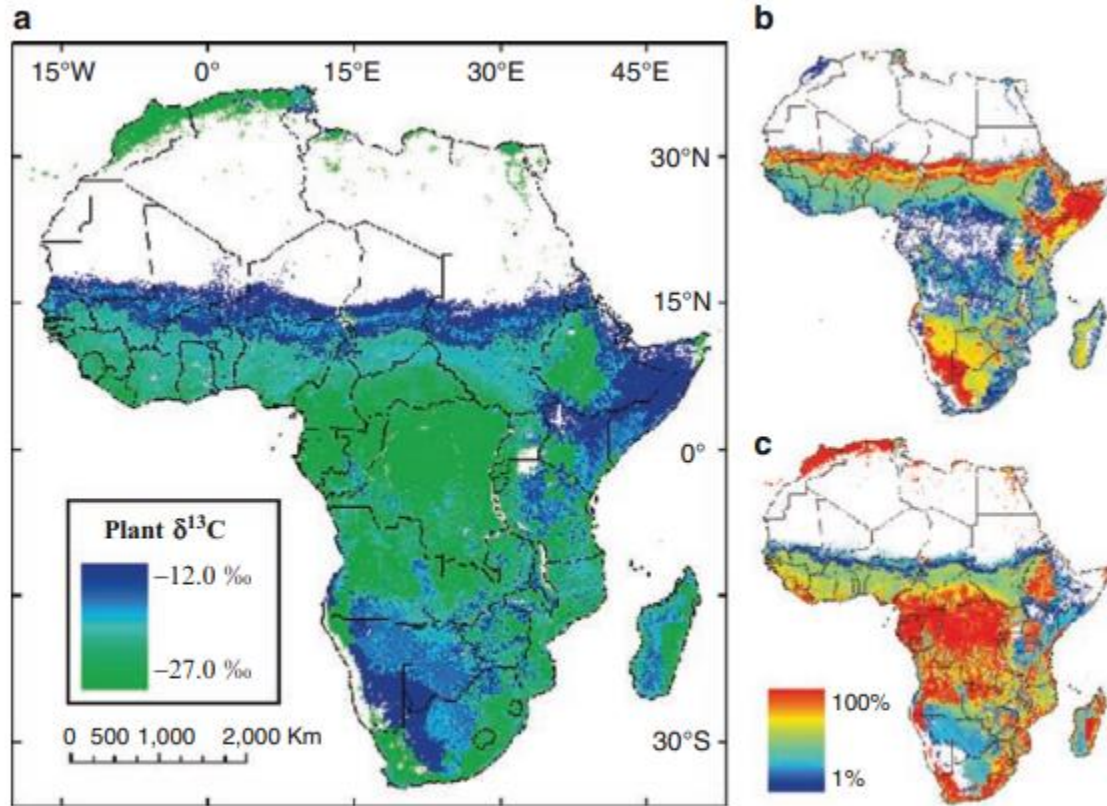
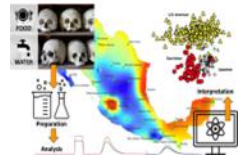
- 74 cling films were analysed for carbon and hydrogen isotope ratio compositions.
- Discrimination of isotope ratio values were calculated using measurement uncertainty.
- Isotope ratio provides a discriminating power of 95% using carbon and hydrogen.







