

## REVIEW

### Isotopes in bioarchaeology - Review

Gabriel Dorado <sup>1</sup>, Teresa E. Rosales <sup>2</sup>, Fernando Luque <sup>3</sup>, Francisco Javier S. Sánchez-Cañete <sup>4</sup>, Isabel Rey <sup>5</sup>, Inmaculada Jiménez <sup>6</sup>, Arturo Morales <sup>7</sup>, Manuel Gálvez <sup>8</sup>, Jesús Sáiz <sup>9</sup>, Adela Sánchez <sup>9</sup>, Víctor F. Vásquez <sup>10</sup>, Pilar Hernández <sup>11</sup>

<sup>1</sup> Author for correspondence, Dep. Bioquímica y Biología Molecular, Campus Rabanales C6-1-E17, Campus de Excelencia Internacional Agroalimentario (ceiA3), Universidad de Córdoba, 14071 Córdoba (Spain), eMail: <bb1dopeg@uco.es>; <sup>2</sup> Laboratorio de Arqueobiología, Avda. Universitaria s/n, Universidad Nacional de Trujillo, Trujillo (Peru); <sup>3</sup> Laboratorio de Producción y Sanidad Animal de Córdoba, Ctra. Madrid-Cádiz km 395, 14071 Córdoba; <sup>4</sup> EE.PP. Sagrada Familia de Baena, Avda. Padre Villoslada 22, 14850 Baena (Córdoba); <sup>5</sup> Colección de Tejidos y ADN, Museo Natural de Ciencias Naturales, 28006 Madrid; <sup>6</sup> IES Puertas del Campo, Avda. San Juan de Dios 1, 51001 Ceuta; <sup>7</sup> Dep. Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Cantoblanco (Madrid); <sup>8</sup> Dep. Radiología y Medicina Física, Unidad de Física Médica, Facultad de Medicina, Avda. Menéndez Pidal s/n, Universidad de Córdoba, 14071 Córdoba; <sup>9</sup> Dep. Farmacología, Toxicología y Medicina Legal y Forense, Facultad de Medicina, Avda. Menéndez Pidal, s/n, Universidad de Córdoba, 14071 Córdoba; <sup>10</sup> Centro de Investigaciones Arqueobiológicas y Paleoeológicas Andinas ARQUEOBIOS, Apartado Postal 595, Trujillo (Peru); <sup>11</sup> Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14080 Córdoba

#### Abstract

The elements of the periodic table are made of populations of isotopes (atoms with the same number of protons, but different number of neutrons). Interestingly, such isotopes may be differentially distributed in the nature. On some instances, such selective fractionation is carried out inside the living organisms, due to their peculiar physiologies. Such is the case of the carbon isotopes in the C3, C4 and CAM plants. Therefore, it is possible to ascertain the diet of ancient remains, since such isotopic differential distribution is transmitted through the trophic chain. Other examples of isotopes used in archaeology include the nitrogen, oxygen, hydrogen, sulfur, strontium, calcium, lead, selenium, zinc, potassium and aluminium. Some isotopes are stable, but others are radioactive and thus decay with time, being therefore an invaluable tool for dating archaeological remains. This way, it is not only possible to determine the age of inorganic and organic material, but also to study paleodiets, paleomobilities and paleoclimates in paleoecological contexts.

*Key words:* atomic number, mass number, nuclide, mass spectrometry, artifacts, tools.

