Application of heavy stable isotopes in forensic isotope geochemistry: A review

Jugdeep Aggarwal a,∗, Judith Habicht-Maucheb, Chelsey Juarezb

a W.M. Keck Isotope Laboratory, Department of Earth Sciences, University of California, Santa Cruz, CA 95064, USA
b Department of Anthropology, University of California, Santa Cruz, CA 95064, USA

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Abstract

Light stable isotopes have been used for many years to characterize the source and transport of materials. More recently heavy isotope systems such as Sr, Nd and Pb have been added to this list in order to aid source identification. With the advent of multiple collector ICP-MS, the range of isotopic tools now available has increased considerably, however, until the isotope systematics of these new non-traditional isotope systems have become better understood, they will not be as useful in characterizing material source and transportation. Applications using heavy metal stable isotopes (mostly traditional heavy isotopes) have reached most avenues in science, including earth sciences, archaeology, anthropology, animal physiology, ecology and toxicology. This field will continue to grow as new applications are developed and techniques become simpler and quicker. This paper provides a review of how this field has grown and presents two new applications using Pb and Sr isotopes in glazes to determine the source of ore used in glazes, and using Sr isotopes to determine the origin of undocumented deceased Mexican border crossers.

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1. Introduction

In the strictest sense forensic science usually applies to litigious circumstances where it is vital to understand the source of a material. It has however been extended to determining sources of materials not only for legal cases but also for academic interest.

In many disciplines of science it is important to be able to determine the source of a material or characterize its transportation history. Examination of fragments of material may help to determine these sources or history by a variety of techniques such as looking at colour, grain sizes, optical properties and perhaps the most definitive – the chemical composition. Chemical composition has been used extensively to determine the source of materials by fingerprinting the chemical composition of the material to be identified and comparing it to the chemical composition of potential sources. This approach has been used extensively for major elements, trace elements (Ulrich et al., 2004), and more recently stable isotopes such as those of C, N, O and H. For a more comprehensive examination of traditional stable isotope and elemental signatures applied to forensic science the reader is encouraged to review the following articles: Hill et al. (2004), Pye (2007), and Murphy and Morrison (2007).

Forensic isotope geochemistry relies on the subtle differences in isotopic abundance of an element to characterize a particular material. These different isotopic abundances give rise to a unique isotopic composition that will identify a material having sourced from a particular region. There are two different types of isotopes that define the unique isotopic composition of a material; those isotopes that originate from the radioactive decay of one isotope, e.g., 87Rb, and those that do not undergo radiogenic decay. In the case of radiogenic isotopes, the daughter isotopes are the ones that are measured to characterize the unique signature of the sources, e.g., Sr, Nd and Pb. In addition to those isotopes formed as a result of radiogenic decay, there are those isotopes whereby natural processes...
There are subtle shifts in N and O isotope ratios indicating different trophic levels within a limited geographic location, the isotopic composition of that rock. However, across different samples derived from a rock (no matter how indirect) will resemble the isotopic composition of the rocks below them. Any plants growing in these soils will tend to a distinctive enough isotopic composition to relate it directly to the soils and any animal eating the plants will similarly carry the same isotopic fractionation. Stable isotopes such as Li, B, Mo and Cd may also show distinct isotopic signatures in rocks from natural mass dependent fractionation processes. These materials can vary from soils and rocks to bones, hair, feathers, wines, chocolate, pottery, metal ores and teeth among other substances.

2. Forensic uses of isotopes

The use of isotopes in forensic applications is relatively new. This may be because many of the techniques are laborious, the instrumentation expensive and complicated to use and the expense for sample preparation high. Isotope ratios have a major advantage over elemental ratios in characterizing a material since isotope ratios are significantly more sensitive tracers than elemental ratios or even elemental concentrations. These materials can vary from soils and rocks to bones, hair, feathers, wines, chocolate, pottery, metal ores and teeth among other substances.