# Isoscapes

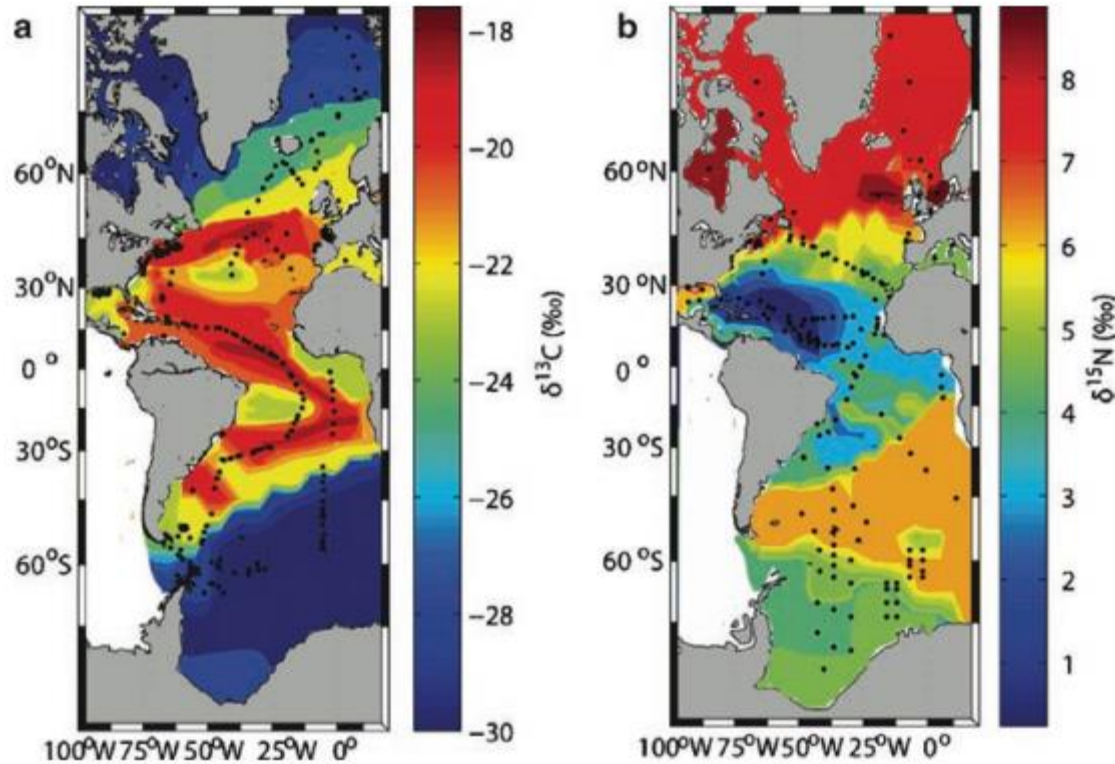
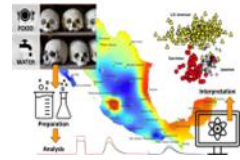


**Fig. 9.2** (a) Stable carbon isotopic distribution for the African continent, (b) percentage of vegetation that uses the C<sub>4</sub> pathway, and (c) percentage of vegetation that uses the C<sub>3</sub> pathway

Isoscapes/Understanding movement, pattern, and process on Earth through isotope mapping. [Jason B. West](#), [Gabriel J. Bowen](#), [Todd E. Dawson](#), [Kevin P. Tu](#)  
ISBN : 978-90-481-3353-6



# Isoscapes

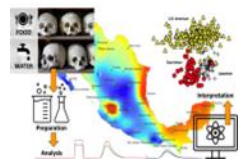


**Fig. 14.1** Contour plots of isotope values in the Atlantic Ocean from a meta-analysis of published data. (a)  $\delta^{13}\text{C}$  values of plankton from the upper ocean (0–500 m;  $n = 425$ ) (b)  $\delta^{15}\text{N}$  values of zooplankton, primarily calanoid copepods, from the upper ocean (0–500 m;  $n = 198$ ). Black dots indicate sample locations (Data are from McMahon et al. (in review))

Isoscapes/Understanding movement, pattern, and process on Earth through isotope mapping. [Jason B. West](#), [Gabriel I. Bowen](#), [Todd E. Dawson](#), [Kevin P. Tu](#)  
ISBN : 978-90-481-3353-6