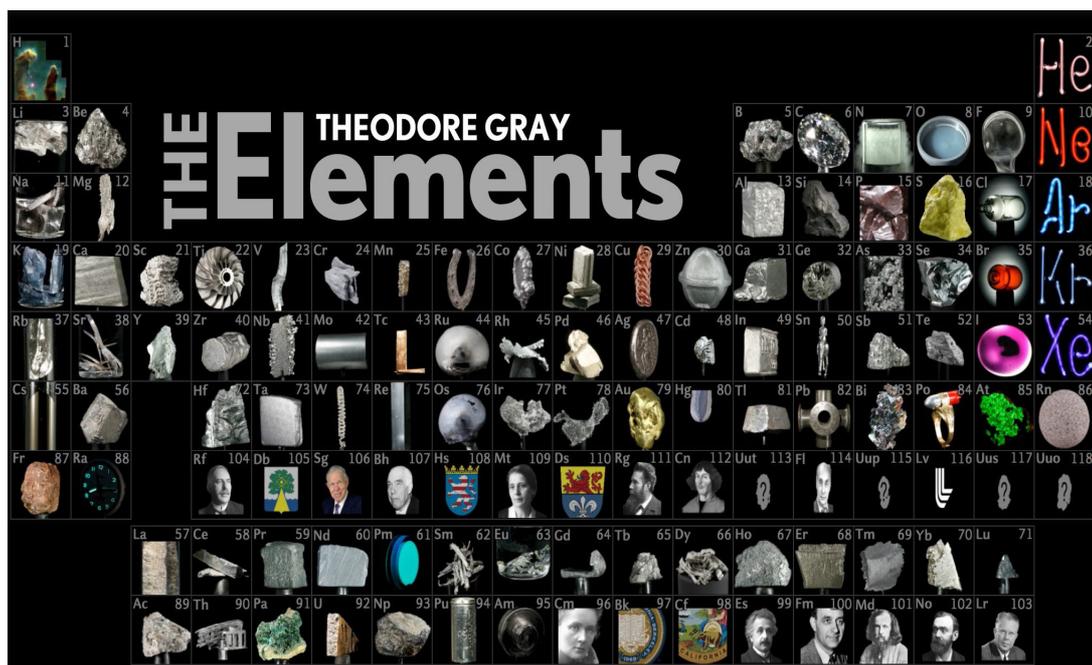
#### Resumen

Los elementos de la tabla periódica están formados por poblaciones de isótopos (átomos con el mismo número de protones, pero diferente número de neutrones). Curiosamente, tales isótopos pueden estar distribuidos de forma diferencial en la naturaleza. En algunos casos, dicho fraccionamiento selectivo se lleva a cabo dentro de los organismos vivos, debido a sus fisiologías peculiares. Tal es el caso de los isótopos del carbono en las plantas C3, C4 y CAM. Por tanto, es posible determinar la dieta de restos antiguos, ya que dicha distribución diferencial de los isótopos es transmitida a través de la cadena trófica. Otros ejemplos de isótopos usados en arqueología incluyen el nitrógeno, oxígeno, hidrógeno, azufre, estroncio, calcio, plomo, selenio, cinc, potasio y aluminio. Algunos isótopos son estables, pero otros son radiactivos y por tanto decaen con el tiempo, siendo por tanto una herramienta inestimable para datar restos arqueológicos. De este modo, es posible no sólo determinar la edad de material inorgánico y orgánico, sino también estudiar paleodietas y paleoclimas en contextos paleoecológicos.

*Palabras clave:* número atómico, número másico, nucleido, espectrometría de masas, artefactos, herramientas.

## Introduction

The archaeology in a broad sense involves the study of the past. This means analyzing evidence that may be partial and somehow modified. That may represent a serious handicap in some instances, where most or even all of the direct evidence may be lost. Fortunately, there is a wonderful tool for such studies that stems from the fact that the Universe is made of the elements of the periodic table first published by Dmitri Mendeleev in 1869 (based on previous proposals), which was widely recognized and further increased with new elements, currently being a work in progress (Fig. 1). The chemical elements are classified after their atomic number (also known as proton number; corresponding to the protons of the atom), electron configuration and chemical properties. Such elements have been discovered in the nature or artificially synthesized.



**Figure 1. Periodic table of the elements.** Classification of the chemical elements after Dmitri Mendeleev (1869), based on previous works and currently in development. Figure credit: The Elements for iPad. © 2012 Theodore Gray - Touch Press <<http://www.touchpress.com/titles/theelements>>.

Most elements are present in the nature as a population of different varieties of atoms, much as the living species are made of populations of individuals showing genetic polymorphisms (mutations or variations in their genomes). Thus, while each chemical element has a unique number of protons, the number of neutrons varies between the different atoms. Such variations are known as isotopes (which is derived from Greek words meaning “same place”, since they are included in the same periodic table site). Therefore, each isotope has a different mass number (number of nucleons: protons plus neutrons; also known as atomic mass, atomic mass number or nucleon number). For instance, the carbon element found in the nature is made of three different types of atoms (all having six protons, but six, seven or eight neutrons, being therefore named after their mass numbers (carbon-12 or  $^{12}\text{C}$ , carbon-13 or  $^{13}\text{C}$ , and carbon-14 or  $^{14}\text{C}$ , respectively). From the semantic point of view, the word isotope is used to group all the atoms of each element, whereas the term nuclide is applied to individual nuclear species. In other words, the term isotope emphasizes the chemical properties over the nuclear ones, while the term nuclide (for instance, an atom with a specific number of

protons and neutrons, like the  $^{13}\text{C}$ ) emphasizes the nuclear properties over the chemical ones.

In any case, since both names correspond to the same elements, they are sometimes used as synonyms, without any particular distinction. The letter “m” is added after the mass number, to identify metastable nuclear isomers (eg.,  $^{131\text{m}}\text{Xe}$ ). They represent energetically-excited nuclear states, which are less-stable than the lower-energy ground states.

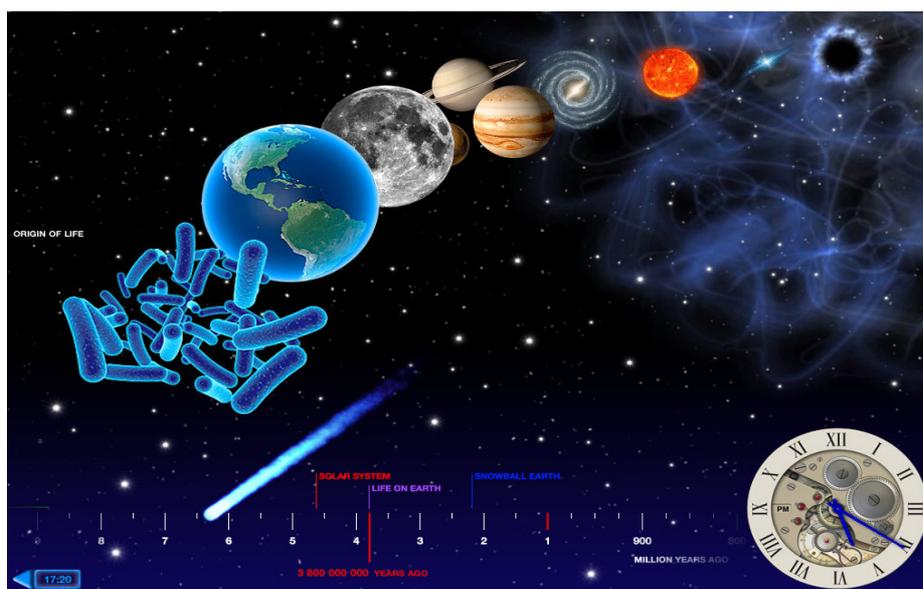
The isotopes (or nuclides) are classified as stable and radioactive (radioisotopes or radionuclides) that undergo radioactive decay. For instance, both the  $^{12}\text{C}$  and  $^{13}\text{C}$  are considered as stable isotopes, while the  $^{14}\text{C}$  is a radioactive form of carbon. When an element has no stable isotope(s), then the atomic mass of its less unstable (most “stable”) isotope is shown in parentheses in the periodic table. Thus, some elements are represented by a stable isotope, others by a radioactive one, and yet others by both. Usually, the most abundant isotopes of a particular element in the nature are usually the stable ones. Yet, many isotopes classified as stable are predicted to be radioactive, albeit with extremely long half-lives. For instance, 35 primordial nuclides (present at the formation of the solar system; ~4.6 milliards of years ago) have extremely long half-lives (more than 80 million years). There are 27 radionuclides with predicted half-lives longer than the age of the universe (~13.75 milliards of years). Extremely long-lived radioisotopes are the tellurium, indium, and rhenium. For instance, the  $^{128}\text{Te}$  has the longest half-life among the radionuclides, being about  $160 \times 10^{12}$  times the age of the universe. Besides, if the proton decay is considered, all elements would be ultimately unstable, given enough time. Yet, the stable/unstable classification is used for practical reasons. On the other hand, the radiogenic nuclides (which can be stable or radioactive) are the ones being generated during the radioactive decay processes.