2.1. Rocks and soils

Many rocks composed of different minerals have distinctive isotopic compositions and their unique composition can be used to fingerprint them. This distinctive rock/mineral composition usually arises from the decay of radiogenic elements e.g., $^{87}\text{Rb}$ to $^{87}\text{Sr}$; the transuranic elements to $^{208}\text{Pb}$, $^{207}\text{Pb}$, $^{206}\text{Pb}$; $^{147}\text{Sm}$ to $^{143}\text{Nd}$. Measurement techniques have tended to normalize out any naturally occurring mass dependent fractionation, however, insights from Aggarwal (submitted for publication) indicates that even these elements show mass dependent isotopic fractionation. Stable isotopes such as Li, B, Mo and Cd may also show distinct isotopic signatures in rocks from natural mass dependent fractionation processes. Soils above any of these rocks will tend to carry the distinctive isotopic composition of the rocks below them. Any plants growing in these soils will tend to a distinctive enough isotopic composition to relate it directly to the soils and any animal eating the plants will similarly carry the same isotopic composition of the rocks. Hence any natural material derived from a rock (no matter how indirect) will resemble the isotopic composition of that rock. However, across different trophic levels within a limited geographic location, there are subtle shifts in N and O isotope ratios indicating isotopic fractionation between different trophic levels (Hobson, 1999).

There have been many cases of using isotopes to distinguish sources of materials. In the case of concrete failures, for example, it can be useful to understand the source of the principal components, so that other concrete failures may be anticipated. Graham et al. (2000) used a combination of Ca, Sr and Mn elemental concentrations along with Sr isotopes to identify the sources of the components.

2.2. Police forensics

In many cases of homicides there is often a lack of physical evidence to identify the perpetrator. Lead isotopic analysis of small fragments of bullet material can often provide a positive identification to the culprit assuming that the bullet cartridges within a single batch all have the same isotopic composition. Stupian et al. (2001) and Puttigieg et al. (2003) showed that this was not always the case since during the manufacture of bullets different sources of Pb can often be added smearing any distinctive isotopic composition. Occasionally where sample evidence is small, over handling of samples in non-pristine environments can compromise any isotopic evidence and yield results that can not be used for a positive conviction (Gulson et al., 2002).

2.3. Animal migration

The origins and migration of wildlife may be determined using isotopic fingerprinting techniques. Hobson (1999) provides a detailed review of how C, N, S and H isotopes can be used to show the origin and migration of animals. Organisms moving between distinctly different foodwebs will carry with them the isotopic composition of previous feeding grounds. This technique can be applied to a range of different animals from insects to birds to large mammals. For example, Hoppe et al. (1999), determined the migration behavior of the mammoths and mastodons using Sr isotopes. The use of isotopes for human migration has been used with some degree of success. Gulson and Gillings (1997) examined Pb isotopes in enamel and dentine and showed that enamel preserved the Pb isotopic composition whereas the dentine showed isotopic exchange over time. Studies on teeth enamel (Gulson et al., 1997) showed distinct difference between Europeans and Australians further demonstrating the powerfulness of isotopes for showing countries of origin. Beard and Johnson (2000) showed that Sr isotopes in teeth could also be used since the Sr is incorporated into teeth at infantile ages without subsequent fractionation and applied this to identification of commingled human remains from the Vietnam conflict.

Vogel et al. (1990) used a combination of N, C, Sr and Pb isotopes in elephant ivory and bones to determine the origin of elephant history and for examining different elephant populations. In this case the isotopic composition of the ivory and bone reflect the isotopic composition of the foods that the elephant ate and therefore the isotopic composition of the soils in which the plants grew.
2.4. Archaeology

English et al. (2001) examined Sr isotopes in roof timbers at the Native American site of Chaco Canyon to determine the source of the trees felled. The Sr isotopes provided enough resolution to show that 2/3 of the logs originated in the Chuska mountains and 1/3 from the San Mateo mountains. Using both archaeological and geochemical data they were able to show that both sources of timber were used at the same time. This provided great insights into the sociological behavior of the native Indians.