# Isoscapes



Isoscapes/Understanding movement, pattern, and process on Earth through isotope mapping. [Jason B. West](#), [Gabriel J. Bowen](#), [Todd E. Dawson](#), [Kevin P. Tu](#)  
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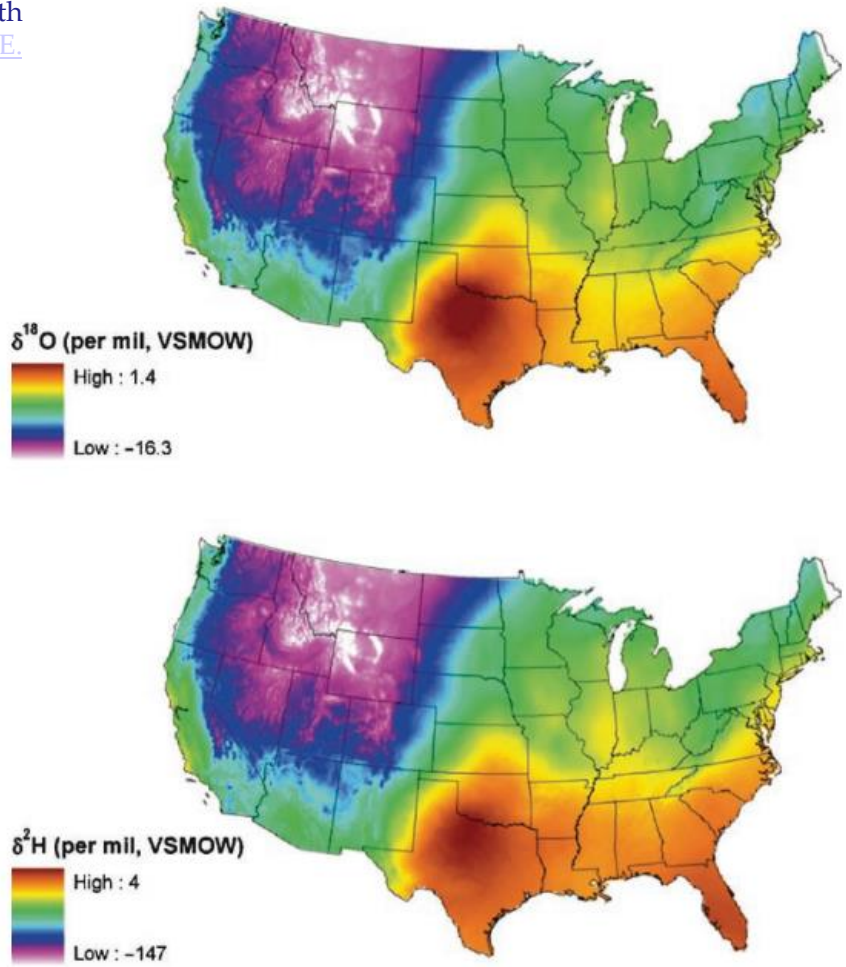


Fig. 17.4 Predicted average hydrogen and oxygen isotope ratios of body water across the USA (After Podlesak et al. in review)





# Isoscapes

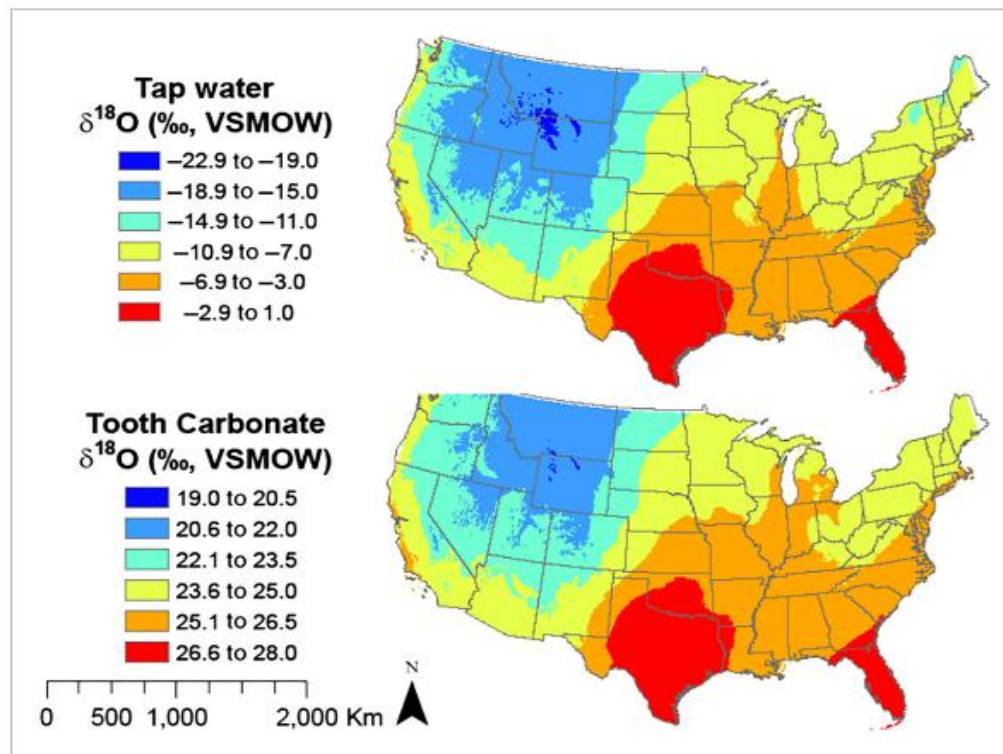
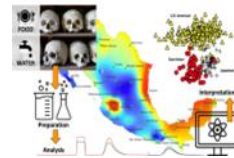