There are more than 5,172 known nuclides to date, including natural (971) and synthetic (4,201) ones: i) 90 theoretically stable to all but proton decay (albeit, such phenomenon has not been observed so far), including the first 40 elements of the periodic table); ii) 254 also considered stable, since no decay has been detected so far, yet being energetically unstable to one or more known decay modes; iii) 288 radioactive primordial nuclides; iv) 339 radioactive nuclides found in the nature (but non-primordials), known as daughter products, daughter isotopes or daughter nuclides because they are generated by cosmic rays and as remaining nuclides left over from radioactive decay products of the radioactive primordials; v) 901 radioactive synthetic nuclides with half-lives of more than one hour and short-lived natural isotopes, including the most useful radiotracers (also known as radioactive tracers or radioactive labels); and vi) 3,300 well-characterized radioactive synthetic nuclides with half-lives of less than an hour.

In relation to the origin of the elements of the periodic table, the Big Bang theory describes the origin and early development of the universe. It states that there was an initial expansion of energy from a singularity. The subsequent expansion caused the early universe to cool, allowing the condensation of energy into matter. Thus, subatomic particles were formed, including protons, neutrons (which combined to form the first atomic nuclei a few minutes after the Big Bang) and electrons (which, after thousands of years, eventually combined with the nuclei to create electrically-neutral atoms). This way, the first hydrogen atoms were generated (together with trace amounts of other

elements like helium and lithium), producing Giant Molecular Clouds (GMC) made of such primordial elements. The GMC coalesced by gravitation in some areas, forming smaller and denser clumps, eventually collapsing to form stars.

The modern variant of the nebular hypothesis, known as the Solar Nebular Disk Model (SNDM) or Solar Nebular Model (SNM), is generally accepted as the model explaining the formation and evolution of the Solar System and other star systems in the universe. Thus, after the gravitational collapse of the GMC, fusion nuclear reactions are ignited due to the high temperature and pressure inside the forming star, generating one atom of helium from each two fusing atoms of hydrogen, further liberating the mass difference as huge amounts of electromagnetic radiation (including the visible light), after the famous Einstein's formula that links the energy with the matter and the speed of the light ( $E = m \cdot c^2$ ). During the life of the star, other heavier elements are also formed. If the star is large enough, it will eventually collapse due to the gravitation, generating a black hole. But if the star has a lower mass, it will eventually explode as a supernova, further synthesizing more heavier elements, being eventually dispersed across the surrounding space, generating a new cloud of matter (now containing also the heavier elements previously formed in the star) that may eventually form planets on different star systems. Indeed, gaseous protoplanetary disks including such cloud matter may be produced around the young stars, and may eventually become planets, which, as in the case of the planet Earth, may generate life (Fig. 2).



**Fig. 2. Evolution of the universe.** From the Big Bang to the life on the planet Earth. Figure credit: Back in Time for iPad. © 2011 Landka <<http://landka.com/backintime>>.

### Isotope identification, quantification and application

The radioactive isotopes can be analyzed by Gamma-Ray Spectrometry (GRS). Such nuclides, as well as the stable isotopes can be quantified using Mass Spectrometry (MS), which is based on the measurement of the mass-to-charge ratio ( $m/z$ ) of charged particles. This way, the isotopic composition of the elements present in a sample can be determined using Isotope-Ratio Mass Spectrometry (IR-MS). In short, the samples are injected, vaporized and electrically charged (ionized). Then, the ions are collected and

accelerated (with the aid of magnets) into a detector, determining their  $m/z$  ratios. As an example, the Flowing Afterglow Mass spectrometry (FA-MS) can be used to determine the deuterium content of water.

To separate rare isotopes from an abundant neighboring mass, the ions are accelerated to extraordinarily high-kinetic energies before the mass analysis by means of Accelerator Mass Spectrometry (AMS), achieving sensitivities (known as “abundance sensitivity”) three orders of magnitude higher than previous methodologies for radioisotope decay counting. This outperforms the alternative methodologies of isotope decay counting when their half-lives are long enough.

