

# Stable Isotope Forensics for identifying Residence Patterns

**E Dotsika\***

Stable Isotopes Unit, N.C.S.R. “Demokritos”, Institute of Nanoscience and Nanotechnology, 15310, Ag.Paraskevi Attikis, Greece

Corresponding author and e-mail: E Dotsika, *e.dotsika@inn.demokritos.gr*

**Abstract.** In this study we investigate a method for determine the “residence patterns” of human by comparing the oxygen isotopic composition of the meteoric water that an individual was drinking, during his living period, with the oxygen isotopic composition of the teeth enamel. Teeth samples were collected from two areas (Athens and Chalkidiki) of Greece from individuals that never travelled and drank tap water. For these two regions the isotopic method is a promising tool for discriminating the provenance.

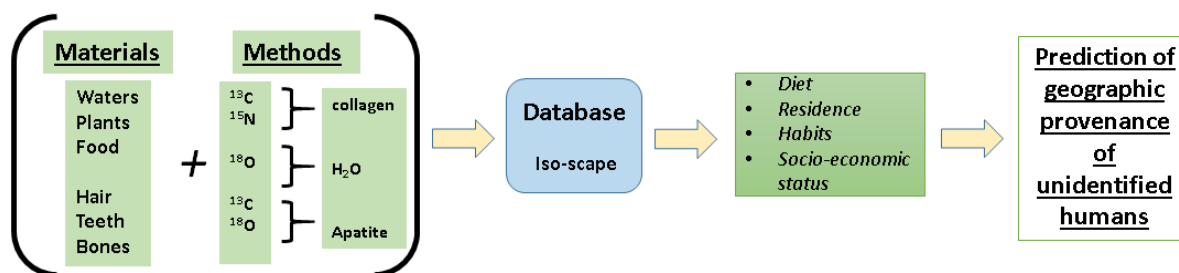
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## 1. Introduction

Methods for identifying remains include examination of the biological profile, fingerprint analysis, dental records, artifacts from medical procedures, and DNA analysis. In cases where partial humans' remains are found the above methods are inconclusive in identifying humans and investigators need additional techniques for collecting characteristic information about the life history of the individual before his death. The only method capable to provide information about the diet and residence patterns of unidentified individuals (by examining bone, teeth, hair, and nails) is the isotope analysis.

The measurement of stable isotope abundances contained within materials, like human remains, can provide information about the history and source of the materials. Stable isotope compositions are used to discriminate between two materials by measuring the distinctive “isotopic signature” of samples: two identical chemical molecules are isotopically different. As such, stable isotope abundances play an increasingly important role in forensics analysis [1].

The use of stable isotopic analysis for predicting the life history of unidentified human remains has generally proceeded on two fronts: evaluation of stable isotopes compositions in various natural systems for understand biological and geological processes and subsequently generation of predictive models. These models incorporate spatially variable isotopic parameters to construct isotope “isoland-scapes” and has been previously used for tracing the origin of unknown ecological (plants, natural products, animals) and geological (soil, minerals and water) samples [1-4]. This isoscape approach has provided an empirical framework from which is possible to predict the geographic origin of unknown samples and so to identify the residence patterns of unidentified human remain based on their isotopic signatures (bone, teeth, hair, and nails) [1, 5-7] (see Figure 1 for a schematic presentation of the method).



**Figure 1.** Stable isotope ratio analysis in forensic investigations.

Also the scientific and technological progress in human epigenetics has begun to play an increasingly important role in the establishment of “epigenetic fingerprint” from crime scene traces to various forensically purposes and questions that cannot be answered through genetics [8-10]. These purposes include the prediction of forensically informative lifestyle and environmental information of an unknown trace donor. Thus isotopic fingerprint, in conjunction with the biological information from the skeleton, and the epigenetic fingerprint may aid in the investigation of missing persons primary by limited the geographic region from which a person is originating (for example, where he previously lived or travelled) and secondary predicting the lifestyle and environmental exposures (socioeconomic status, activity, body size, alcohol and drug abuse, smoking, habits...) of unidentified humans. In this framework we conducted stable isotope analysis of teeth samples of known origin (Athens and Chalkidiki area) and compared them to the isoscape models of  $^{18}\text{O}$  of water from these areas. This method is a promising tool for predicting region of origin and residence patterns of unidentified human remains.

## 2. Sampling and methods

The isotopic composition of carbonate and phosphate minerals in tooth enamel and bone bioapatite reflects the isotopic composition of diet water (drinking water or water contained within food) [11]. There is a strong relationship between the oxygen isotope ratios of carbonate and phosphate minerals and water consumed by an individual. Thus, for big mammals (like humans) the impact of food on tooth and bone bioapatite oxygen isotope ratios is much less than the impact of drinking water.