Figure 5

[Open in figure viewer](#)

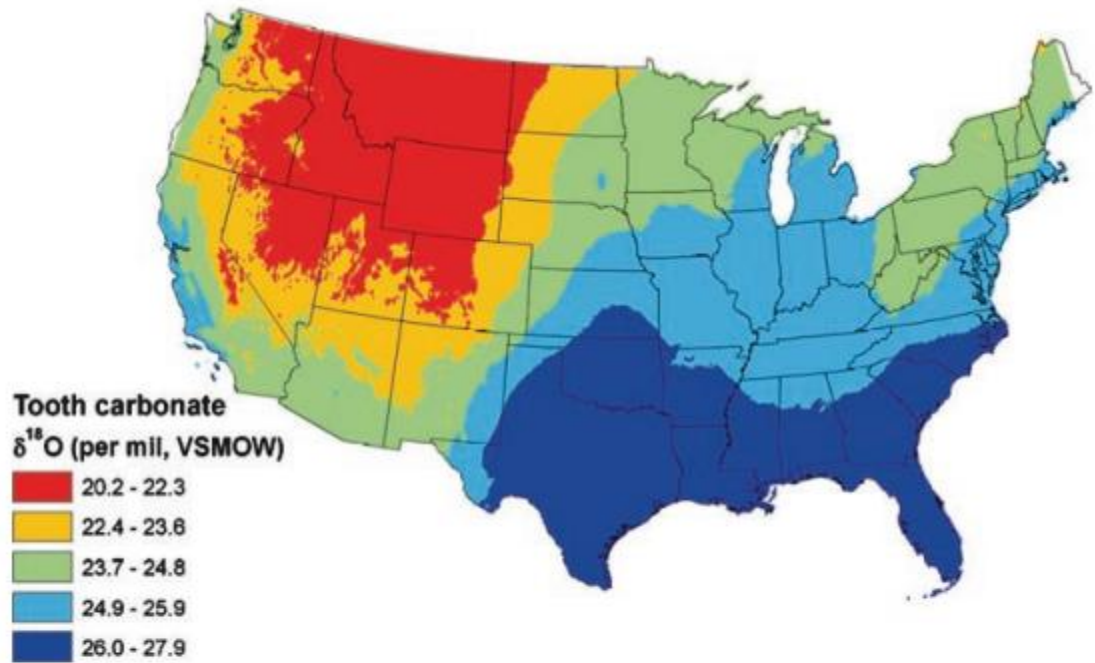
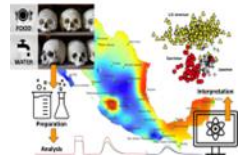
[PowerPoint](#)

Isoscape of  $\delta^{18}\text{O}$  values in tap water across the contiguous USA (top) and isoscape of  $\delta^{18}\text{O}$  values in the carbonate fraction of tooth enamel bio-apatite throughout the USA (bottom). Reproduced with permission of John Wiley & Sons Ltd, from "Stable Isotope Forensics—Methods and Forensic Applications of Stable Isotope Analysis 2ed." Meier-Augenstein (2018)

WIREs Forensic Sci.2019;1:e1339

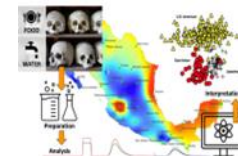


# Isoscapes



**Fig. 17.6** The predicted distribution of oxygen isotope ratios of carbonates in enamel from human teeth across the contiguous USA

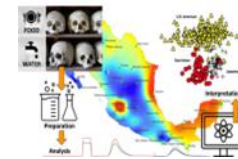
Wolfram Meier-Augenstein, Forensic stable isotope signatures: Comparing, geo-locating, detecting linkage , WIREs Forensic Sci. 2019; 1: e1339.