The isotopes have a wide range of applications, including: i) radiotracers for metabolism studies (anabolism and catabolism). Indeed, the metabolic reactions and networks have been deciphered that way (fermentations, photosynthesis, Krebs cycle, etc); ii) radiotracers in biomedicine to reveal and diagnose physiological conditions (health and disease) in cells, tissues, organs and complete organisms; iii) radioactive drugs for cancer treatment, by means of radiotherapy; iv) substrate dating in geology (eg., geochemistry, paleoclimatology and paleoceanography); thus, naturally-occurring and long-lived radio-isotopes such as the  $^{10}\text{Be}$ ,  $^{26}\text{Al}$ ,  $^{36}\text{Cl}$  and  $^{14}\text{C}$  are used for Surface-Exposure Dating (SED) in geology and the  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{36}\text{Cl}$ , and  $^{129}\text{I}$  are used as hydrological tracers; v) traceability of the nuclear-reaction debris (“hot particles”); thus, the isotopic signatures of  $^{152}\text{Eu}/^{155}\text{Eu}$ ,  $^{154}\text{Eu}/^{155}\text{Eu}$ , and  $^{238}\text{Pu}/^{239}\text{Pu}$  are different for fission and fusion nuclear reactions, which may also show distinctive ratios for  $^{60}\text{Co}/^{59}\text{Co}$ ,  $^{125}\text{Sb}/^{121}\text{Sb}$ ,  $^{144}\text{Ce}/^{133}\text{Ce}$ ,  $^{240}\text{Pu}/^{239}\text{Pu}$  and  $^{133}\text{Xe}/^{131\text{m}}\text{Xe}$ , among others (as in the Chernobyl and Fukushima nuclear accidents), whereas underwater bursts will be mostly made of irradiated sea salts; and vi) tools for dating archaeological samples, including the remains of living organisms (microorganisms, plants and animals) and their contexts.

### Isotopes in archaeology

Different isotopes can be used in archaeological studies, depending on the specific application (Knudson and Stojanowski 2008; Lee-Thorp 2008). Although the isotopes of a particular element have identical chemical properties, they may show a differential distribution (fractionation) due to their different masses. Thus, they can be differentially used by the cellular enzymes involved in the metabolism of the living organisms, reflecting also their percentages in the nature. Therefore, the isotope analyses allow the identification of the isotopic signatures (also known as isotopic fingerprints), which are usually calculated as the Isotope Ratios (IR) of stable or unstable isotopes of particular elements of the periodic table in a specific material. Thus, the Stable Isotope Analysis (SIA) and the Radioactive Isotope Analysis (RIA) can be used to determine the isotopic signature by means of the IR, which allows to calculate the distribution of different isotopes in a sample.

This way, it is possible not only to determine the ages of the samples, but also to infer diets (paleodiets), trophic levels, weather patterns, mobility of individuals and populations, etc. Indeed, the AMS technology is so powerful that it can tell apart, for instance, stable  $^{14}\text{N}$  from radiocarbon  $^{14}\text{C}$  (effectively separating atomic isobars), and that from stable  $^{12}\text{C}$ . As a consequence, the AMS is usually applied for  $^{14}\text{C}$  radiocarbon dating. Albeit, significantly large samples are required for decay counting, due to the

long  $^{14}\text{C}$  half-life. The most used isotopes in archaeology are described next, including carbon, nitrogen, oxygen, hydrogen, sulfur, strontium, calcium, lead, selenium, zinc, potassium and aluminum.

### A. Carbon isotopes

The carbon element population in the nature is made of two stable isotopes ( $^{12}\text{C}$ ,  $^{13}\text{C}$ ) and a radioactive one ( $^{14}\text{C}$ ). They show a natural abundance of 98'89, 1'11 and 0'00000000010%, respectively. The latter are constantly generated by neutron bombardment of the nitrogen atoms in the upper atmosphere. Then, they are oxidized by the atmospheric oxygen, producing carbon dioxide ( $\text{CO}_2$ ), which is fixed into organic matter by the photosynthetic organisms, and is subsequently distributed across the trophic pyramid. The  $^{14}\text{C}$  is a beta-emitter with a half-life of 5,730 years. As any other radionuclide, the  $^{14}\text{C}$  starts decaying immediately after being formed. It is constantly replenished in the living organisms, but stops being further furnished after their death. Therefore, the percentage of the  $^{14}\text{C}$  isotope present in a sample can be used to determine its age and origin up to about 50,000 years. Such radioisotope is below detectable limits in much older material, as is the case of fossil fuels like the coal or petroleum, as well as the products artificially made from them.

The isotopes are quantified using a standard as a reference value. In the case of the carbon, such standard was originally the  $\text{CO}_2$  obtained from the Pee Dee Belemnites at the Pee Dee Formation (Cretaceous) of South Carolina (USA), known as PDB standard. Currently, calibrated  $\text{CO}_2$  from other sources is being used as standard. The carbon isotope ratio is calculated as a delta notation in parts per thousand (per mil; ‰):

$$^{13}\text{C}/^{12}\text{C} \text{ ratio} = \delta^{13/12}\text{C} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

where the standard is the PDB value (0.0112372‰). Thus, samples with higher or lower  $\delta^{13}\text{C}$  values than the PDB standard have positive or negative  $\delta^{13}\text{C}$  values, respectively, being zero for ratios equal to the PDB standard.

The carbon found in inorganic carbonates exhibits little isotopic fractionation, whereas the organic products generated via the photosynthesis are depleted of the heavier isotopes. Besides, there are three types of plants in relation to their photosynthetic carbon-fixation pathways: i) the C3 photosynthesis (eg., rice, wheat, barley, soybean, potato and trees) has a single  $\text{CO}_2$ -fixation step, generating a three-carbon molecule; ii) the Crassulacean Acid Metabolism (CAM; eg., pineapple, some orchids and cacti) photosynthesis has two  $\text{CO}_2$ -fixation steps: the first one fixes atmospheric  $\text{CO}_2$  into malic acid at night (which is stored in vacuoles), and the second one breaks down such product at day to generate  $\text{CO}_2$  for the photosynthesis; and iii) the C4 photosynthesis (eg., maize, sugar cane, millet and sorghum) has also two  $\text{CO}_2$ -fixation steps: the first one fixes atmospheric  $\text{CO}_2$  into a four-carbon molecule (malic acid; which is moved to bundle-sheath cells on the leaf interior), and the second one breaks down such product in such cells to generate  $\text{CO}_2$  for the photosynthesis.