By keeping in mind the existing relationship between oxygen isotope of water and oxygen ratio of teeth and supposing no other identifying information or evidence available, we apply stable isotope analyses to investigate potential geographic origins of the individuals in the years before death and adolescence. In fact, teeth and bone can be considered “historic” recorders because teeth enamel is formed when an individual is juvenile while the bone is continuously remodelled during the individual’s lifetime [12]. Therefore, from the isotopic composition of diet water (measured in teeth) of an individual it is possible to correlate with the isotopic composition of water of the region where he lived before his death, and consequently to reconstruct his residence patterns. In this work we analysed sprig water and enamel collected from molar, for both oxygen and carbon isotope ratios, of known persons. In total, 15 teeth samples and 15 water samples were collected from the two areas. Especially for the teeth samples, they are from individuals that they never travelled.

Any human tissue could be measured with Isotope Ratio Mass Spectrometer (IRMS). Abundances of stable isotopes of an element are described as the ratio (R) of the rare or heavy form to the common or light form (e.g.,  $R = ^{18}\text{O}/^{16}\text{O}$  or  $^2\text{H}/\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$ ). The results are expressed in  $\delta$ -notation as parts per thousand (‰) difference from an accepted reference point, where:  $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)$ . This  $\delta$ -notation is also used for expressing the stable isotope ratios of hydrogen (H), carbon (C), nitrogen (N), and sulfur (S), collectively described as the bio-elements.

The error for  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$  is  $\pm 0.2\text{‰}$  and for  $\delta^2\text{H}$   $\pm 1\text{‰}$ . Isotopic analyses were conducted at the Unit of Stable Isotopes, Institute of Nanoscience and Nanotechnology, N.C.S.R. “Demokritos”.

### 3. Results

- The “residence patterns” approach of this study is based on the relationship of the oxygen isotopic composition of the water that a known individual was drinking, during his living period, with the respective oxygen isotopic composition of its teeth.
- To predict potential origins from the  $\delta^{18}\text{O}$  value determined for the tooth enamel, we first converted carbonate measurements to equivalent phosphate data [13] and then predicted drinking water [14] from the phosphate data. In order to achieve that, several equations from international literature may be used, which practically convert the  $\delta^{18}\text{O}$  of the carbonate component of the bioapatite ( $\delta^{18}\text{O}_\text{C}$ ) to  $\delta^{18}\text{O}$  of the phosphate component ( $\delta^{18}\text{O}_\text{P}$ ) and finally to  $\delta^{18}\text{O}$  of water ( $\delta^{18}\text{O}_\text{w}$ ). As so, the  $\delta^{18}\text{O}_\text{C}$  (vPDB) values need to be converted into  $\delta^{18}\text{O}_\text{C}$  (vSMOW) using the established equation:
- $\delta^{18}\text{O}_\text{vSMOW} = 1.03091 * \delta^{18}\text{O}_\text{vPDB} + 30.91$  [15].
- According to Iacumin et al. (1996) the relationship between  $\delta^{18}\text{O}_\text{P}$  and  $\delta^{18}\text{O}_\text{C}$  values is expressed by the equation  $\delta^{18}\text{O}_\text{C} = 1.015(\pm 0.043) * \delta^{18}\text{O}_\text{P} + 8.79(\pm 0.79)$ , resulting through studies on bone and tooth samples of modern mammals.
- For the conversion of  $\delta^{18}\text{O}_\text{P}$  in  $\delta^{18}\text{O}_\text{w}$  Hoppe (2006) suggested an equation that combines the structural oxygen with the consuming water:  $\delta^{18}\text{O}_\text{P} = 21.28(\pm 0.51) + [0.68(\pm 0.04) * \delta^{18}\text{O}_\text{w}]$ .

The sampling for Athens (Figure 2), with  $\delta^{18}\text{O}$  of enamel between  $-6.5\text{‰}$  and  $-6.3\text{‰}$  suggest that the individuals drunk water from the region of Athens ( $-6.5\text{‰}$  to  $-5.0\text{‰}$  with the majority being to  $-6.5\text{‰}$ ) where they passed all their lives (Athens Teeth 1). Teeth samples of individuals, which drunk bottled water (Athens Teeth 2), indicate more negative isotopes values ( $-9.0\text{‰}$  to  $-8.0\text{‰}$ ). This is expected because the measured stable isotope ratios of bottled water in Greece range from  $-9.2\text{‰}$  to  $-7.2\text{‰}$  for  $\delta^{18}\text{O}_\text{w}$ .

Teeth samples coming from Chalkidiki present more negative oxygen values ( $-8.1\text{‰}$  to  $-7.5\text{‰}$ ) in accordance with the isotopic values measured in spring water of the Chalkidiki ( $-9.5\text{‰}$  to  $-6.5\text{‰}$  with the majority being to  $-8.0\text{‰}$ ).