2.5. Food authentication

Food authentication is an important and growing field that has used many of the tools available to forensic scientists to authenticate foods. This is important since some foods sourced from established areas tend to be more valued than those from other less established areas. There have been many studies characterizing foods using a suite of different isotopes. Fortunato et al. (2004) used Sr isotopes in cheeses to characterize different source regions, and Kawasaki et al. (2002) examined rice provenance using Sr isotopes. A suite of different isotopes (C, N, O, S and Sr) were used to characterize butter from a variety of different worldwide sources (Rossmann et al., 2000). Calderone et al. (2004) examined the C isotopic composition of glycerol in wines to examine if the wine had been adulterated by the addition of glycerol. Classically Sr isotopes have been used to distinguish between different source regions for food products. Almedia and Vasconcelos (2004) examined wines, grapes and soils and showed that in wine production the Sr isotopic composition of the wine did reflect that of the soil, showing that provenance could be determined from the wine itself and that during the wine making process the Sr isotopic composition of the wine remained unchanged. Many studies have been carried out using Sr isotopes in wine to show provenance and thereby authenticate (Horn et al., 1998; Almedia and Vasconcelos, 2001; Martin et al., 1999; Barbaste et al., 2002). Studies of Pb isotopes in wines, however, has shown that the Pb isotopic composition of wines may be changed during processing depending on the age of the wine making equipment (Almedia and Vasconcelos, 2003). Those vineyards with new stainless steel processing equipment tend to leave the wine with the isotopic composition of the soils from where the grapes were harvested and those wines manufactured with equipment containing brass has a significant effect on altering the Pb isotope ratio of the wine. In addition to contamination from processing, Voellkopf et al. (1992) found that Pb from the Pb capsule of the closure piece can influence the isotopic composition of the wine. Care must therefore be employed when assessing the provenance of food, or determining the authenticity of foods since contamination from source may be prevalent. In addition to being able to determine source region using Pb isotopes the widespread use of Pb in gasoline up to the 1980s also allows an assessment of the age of the wine since atmospheric Pb content has been gradually diminishing (Medina et al., 2000).

2.6. Groundwater contaminants

Contamination into aquifers and groundwaters has been an important issue that has relied on the use of isotopes to identify particular point sources. Many different isotope systems have been employed to address point source discharges and these include O and N isotopes in nitrates by Buzek et al. (2006) to show the influence of landfills, local sewage systems and fertilizers in aquifers. Pennisi et al. (2006) used B and Sr isotopes measured in groundwaters to show an anthropogenic influence from previous B mineral processing in addition to geothermal release. Ader et al. (2004) showed that Cl isotopes in chlorates and perchlorates had distinctive enough signatures to be able to be traced as anthropogenic sources from herbicides, solid fuel propellants and explosives in groundwaters.

The highly toxic nature of Pb and the extensive use of Pb in gasoline have given rise to careful controls on the amount of Pb in foods. Rankin et al. (2005) showed by examining Pb isotopes in chocolate that elevated levels of Pb were most likely due to contamination during processing rather than from the sources of the cocoa and milk.

3. Non traditional heavy isotope systems

There has been an explosion in isotope geochemistry in the last 10 a through the advent of multiple collector ICP-MS instrumentation. It is now possible to measure with great accuracy and precision isotopes ratios of elements that have been difficult by TIMS techniques. However, since this field is relatively new, the isotope systematics of these new isotopes (e.g., Cr, Fe, Mo, Hg, Tl, Si, Se, Cu, Zn, Se and Te) are not well understood, it is premature to assess how useful these isotope systems are in forensic isotope geochemistry. In situations where identification of the origin can be deduced by simple comparison of the isotope ratio of the artifact to source material and where there has been no aqueous transport mechanism, these isotope systems offer potential as powerful tools. However, it has been noted by several workers (e.g., Anbar et al., 2000; Johnson and Bullen, 2004) that during transport processes, fractionation of isotopes can occur by redox and sorption processes. Dolgopolova et al. (2006) discovered that lichens growing close to a metal ore processing plant showed fractionated Zn isotopes from the Zn ore, indicating that either the lichens fractionated the Zn isotopes or that during aerosol transportation the Zn was isotopically fractionated. In these cases the forensic use of these isotopes can be quite limited unless these fractionation processes from the source are well understood. A study by Ellis et al. (2002) highlights this issue in the case of Cr isotopes. Chromium plating baths are extensively used in heavy plant machinery. The waste from these Cr plating operations discharges Cr into groundwaters, with the Cr being isotopically enriched in the heavy isotope as a result of Cr reduction and the subsequent loss of light Cr in the groundwater (Ellis et al., 2002). Hence there is no direct correlation of the Cr source with that measured in the groundwaters since redox effects give rise to isotopic
fractionation modifying the isotopic composition of the groundwaters, thus making it difficult to identify a point source contaminant into the aquifer. Other elements show isotopic shifts from their source during processing. Cloquet et al. (2006) studied Cd isotopes in soils around a Pb–Zn refinery plant and found isotopically fractionated Cd in the soils compared to the ore. Despite this fractionation, the soil Cd showed a distinct dispersal pattern indicating refinery derived Cd that was isotopically light compared to the ore source.