The C3 plants arose during the early Paleozoic era (550 million years ago) with the land plants, and are the dominant ones (~95% of the plant biomass and ~90% of the known plant species on the plant Earth, accounting for ~70% of the terrestrial carbon fixation), yet, they lose by transpiration about 97% of the water that they absorb from the soil, and

therefore are not adapted to hot and dry climates that the Earth has experienced along its evolution.

Thus, some C3 plants evolved in the Paleocene epoch (about 65 millions years ago) into CAM plants (less than 1% of the plant biomass and ~7% of the known plant species, accounting for less than 1% of the terrestrial carbon fixation) in areas with drought, high temperatures and nitrogen or CO<sub>2</sub> limitation (CAM may lose only 35 to 20% of water as compared to C3 or C4 (see below), respectively, for a given degree of stomatal opening). No wonder that most of CAM plants are epiphytes or succulent xerophytes, for which the water savings are of paramount importance for survival.

Some C3 plants also evolved into C4 plants in at least 40 independent events (convergent evolution), involving different plant families. The C4 plants arose in the Oligocene epoch (25 to 32 million years ago) and became ecologically significant in the Miocene Period (6 to 7 million years ago). (~5% of the plant biomass and ~3% of the known plant species, yet accounting for ~30% of the terrestrial carbon fixation). The current C4 plants are concentrated at latitudes below 45° (tropics and subtropics), where they are more efficient than C3 plants. This is due to the fact that the high temperatures significantly increase the oxygenase activity of the Ribulose-1,5-Bisphosphate Carboxylase Oxygenase (RuBisCO) and thus the photorespiration levels of the C3 plants in comparison to the C4 ones, rendering the former less competitive than the latter in such areas (Osborne and Beerling 2006).

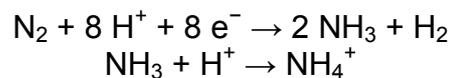
Interestingly, the physiological and metabolic differences also generate different  $\delta^{13}\text{C}$  values: i) more pronounced isotope-separation effect in C3 (-24 to -33‰); less-depleted <sup>13</sup>C isotope in CAM (-10 to -20‰) and even less-depleted <sup>13</sup>C isotope in C4 (-10 to -16‰). Likewise, the freshwater fish contain lower  $\delta^{13}\text{C}$  ratios (similar to the C3 plants) than in the marine fish (similar to the C4 plants). Since such ratios are propagated through the food chain, it is possible to make inferences in relation to the diet, the trophic level and the subsistence of different organisms (Brown and Brown, 2011; Szpak et al, 2013). For instance, it is possible to ascertain the main plant or fish diet of the humans or other animals analyzing the  $\delta^{13}\text{C}$  ratio of their bodies or remains like flesh, bone and dentine collagen. Obviously, it is not possible to determine with such methodology if the principal diet was corn or, say, corn-fed beef. There are also plants capable of switching between different methods of carbon fixation. For instance, the dwarf jade plant (*Portulacaria afra*) normally uses the C3 pathway, yet can use CAM if it is drought-stressed. Likewise, the purslane (*Portulaca oleracea*) normally uses the C4 photosynthesis, but can switch to CAM when drought-stressed.

On the other hand, the ratio <sup>13</sup>C/<sup>12</sup>C is used in geochemistry, paleoclimatology and paleoceanography to analyze methane sources and sinks, since they have different affinity for such isotopes. Likewise, the calcite is the most stable polymorph mineral of calcium carbonate (CaCO<sub>3</sub>), besides aragonite and vaterite, which are less stable. The calcite or of salt domes is produced from the CO<sub>2</sub> after petroleum oxidation, being therefore <sup>13</sup>C-depleted due to its photosynthetic origin, whereas the limestones (sedimentary rock largely made of calcite and aragonite) formed in seas and oceans from the atmospheric carbon dioxide precipitation contain normal proportions of <sup>13</sup>C. Interestingly, many limestones contain skeletal fragments or shells of dead marine organisms, such as mollusks, corals or foraminifers.

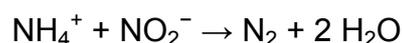
## B. Nitrogen isotopes

The nitrogen element has two stable isotopes ( $^{14}\text{N}$  and  $^{15}\text{N}$ ), with a natural abundance of 99'63 and 0'37, respectively. Most plants absorb nitrate anions ( $\text{NO}_3^-$ ) or ammonium cations ( $\text{NH}_4^+$ ) from the soil, which can be used to synthesize biomolecules like amino acids (peptides), nitrogenous bases (nucleic acids) and chlorophylls. Some plants contain rhizobia bacteria inside root nodules. This is typical of the legumes (alfalfa, clover, peas, beans, lentils, lupins, mesquite, carob, soybeans, peanuts, etc) and also a few non-legumes.

Such bacteria are capable of fixing atmospheric nitrogen ( $\text{N}_2$ ) into ammonia ( $\text{NH}_3$ ), which then is protonated into ammonium cation ( $\text{NH}_4^+$ ), which can be used by the plants:



A few (carnivorous) plants obtain nitrogen capturing animals, which, and thus obtain their nitrogen-containing biomolecules from other organisms, like the heterotroph organisms do (eg., fungi and animals). When the organisms die or release waste, the organic molecules are metabolized by the soil bacteria (and some fungi), becoming ammonium (ammonification or mineralization). Additionally, the nitrification takes place in two steps. In short, the ammonia is oxidized by some bacterial species like the ones of the genus *Nitrosomonas* into nitrites (which in high concentrations are toxic to the plants). Then, other bacterial species like the ones of the genus *Nitrobacter* further oxidize the nitrites into nitrates, which can be used for the plants. The nitrates can be also reduced back into nitrogen gas in anaerobic conditions by bacterial species like *Pseudomonas* and *Clostridium* (denitrification). Besides, the nitrite and ammonium can be directly converted in anaerobic conditions into molecular nitrogen, mostly in the oceans, by the bacterial phylum Planctomycetes. Such a process is known as Anaerobic ammonium oxidation (Anammox), representing about 50% of the  $\text{N}_2$  produced by the oceans, thus closing the nitrogen cycle in the nature:



