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RECONSTRUCTING FIRE HISTORY FROM STALAGMITE ORGANIC COMPOUNDS: A HIGH RESOLUTION STUDY FROM NORTHWESTERN AUSTRALIA

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Contents

PREMISE

Wildfires represent one of the biggest issues that Australia has to face. These events occur pretty often, and the population is regularly alert about whether these dangerous situations will happen. Wildfires are enhanced especially when particular climatic conditions are present in the affected area. For this reason, gaining knowledge about past episodes, in terms of frequency and intensity of past bushfires, is a highly valuable tool both for understanding the climatic and environmental history of the area and learning how to predict and manage future events.

The present work aims to contribute to the investigation of these events by using a new and original approach in paleoresearch. The purpose of this dissertation is to determine whether fire-derived polycyclic aromatic hydrocarbons and *n*-alkanes can be preserved in speleothems, and particularly in stalagmites, and thus if they can be used as novel proxies for paleofires. Speleothems are very reliable in their high temporal resolution, enabling a record of fire events at annual scale.

Analytical chemistry is the tool of choice for this study; because of the low concentration of organic compounds present in speleothems, much effort was put in decreasing the possible contamination and increasing the analytical signal. This has been possible thanks to the rigorous analytical method applied in this study, and to the highly sensitive instruments used for the qualitative and quantitative analysis.

1 INTRODUCTION

1.1 Climate and climate archives

Planet Earth is and has always been a dynamic system. One of the elements that plays a fundamental role in the continuous transformation of our planet is the climate.

We can define climate as a wide range of weather factors, e.g. precipitation and temperature, which contribute to the conditions of a region, and climate is distinct from the weather for the time scale considered. While weather accounts for the fluctuations of the atmospheric conditions from hours to weeks, climate refers to long term changes, from years to millennia or longer. The close connection between climate and time is the reason why climate scientists are not just interested in what is happening in the present, e.g. warming of the oceans, atmosphere, and cryosphere, but also in reconstructing the climates of the past. It is tremendously important to gain a better understanding of past climate and especially the changes that occurred. Gaining this knowledge helps to contextualize the changes that are occurring right now and to predict future events.

Acquiring data and information about the climate of the past is not always an easy task. Which kind of components and phenomena do we need to examine if we want to reconstruct past climate conditions? How can we acquire data about climate in times when there were no human records or measuring instruments? These two questions are the basis of the discipline called paleoclimatology, which studies climate variations prior to instrumental recording. Humankind's regular collection of data only covers the last two-to-three centuries, while Earth's climate has been in an almost stable condition and has remained quite stable for at least the last 1000 years. Studies of ancient climates reveal long periods of ice in the Sahara Desert and vegetation at the poles [1]. This is why it is staggeringly important for us to better understand what occurred thousands and even millions of years ago. It is by understanding the causes of past climatic variations and through the integration of increasingly accurate models that we can predict future climate changes [1, 2]. In order to have a precise forecast, the model must be informed with the factors and the natural variability which act as a “background”. This background is provided by paleoclimatic studies [2]. Moreover, we know that changes in climate follow different temporal scales, from years to millions of years. Part of the reconstruction can be based on historical manuscripts that report the frequency and severity of volcanic eruptions, flood events, and winters, but these only span the last 7000 years (when writing was invented), and in a discontinuous way. Therefore, direct measurements or historical findings cannot provide a complete description and comprehension of the phenomena that influenced past climate [2]. For this reason, what we know about the climate system comes from indirect measurements and observations that help us in this difficult task of reconstructing the past, when Earth was very different from what we can observe today.

The knowledge about early Earth flows through evidence in climate archives that traces climate phenomena during different periods. It is fundamental to understand that each archive interacts with the climatic signal, and thus is affected by natural disturbance, e.g. wind, temperature, water, fire, etc., which have to be taken into account when reconstructing past events [1]. Paleoclimatologists use many different archives, which vary in spatial and temporal coverage, and in composition, and thus in how information is accumulated and stored. Examples of archives include tree rings, ice cores from glaciers, sediments, corals, and speleothems. In order to use an archive as a source of climate information, we must understand how the Earth's climatic history is recorded in each archive, what temporal resolution we can obtain from each, and the difficulties linked with the extraction of these climate data. One of the main factors that influences all the features stated above is the deposition mechanism, which varies among archives. Many factors, such as chemical reactions or erosion, may interfere and transform the climatic signal in a non-climate dependent signal; the archive is a complex system that interacts with the surrounding environment, and while the growth of the archive, e.g. a sedimentary deposition, proceeds, it can incorporate climate signals but also be affected by non-climate effects. Wave action that erodes the layers of marine sediments is an example of disturbances that cause the interruption of deposition, and thus a lack of information. Animals or microorganisms which interact with the sediments and change the chemical and physical composition, turning over the sediment or transforming the molecules that are aggregated in the sediment particles is another example. All these effects create a "noise" that may interfere with reconstructing the climate of the past. For this reason, specific methods are needed to enhance the quality and amount of information that these "natural time capsules" can provide [1, 2].