*Danger!*

*Miracles?*





# To the field of stars: Stable isotope analysis of medieval pilgrims and populations along the Camino de Santiago in Navarre and Aragon, Spain

Patxi Pérez-Ramallo<sup>a, b, \*</sup>, José Ignacio Lorenzo-Lizalde<sup>c</sup>, Alexandra Staniewska<sup>d</sup>, Mattin Aiestaran<sup>e, f</sup>, Juanxo Aguirre<sup>f</sup>, Jesús Semas Sesma<sup>g</sup>, Sara Marzo<sup>h</sup>, Mary Lucas<sup>b, g</sup>, Jana Ilgner<sup>o</sup>, David Chivall<sup>j</sup>, Tom Higham<sup>k</sup>, Ricardo Rodríguez-Varela<sup>l, m</sup>, Anders Götherström<sup>l, m</sup>, Francisco Etxebarria<sup>n</sup>, Aurora Grandal-d'Anglade<sup>n, r</sup>, Michelle Alexander<sup>h</sup>, Patrick Roberts<sup>b, o, p</sup>

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## Research Article

Received: 3 March 2012 Revised: 3 May 2012 Accepted: 4 May 2012 Published online in Wiley: 10 April 2013

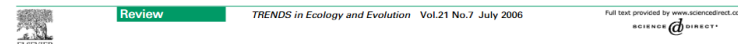
Rapid Commun. Mass Spectrom. 2012, 26, 1596–1602 (wileyonlinelibrary.com) DOI: 10.1002/rcm.6267

### Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans

A. Borrell<sup>a</sup>, N. Abad-Oliva, E. Gómez-Campos, J. Giménez and A. Aguilar

Biodiversity Research Institute (IrBio) and Department of Animal Biology, Faculty of Biology, University of Barcelona Diagonal, 643 (08028), Barcelona, Spain

**RATIONALE:** In stable isotope research, the use of accurate, species-specific diet-tissue discrimination factors (i.e.  $\Delta^{15}\text{N}$ ) is central to the estimation of trophic position relative to primary consumers and to the identification of sources of an individual. Previous research suggested that the diet of fin whales from the western off northwestern



### Stable isotopes as one of nature's ecological recorders

Jason B. West<sup>1</sup>, Gabriel J. Bowen<sup>2</sup>, Thure E. Cerling<sup>3</sup> and James R. Ehleringer<sup>1</sup>

<sup>1</sup>Department of Biology, University of Utah, Salt Lake City, UT 84115, USA  
<sup>2</sup>Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, IN 47907, USA  
<sup>3</sup>Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84115, USA

Analyses of the natural variation in stable isotopes of components of ecological systems have provided new ( $\delta^{15}\text{N}$ ) record dietary and trophic-level information in animals [7] and nitrogen fixation in plant-microbe



Forensic Science International 106 (1999) 27–35

Stephen J. Morewitz · Caroline Sturdy Colls Editors

### Handbook of Missing Persons

Forensic Science International

[www.elsevier.com/locate/forsciint](http://www.elsevier.com/locate/forsciint)

Springer

### Geo-location of heroin and cocaine by stable isotope ratios

James R. Ehleringer<sup>a, \*</sup>, Donald A. Cooper<sup>b</sup>, Michael J. Lott<sup>c</sup>, Craig S. Cook<sup>c</sup>

<sup>a</sup>Stable Isotope Ratio Facility for Environmental Research (SIRFER), Department of Biology, University of Utah, Salt Lake City, UT 84112-0840, USA

<sup>b</sup>Drug Enforcement Administration, Special Testing & Research Laboratory, McLean, VA 22102, USA