As with the carbon isotopes, the  $^{15}\text{N}/^{14}\text{N}$  ratio present in the different organisms also depends on their metabolisms or the previous metabolisms of their food:

$$^{15}\text{N}/^{14}\text{N} \text{ ratio} = \delta^{15/14}\text{N} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

This is an invaluable tool for archaeologists, since the remains like the hairs, teeth and bones can be used to determine ancient diets. Thus, the nitrogen isotope signature of the animals that feed from plants (herbivorous) is typically different from the ones that feed from other animals (carnivorous), with omnivorous showing variable intermediate profiles, depending on their actual diets, which may also depend on the time of the year. The terrestrial- and marine-based diets generate also different nitrogen isotope ratios, offering the possibility to study the ancient cultural attitudes towards different food sources. Besides, such ratio may increase 3 to 4‰ with each link upwards on the food chain. Thus, the herbivorous tissues (including the ones from vegans) usually have significantly less  $^{15}\text{N}$  than the carnivorous ones. Indeed, the terrestrial plants (except the

legumes) have nitrogen isotopic ratios of 2 to 6‰. This way, it has been found that the Neanderthals (*Homo sapiens neanderthalensis*) were mostly predators of large terrestrial herbivores, whereas the modern humans (*Homo sapiens sapiens*) had a varied diet, as shown by their wider range of nitrogen isotopic values, also indicating the consumption of aquatic (marine and freshwater) resources (Richards and Trinkaus 2009; Brown and Brown, 2011; Szpak et al, 2013).

### C. Oxygen isotopes

There are three oxygen isotopes ( $^{16}\text{O}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$ ), with a natural abundance of 99'76, 0'04 and 0'21%, respectively. Thus, the following isotopic ratio is generally used:

$$^{18}\text{O}/^{16}\text{O} \text{ ratio} = \delta^{18/16}\text{O} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The water from different origins (eg., atmospheric vapor, seas and oceans, ice poles, and meteoric water) typically shows different oxygen isotopic profiles. On the other hand, the oxygen isotopic signature of the atmosphere depends on several factors, including climatic ones like the temperature (determining the evaporation and the precipitation profiles) and the isotopic exchange rates. Thus, the oxygen isotopes present in the water show a differential evaporation, depending on their mass. Indeed, the oxygen isotopic profile of the rainwater is correlated to different factors, like latitude, altitude and distance from the seas and oceans. On the other hand, the vapor-pressure of the water decreases with the concentration of dissolved salts. There is also a differential precipitation depending on the condensation temperature and the amount of vapor already condensed into precipitation. Interestingly, the different oxygen isotopes are incorporated into the carbonate minerals, including the calcium carbonate of the skeletons of the organisms. Therefore, the oxygen isotopic signature can be used as a valuable record of paleohydrologic and paleoclimatic information for archaeological studies (eg., evaporation, temperature and salinity of the water, etc), as well as mobility of species (Brown and Brown, 2011).

### D. Hydrogen isotopes

The hydrogen element is made of three isotopes, including two stable ( $^1\text{H}$  or protium and  $^2\text{H}$ , deuterium or D) and one radioactive ( $^3\text{H}$ , tritium or T) of both cosmogenic and anthropogenic origin. The natural abundance of  $^1\text{H}$ : $^2\text{H}$  is 99'985:0'015%. Thus, the ratio of stable hydrogen isotopes is expressed as:

$$^2\text{H}/^1\text{H} \text{ ratio} = \delta^{2/1}\text{H} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^2\text{H}/^1\text{H})_{\text{sample}}}{(^2\text{H}/^1\text{H})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The hydrogen and oxygen of the water are strongly associated ( $r^2 \geq 0'95$ ) and therefore, they are usually analyzed together, for instance, for dating probes of ancient ice in the Artic Ocean or in the Antarctica. Additionally, since most clouds are generated from the evaporation of low-latitude oceans, their precipitation is enriched in  $^2\text{H}$  and  $^{18}\text{O}$ , becoming lighter as the rain continues, which is usually associated with cloud movement across the continents. Due to these isotopic discriminations during the evaporation and condensation phase changes of the water, and the fact that such variations are transferred to the organism tissues via their diet (including the drinking water), the isotopic profiles can be exploited to discover animal migrations, since they

are related to latitude and altitude, as well as the distance from the sea or ocean, the season and the pluviocity (Crawford et al. 2008; Lee-Thorp 2008).

Furthermore, the hydrogen isotopic signatures of the bone collagen from humans and other animals have been used for paleodietary and paleoenvironmental reconstructions. Thus, the data obtained from ancient material shows species-specific isotopic ratios, increasing in steps of 10 to 30‰ from herbivores to omnivores (excluding humans) and from them to humans, demonstrating that the latter were mainly carnivorous at the time (Lee-Thorp 2008; Reynard and Hedges 2008; Arnay-De-La-Rosa et al. 2010).

On the other hand, since the half life of  $^3\text{H}$  is 12'43 years (decaying to  $^3\text{He}$ ), it can be used to date material with less than 100 years.