As more work is carried out on these novel isotope systems to fully understand the isotope systematics, their utility and application will become more widespread in tracing sources and transport, and in their use as forensic tools.

4. Case studies

The heavy metal isotopes have been applied to two case studies to determine provenance. In both cases it is not the isotope system being utilized that is unique but the application to the specific problem. Whilst these studies may not be definitive, they give us strong tools to make assessments and lay the groundwork for future studies.

4.1. Case study 1. Source of ores for the Pueblo Indians in South west USA

4.1.1. Background

The prehistoric Pueblo potters in the SW USA (Fig. 1) were unique in their technology of pottery ornamentation using a mix of silica, alumina and a Pb flux to create glaze-paint decorations (Shepard, 1942). Exposures of Pb ore in north central New Mexico are limited suggesting that certain potting communities may have dominated the production and exchange of these Rio Grande glazed pots (Habicht-Mauche, 1995). Mineralogical studies (Shepard, 1956; Warren, 1979) have indicated that these glazed pots were widely traded through the Rio Grande Valley during the Intermediate Glaze period (AD 1425-1515). Studies...
Lead has 4 isotopes, 3 of these are formed as a result of radiogenic decay of the U and Th isotopes, $^{204}$Pb is non-radiogenic. Different ore bodies are likely to have different Pb isotope ratios reflecting different ages of formation and different sources of Pb during the formation of the ore body itself. By measuring the Pb isotopic composition of the ore bodies and comparing it to the isotopic composition of the glaze-paints it is possible to determine the source of the ore in the glaze material. Habicht-Mauche et al., 2000; Habicht-Mauche et al., 2002 carried out an extensive study on the glazes and ores to show isotopically distinctive populations of Pb ores and glazes (Fig. 2). There is however, a population where the Pb isotope ratios are not distinctive enough to distinguish between closely related ore sources (Cerrillos Hills and Magdalena Mountains), both of which have been shown to have been used for glaze-paint production. These are where the $^{207}$Pb/$^{204}$Pb and $^{206}$Pb/$^{204}$Pb ratios are particularly low. To progress any further requires additional tools.

4.1.3. Copper and strontium isotopes

Commonly associated with Pb is Cu at significant concentrations. The use of Cu isotopes to distinguish the different Pb ores bodies and in glazes was investigated. Larson et al. (2003) showed that different Cu ores from the same ore bodies showed different Cu isotopic signatures indicating that Cu isotopes could not be used to distinguish different ore bodies sources. Strontium is well known to have significant variations in the $^{87}$Sr/$^{86}$Sr ratio through the radiogenic decay of $^{87}$Rb. However, Sr concentrations in Pb ore can be particularly low.

4.1.4. Method

As a trial for the technique several ore samples from the Cerrillos and Magdalena ore districts whose Pb isotopic ratios significantly overlap were taken and digested using a mix of HNO₃ and HCl. Once completely digested the samples were dried down and then re-dissolved in 1 mL of 2.5 M HCl. The sample was passed through a cation exchange resin to separate the Sr from other elements and the Sr fraction collected. (Complete details for the procedure can be found at the Keck Isotope Laboratory website: http://keckisotope.ucsc.edu/Chemistry.html). The Sr fraction was then dried down before being loaded onto a Ta outgassed filament for running on the Sector 54 thermal ionization mass spectrometer. In addition to the ore samples, one sample of glaze-paint was also analysed. The glaze was recovered from a pottery sherd from the site of Kuapa in north central New Mexico by use of a dentist drill. Sample processing was identical to that performed for the ore samples.