<sup>c</sup>Stable Isotope Ratio Facility for Environmental Research (SIRFER), Department of Biology, University of Utah, Salt Lake City, UT 84112-0840, USA

Received 21 April 1999; received in revised form 31 August 1999; accepted 6 September 1999

### Forensic Stable Isotope Biogeochemistry

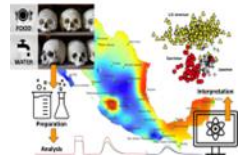
Annual Review of Earth and Planetary Sciences  
Vol. 44:175-206 (Volume publication date June 2016)  
First published online as a Review in Advance on April 27, 2016  
<https://doi.org/10.1146/annurev-earth-060115-012303>

Thure E. Cerling,<sup>1,2,3</sup> Janet E. Barnette,<sup>3</sup> Gabriel J. Bowen,<sup>1</sup> Lesley A. Chesson,<sup>3</sup> James R. Ehleringer,<sup>2,3</sup> Christopher H. Remien,<sup>4</sup> Patrick Shea,<sup>2</sup> Brett J. Tipple,<sup>3,5</sup> and Jason B. West<sup>2</sup>

<sup>1</sup>Department of Geology and Geophysics, University of Utah, Salt Lake City, Utah 84112, email: thure.cerling@utah.edu  
<sup>2</sup>Department of Biology, University of Utah, Salt Lake City, Utah 84112  
<sup>3</sup>IsoForensics, Salt Lake City, Utah 84108  
<sup>4</sup>Department of Mathematics, University of Idaho, Moscow, Idaho 83844  
<sup>5</sup>Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas 77843



# Curiosity....



Human 50 kg

*You are what you eat*

$^1\text{H}$  5,0 kg

$^2\text{H}$  1,5 g

$^{12}\text{C}$  11,4 kg

$^{13}\text{C}$  137 g

$^{14}\text{N}$  1,3 kg

$^{15}\text{N}$  5,1 g

$^{16}\text{O}$  30,4 kg

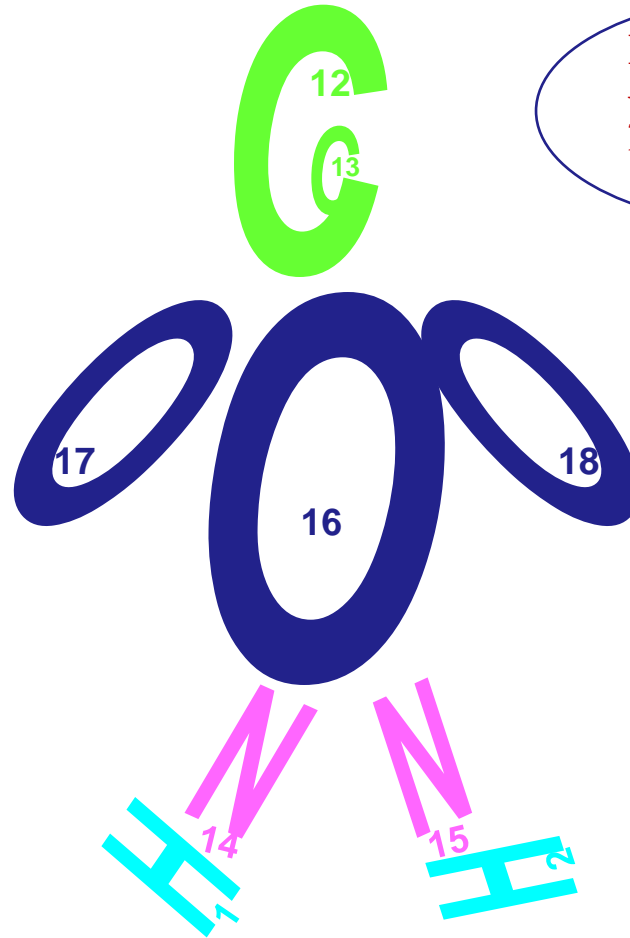
$^{17}\text{O}$  12,3 g

$^{18}\text{O}$  68,6 g

---

~50 kg

Have you had your isotopes today?



(Wada and Hattori, 1990.)