### E. Sulfur isotopes

The sulfur element (or sulphur) has 16 isotopes, including four stable ones:  $^{32}\text{S}$ ,  $^{33}\text{S}$ ,  $^{34}\text{S}$  and  $^{36}\text{S}$ , with a natural abundance of 95'02, 0'75, 4'21 and 0'02%, respectively. The ratio of the most abundant sulfur stable isotopes in the nature is:

$$^{34}\text{S}/^{32}\text{S} \text{ ratio} = \delta^{34/32}\text{S} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{34}\text{S}/^{32}\text{S})_{\text{sample}}}{(^{34}\text{S}/^{32}\text{S})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The sulfur is present in the lithosphere (mostly metamorphic and sedimentary rocks, with little contributions from the igneous ones), being eventually transported to the rivers, lakes, seas and oceans (the largest sulfur sink) by means of the eroding agents. The water splashes supply sulfate to the atmosphere, which eventually returns by precipitation. Besides the modern anthropogenic activities, the volcanoes also contribute to the atmospheric sulfur.

The sulfur isotopic profiles can be used to trace their natural and anthropogenic sources in hydrology related to agricultural practices, for instance. On the other hand, the organically-bound sulfur present in fossil fuels can be used to determine the age of the source rocks and the one on Precambrian rocks can be used to ascertain the origin of the life and its evolution. The sulfur isotopic profile have been also used for paleodiet studies.

### F. Strontium isotopes

The strontium element has four stable isotopes:  $^{84}\text{Sr}$ ,  $^{86}\text{Sr}$ ,  $^{87}\text{Sr}$  and  $^{88}\text{Sr}$ , with a natural abundance of 0'56, 9'86, 7'00 and 82'58%, respectively. The  $^{87}\text{Sr}$  is radiogenic, being generated by the of  $^{87}\text{Rb}$  decay. The Sr isotopic signature most used is:

$$^{87}\text{Sr}/^{86}\text{Sr} \text{ ratio} = \delta^{87/86}\text{Sr} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{87}\text{Sr}/^{86}\text{Sr})_{\text{sample}}}{(^{87}\text{Sr}/^{86}\text{Sr})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The Sr profiles usually correspond to the ones of the original source, since such element does not fractionate in general, and in any case the  $^{88}\text{Sr}/^{86}\text{Sr}$  ratio is constant, being therefore an excellent internal standard. Interestingly, the Sr can replace the Ca in the mineral lattices and in the plants and animal cellular structures. Thus, the Sr isotopic profiles can be used for hydrological studies, to trace the Ca sources and cycling in terrestrial and oceanic ecosystems. It is also useful to determine the pathways and availability of nutrients in ecosystems. As an example, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in tooth are

correlated to the ones in the area where they were formed during childhood. Therefore, this isotope represents an excellent tool to determine the mobility of animals, including humans. In fact, the strontium isotope profiles have been applied to determine kinship, specially when coupled with studies of ancient DNA (aDNA) (Dorado et al, 2007-2011), revealing exogamy with patrilocal marriages, in which the women moved from one area to another (Brown and Brown, 2011).

### G. Calcium isotopes

The calcium element has 24 isotopes ( $^{34}\text{Ca}$  to  $^{57}\text{Ca}$ ), including five stable ( $^{40}\text{Ca}$ ,  $^{42}\text{Ca}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$  and  $^{46}\text{Ca}$ ). The  $^{48}\text{Ca}$  is radioactive, albeit with a half-life so long ( $4.3 \times 10^{19}$  years), that it can be considered stable from a practical point of view. Their abundance in nature is 96'941, 0'647, 0'135, 2'086, 0'004 and 0'187%, respectively.

The following Ca isotopic signature is generally used in archaeology:

$$^{44}\text{Ca}/^{42}\text{Ca} \text{ ratio} = \delta^{44/42}\text{Ca} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{44}\text{Ca}/^{42}\text{Ca})_{\text{sample}}}{(^{44}\text{Ca}/^{42}\text{Ca})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

This isotopic profile is useful for paleodiet studies (Brown and Brown, 2011).

### H. Lead isotopes

The lead element is made of four stable isotopes ( $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$  and  $^{208}\text{Pb}$ ). The later three are radiogenic, being generated through the decay of uranium and thorium. Their natural abundance is 1'4, 24'1, 22'1 and 52'4%, respectively. One of the Pb isotopic signatures used is:

$$^{207}\text{Pb}/^{206}\text{Pb} \text{ ratio} = \delta^{207/206}\text{Pb} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{207}\text{Pb}/^{206}\text{Pb})_{\text{sample}}}{(^{207}\text{Pb}/^{206}\text{Pb})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The Pb isotopic profiles can be used to trace the environmental pollution by such metal and to trace the ores used in artifacts in archaeological studies, as well as to determine individual and population mobilities, being incorporated as environmental contaminants into bones and teeth (Brown and Brown, 2011).

### I. Selenium isotopes

The selenium element has six stable isotopes ( $^{74}\text{Se}$ ,  $^{76}\text{Se}$ ,  $^{77}\text{Se}$ ,  $^{78}\text{Se}$ ,  $^{80}\text{Se}$  and  $^{82}\text{Se}$ ), with natural abundances of 0'89, 9'37, 7'63, 23'77, 49'61 and 8'73, respectively. One of the isotope ratios used is:

$$^{82}\text{Se}/^{76}\text{Se} \text{ ratio} = \delta^{82/76}\text{Se} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{82}\text{Se}/^{76}\text{Se})_{\text{sample}}}{(^{82}\text{Se}/^{76}\text{Se})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The Se isotope transformation may occur through the changes in the redox state, making them useful tracers of redox processes in the ecosystems (Mitchell et al. 2012). They can be also used to reconstruct paleodiets, in a similar way than zinc isotopes.