4.1.5. Results and discussion

Results are shown in Fig. 2 and these show that while there is no overlap in the Pb isotope signatures for the Cerrillos and Magdalena ore sources, a plot of Sr isotopes against $^{206}$Pb/$^{204}$Pb reveals clear variation between the two ore bodies (Fig. 3). Since the number of ore samples analysed for both Sr and Pb isotopes is limited it is not definitive where the Pb glaze was sourced, however, the data do suggest that the sherd is ornamented with Pb-based paint derived from the Cerrillos mining district. A combination of isotope systems has been able to shed more light on the source of Pb glazes for these Pueblo potters and therefore shed light on their trading routes. The data indicate that the potters' preference and existing networks of inter-community interaction were more important than proximity and ease of access in determining where to acquire ore from.

![Fig. 2](image-url) Lead isotope analyses of lead ores from the north central New Mexico. Analyses were made by high resolution ICP-MS and typical errors are $\pm 0.02$.

![Fig. 3](image-url) Variations in Sr and Pb isotopes for several ore samples and a pottery sherd. Errors for the Sr isotope ratios measured by thermal ionization mass spectrometry are typically $\pm 0.00001$ and for the Pb isotopes measured by high resolution ICP-MS are $\pm 0.02$. 
This study clearly shows that in many cases the use of one isotopic system may not provide enough resolution and that a multi-isotopic approach may be required to characterize the materials with adequate source resolution.

4.2. Case study 2. Strontium isotopic ratios as birthplace markers, tracking region of origin for forensic identification

The USA–Mexico border has long been a popular gateway of entry for immigrants attempting to enter the US economic system and has consequently been the site of a number of crossing-related deaths. Studies estimate that approximately 700 men, women, and children die each number of crossing-related deaths. Studies estimate that 1/3 are never identified (Eschbach et al., 2000; Burton et al., 2003; Hoppe et al., 1999; Price et al., 2003). Of these, it is estimated that 1/3 are never identified (Eschbach et al., 2003). This case study examines the use of Sr isotopes in tooth enamel to determine the origin of these undocumented Mexicans and to develop this tool for forensic geolocation.

For forensic anthropologists much of the difficulty associated with identifying and repatriating deceased, undocumented border-crossers along the US Mexico border stems from an inability to narrow the search area to precise locations. Moreover, countries in Latin America lack well developed database systems of documentation and identification for missing persons. In order to employ precise identification technologies such as DNA or dental analysis, investigators must first determine a searchable region in which to locate family, personal records and or legal documents. Creation of a map, documenting region of origin via tooth enamel Sr isotope ratios in human tooth enamel has been carried out using thermal ionization mass spectrometry (TIMS). This research has revealed geologically specificSr isotopic ratios within this tissue are a reflection of the geological substrate in which the food ingested was grown. In dental enamel, Sr is incorporated during enamel formation a process that is finished in early childhood (Hillson, 1996). Once the enamel crown of a tooth has been formed it does not recrystalize or remodel. For this reason, Sr isotopes from dental enamel reflect the geochemistry of childhood residence.

The ability to determine region of origin for a deceased individual is a tool of great forensic significance, however, this technology has rarely been applied to modern populations and with mixed result (Beard and Johnson, 2000). This is due in large part to the inclusion of packaged and imported foods in the modern diet. In rural Mexico and Latin America the practice of including locally grown produce and meats in the diet has been retained to a much greater extent than in the USA. Given this information it was theorized that isotopic difference could be delineated in the tooth enamel of individuals originating from these localities and utilized to narrow down a region of origin.

4.2.2. Materials and methods

Nineteen human teeth from 4 Mexican states were analysed: 4 from the District Federal, 2 from Michoacan, 3 from Guanajuato, and 10 from Jalisco. The teeth were donated from 16 Mexican born individuals receiving dental treatment at local Santa Cruz area dental clinics. Donors provided the tooth sample as well as an informational page documenting their age, sex, and birthplace down to the village level. The teeth chosen for the study were removed for dental or orthodontic reasons and maintained at least 80% of their exterior enamel. Each tooth represented one sample and was prepared under clean lab conditions to isolate the Sr in the enamel for analysis.

Tooth enamel was manually separated from the dentine through use of a mounted dental drill. Following acid etching they were rinsed with DI water, dried and crushed and repeatedly leached with acetic acid. Aliquots of 100 mg of freeze-dried sample were digested in a HNO3–HF acid mixture. Digested samples were loaded onto Sr Spec® resin and the Sr eluted with water.