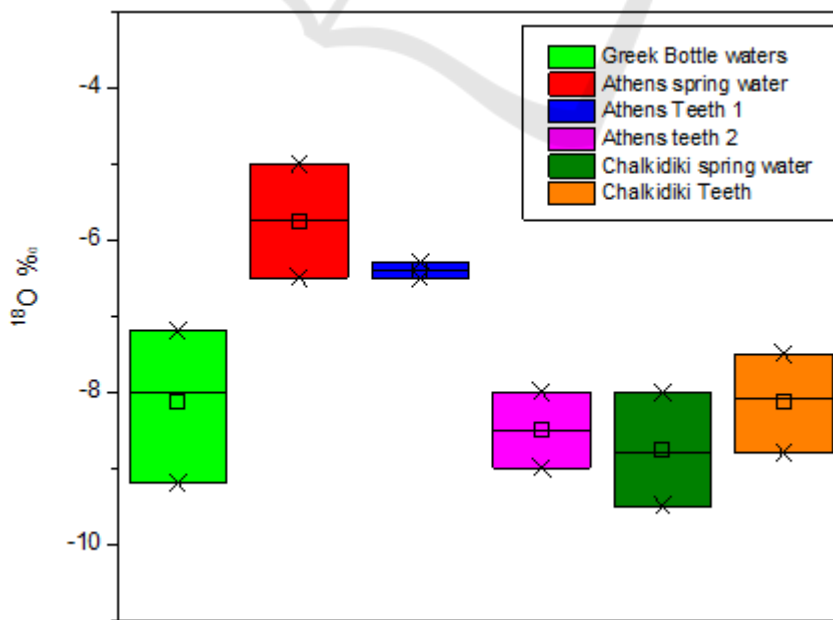


Figure 2.  $^{18}\text{O}$  from spring water and human teeth.

#### 4. Conclusions

The “residence patterns” approach is based on the relationship of the oxygen isotopic composition of the meteoric water and the oxygen isotopic composition of the teeth enamel. For the two regions studied (Athens and Greece) this method (applied on known individuals that were drinking water and never travelled outside of their residence) can discriminate the geographic origin of unidentified humans. This method can potentially be applied for discriminating unknown human samples in a wider region. An isoscape map of the drinking waters (tap water, springs and bottle waters) of the region under investigation is required in order to compare and potentially narrow down or even discriminate the origin of the unidentified human samples.

#### References

- [1] Ehleringer J R and et al 2010 *A framework for the incorporation of isotopes and isoscapes in geospatial forensic investigations*, in *Isoscapes* Springer p 357-387
- [2] Ehleringer J R and et al 2008 *Hydrogen and oxygen isotope ratios in human hair are related to geography*. Proceedings of the National Academy of Sciences 105(8): p 2788-2793
- [3] Dotsika E, S Lykoudis and D Poutoukis 2010 *Spatial distribution of the isotopic composition of precipitation and spring water in Greece* Global and Planetary Change 71(3-4): p 141-149
- [4] Dotsika E and et al 2010 *Palaeo-climatic information from isotopic signatures of fossil teeth in Late Pleistocene from Arkoudospilia Cave (Aridea, N. Greece)* in *EGU General Assembly Conference Abstracts*
- [5] Ambrose S H and L Norr 1993 *Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate in Prehistoric human bone* Springer p 1-37
- [6] Bartelink E, R Berry and L Chesson 2014 *Stable isotopes and human provenancing*. Advances in forensic human identification p 165-192
- [7] Bartelink E J and et al 2014 *Application of stable isotope forensics for predicting region of origin of human remains from past wars and conflicts*. Annals of Anthropological Practice 38(1): p 124-136
- [8] Joehanes R and et al 2016 *Epigenetic signatures of cigarette smoking* CLINICAL PERSPECTIVE Circulation: Cardiovascular Genetics 9(5): p 436-447
- [9] Freire-Aradas, A., C. Phillips, and M. Lareu, *Forensic individual age estimation with DNA: From initial approaches to methylation tests*. Forensic science review, 2017. 29(2)
- [10] Christensen B C and et al 2009 *Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context* PLoS genetics 5(8): p e1000602
- [11] Kohn M J 1996 *Predicting animal  $\delta^{18}O$ : accounting for diet and physiological adaptation*. Geochimica et Cosmochimica Acta 60(23): p 4811-4829
- [12] Stenhouse M J and M S B 1979 *The uptake of bomb  $^{14}C$  in humans* in *Radiocarbon Dating: Proceedings of the Ninth International Conference* Los Angeles and La Jolla: University of California Press, Berkeley
- [13] Iacumin P and et al 1996 *Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate?* Earth and Planetary Science Letters 142(1-2): p 1-6
- [14] Hoppe K A 2006 *Correlation between the oxygen isotope ratio of North American bison teeth and local waters: implication for paleoclimatic reconstructions*. Earth and Planetary Science Letters 244(1-2): p 408-417
- [15] Coplen T B , Kendall C and Hopple J 1983 Comparison of stable isotope reference samples *Nature* 302(5905): p 236