### J. Zinc isotopes

The zinc element has five stable isotopes ( $^{64}\text{Zn}$ ,  $^{66}\text{Zn}$ ,  $^{67}\text{Zn}$ ,  $^{68}\text{Zn}$  and  $^{70}\text{Zn}$ ), with natural abundances of 48'63, 27'90, 4'10, 18'75 and 0'62%, respectively. One of the Zn isotope ratios used is:

$$^{66}\text{Zn}/^{64}\text{Zn} \text{ ratio} = \delta^{66/64}\text{Zn} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{66}\text{Zn}/^{64}\text{Zn})_{\text{sample}}}{(^{66}\text{Zn}/^{64}\text{Zn})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The Zn isotopic signatures can be used for dietary, biological and environmental studies, being a valuable biogeochemical tracer (Cloquet et al. 2008), as indicated for the Se stable isotopes.

### K. Potassium isotopes

The potassium element has two stable isotopes ( $^{39}\text{K}$  and  $^{41}\text{K}$ ) and a radioactive one ( $^{40}\text{K}$ ) with natural abundances of 93'3, 6'7 and 0'01%, respectively.

The isotopic ratio most used is:

$$^{40}\text{K}/^{39}\text{K} \text{ ratio} = \delta^{40/39}\text{K} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{40}\text{K}/^{39}\text{K})_{\text{sample}}}{(^{40}\text{K}/^{39}\text{K})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The K isotopic signature is used for geological dating, weathering and trophic studies

### L. Aluminium isotopes

The aluminium element has one stable isotope ( $^{27}\text{Al}$ ) and a one radioactive one ( $^{26}\text{Al}$ ) with natural abundances of almost 100% and trace amounts, respectively. The isotopic ratio of Al is:

$$^{26}\text{Al}/^{27}\text{Al} \text{ ratio} = \delta^{26/27}\text{Al} \text{ isotopic signature (‰)} = \left\{ \left[ \frac{(^{26}\text{Al}/^{27}\text{Al})_{\text{sample}}}{(^{26}\text{Al}/^{27}\text{Al})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The Al isotopic signature is used to study the surface-exposure ages and the erosion rates.

### Concluding remarks

The nature is made of chemical elements that exist as populations of isotopes that may be stable and radioactive. Such isotopes may be present at different proportions in the nature, and additionally, they may undergo inorganic or organic fractionation. Since there is technology to quantify such isotopes, it is therefore possible to determine the age of inorganic and organic material, as well as to infer paleodata related to diet, mobility and climate in paleoecological contexts.

*Acknowledgements.* Supported by “Ministerio de Economía y Competitividad” (AGL2010-17316); “Consejería de Agricultura y Pesca” of “Junta de Andalucía” (041/C/2007, 75/C/2009 and 56/C/2010); “Grupo PAI” (AGR-248); and “Universidad de Córdoba” (“Ayuda a Grupos”), Spain.

### References

- Arnay-De-La-Rosa M, Gonzalez-Reimers E, Yanes Y, Velasco-Vazquez J, Romanek CS, Noakes JE. 2010. Paleodietary analysis of the prehistoric population of the Canary Islands inferred from stable isotopes (carbon, nitrogen and hydrogen) in bone collagen. *J Archaeol Sci* 37(7): 1490-1501.
- Brown TA, Brown K. 2011. Biomolecular archaeology: an introduction. Wiley-Blackwell, Chichester, West Sussex ; Malden, MA.

- Cloquet C, Carignan J, Lehmann MF, Vanhaecke F. 2008. Variation in the isotopic composition of zinc in the natural environment and the use of zinc isotopes in biogeosciences: a review (vol 390, pg 451, 2008). *Anal Bioanal Chem* 392(1-2): 319-319.
- Crawford K, McDonald RA, Bearhop S. 2008. Applications of stable isotope techniques to the ecology of mammals. *Mammal Rev* 38(1): 87-107.
- Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Gálvez M, Sáiz J, Sánchez A, Vásquez VF. 2009. Ancient DNA to decipher the domestication of dog (REVIEW). *Archaeobios* 3: 127-132.
- Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P, Vásquez VF. 2010. Biological mass extinctions on planet Earth (REVIEW). *Archaeobios* 4: 53-64.
- Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P. 2011. Ancient nucleic acids from maize - A review. *Archaeobios* 5: 21-28.
- Dorado G, Vásquez V, Rey I, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P. 2008. Sequencing ancient and modern genomes (REVIEW). *Archaeobios* 2: 75-80.
- Dorado G, Vásquez V, Rey I, Vega JL. 2007. Archaeology meets Molecular Biology (REVIEW). *Archaeobios* 1: 1-2.
- Knudson KJ, Stojanowski CM. 2008. New directions in bioarchaeology: Recent contributions to the study of human social identities. *J Archaeol Res* 16(4): 397-432.
- Lee-Thorp JA. 2008. On Isotopes and Old Bones. *Archaeometry* 50: 925-950.
- Mitchell K, Mason PRD, Van Cappellen P, Johnson TM, Gill BC, Owens JD, Diaz J, Ingall ED, Reichart GJ, Lyons TW. 2012. Selenium as paleo-oceanographic proxy: A first assessment. *Geochim Cosmochim Acta* 89: 302-317.
- Osborne CP, Beerling DJ. 2006. Nature's green revolution: the remarkable evolutionary rise of C4 plants. *Philos Trans R Soc Lond B Biol Sci* 361(1465): 173-194.
- Reynard LM, Hedges REM. 2008. Stable hydrogen isotopes of bone collagen in palaeodietary and palaeoenvironmental reconstruction. *J Archaeol Sci* 35(7): 1934-1942.
- Richards MP, Trinkaus E. 2009. Out of Africa: modern human origins special feature: isotopic evidence for the diets of European Neanderthals and early modern humans. *Proc Natl Acad Sci U S A* 106(38): 16034-16039.
- Szpak P, White CD, Longstaffe FJ, Millaire J-F, Vásquez-Sánchez VF. 2013. Carbon and nitrogen isotopic survey of northern Peruvian plants: baselines for paleodietary and paleoecological studies. *PLoS ONE* 8(1): e53763. doi:10.1371/journal.pone.0053763 (in press).