The samples were analysed within the W.M. Keck Isotope laboratory using the VG Sector 54 thermal ionization mass spectrometer. Replicate analyses of NBS 987 give a 87Sr/86Sr of 0.710258 +/−0.000024.

4.2.3. Results and discussion

The preliminary results (Fig. 4) reveal the formation of 3 significant and distinct regions in the data set. Region 1 encompassed samples from both Jalisco and Guanajuato.
The samples from region 1 had a high degree of overlap with an average value of 0.70531±0.00021. Region 2 encompassed samples from Michoacan with an average value of 0.70460±0.00028. Region 3 encompasses samples from the District Federal and maintains an average value of 0.70626±0.00015. Fig. 5 plots the values over their geographical origins. The difference between the 3 regions can be attributed to the variation in the Sr isotope ratios of the local geologic environment. While the differences between each region may seem small, they are significant considering the resolution of the instrumental technique.

The use of isotopic ratios to determine region of origin is not new to the discipline of anthropology but historically has been focused on archaeological populations (see, Burton et al., 2003; Ezzo et al., 1997; Lillie et al., 2003; Price et al., 2000, 2002). When dealing with archaeological populations, workers have taken faunal samples (both archaeological and modern) as well as geological samples as the baseline comparative (Price et al., 2002; Burton et al., 2003; Schweissing and Grupe, 2003). With modern populations, this model is less successful due to the introduction of packaged foods in the diet that shifts the Sr signature away from the local geology therefore negating the usefulness of a soil or faunal sample baseline. Analyses must then be directed to within population variation to determine a statistically acceptable baseline. Such variation represents the differences present within a population originating from the same geo-political area, for example, individuals from the state of Jalisco or the Rancho Del Varro. Analyzing isotope ratios in this manner helps to account for the cultural aspects of a particular area and people and their affinity towards certain local and packaged foods. This is not to suggest that boundaries assigned through political means will maintain unique and individual geochemical signatures but rather, from a cultural standpoint these political accumulations of people provide a logical plateau from which to begin the analysis. In fact, as this analysis shows with the formation of region 1, which encompasses individuals from both Jalisco and Guanajuato, geochemical signatures can span multiple established political boundaries. In this case, the key to successful determination of geochemical boundaries is a large evenly spread regional sample base, the incorporation of multiple lines of isotopic evidence and statistical analysis.

4.2.4. Conclusions

The project demonstrates a real potential to identify the region of origin of modern individuals from human tooth enamel. While region of origin identification is not a positive ID it is a much needed stepping stone in an effort to narrow the search for a positive identification. While this effort is focused on the creation of an identification map (Fig. 5) documenting Sr isotopic ratios for populations originating in Mexican regions, it is hoped to extend this technology into south and central America to offer a more inclusive repatriation tool for all undocumented border crossers.

Clearly the use of isotopes such as Sr is a powerful tool in determining provenance for migrant undocumented human populations. Its utility, however, is hampered by the
5. Summary

Heavy stable isotopes are powerful tools with which to understand sources of materials in the environment. This might be for litigative circumstances such as murder scenes, identifying the source of a contaminant in food or water or even examining the migration of animals across large areas. There are a number of heavy metal isotope systems that allow us to determine these sources and transport paths at the moment-Pb, Sr and Nd. In this paper, applications have been presented using Sr and Pb isotopes to determine provenance of Pb ore used to make glazes for the Pueblo Indians and using Sr isotopes to determine geo-location of undocumented deceased Mexicans crossing the US/Mexican border.

New instrumentation has opened up the number of isotope systems that can be measured at high precision, however until the isotope systematics are better understood, these new isotope systems will have limited application to forensic sciences. In the meantime, the isotope systems that are well understood give the ability to trace materials and animals through the environment. As the field grows and the isotope systematics of more elements are understood the challenge will become to determine which isotope system is most applicable.

These are clearly early days for understanding the isotope systematics for these non-traditional stable isotopes. As our understanding increases we have the potential to apply them to progressively more problems. However, since the cost and time required to prepare samples is significant this may hamper the commercial application of these tools for forensic science.

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