During the deposition mechanism, climatic signals can be stored and preserved under favorable conditions. These signals are called proxy indicators. A proxy is a preserved physical, chemical or biological characteristic of the environment that replaces a direct measurement [1, 3]. Therefore, a climate proxy can be used as a conserved indirect measurement of a climate-dependent phenomenon or climatic signal. The knowledge of the mechanism through which these signals are recorded by the proxy is essential to decipher the climate information. Biotic and geological-geochemical proxies are the proxies mostly used by paleoclimatologists. Fossil remains are widely used to reconstruct past climate and represent an example of biotic proxy. For example, plant fossil remains such as palm-like trees found at northern latitudes are used to infer warmer climate conditions. Another example is provided by pollen grains deposited in lake sediments; these can give information about the type of plants and the abundance of vegetation during periods of time. On the other hand, debris and moraines found in warm areas tell us about a cold past and the presence of ancient glaciers (geological proxy), or the isotope composition and distribution through the archive can provide data about the local ocean temperature (geochemical proxies) [1]. A different type of proxy is represented by biomarkers: molecules preserved in archives that provide information about the source which produced them. Polycyclic aromatic hydrocarbons (PAHs) are produced by fires and n-alkanes synthesized by plants are the two classes of biomarkers that will be used in the present work. A detailed description of the two proxies is provided later.

One of the major efforts that has to be made involves calibrating the archives. In the next section, a description of the main methods to calibrate the archives is provided, in particular those used in this study.

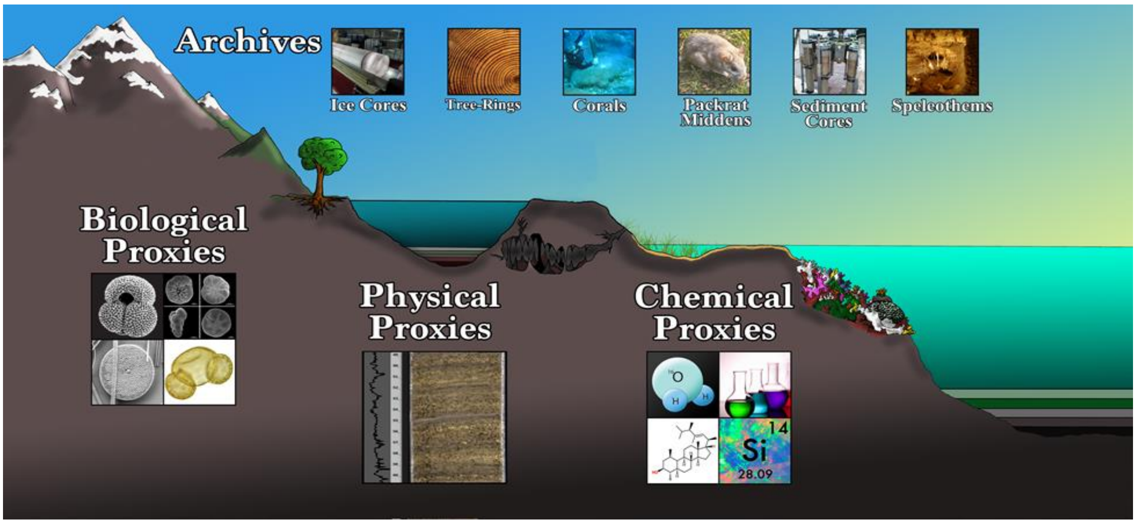


Figure 1.1 Main archives and proxies used for paleoenvironmental studies

1.1.1 How to date climate records

When a climatic signal is identified, it is necessary to match it with a time frame. This can be wide or narrow, depending on the resolution that can be obtained. The resolution depends on the archive and on the technique used.

As stated above, proxies are climate-dependent characteristics; a difference or a perturbance in the climate influences the proxy, causing it to vary according to the climate variation. In order to take advantage of these mechanisms, it is necessary to gain a deep knowledge of how the proxy is climate dependent. To achieve this, the first step is to assume that what climatologists call “the principle of uniformitarianism” is valid [2]. Briefly, this principle states that processes observed today and governed by natural laws occurred also in the past by following the same mechanisms. Therefore, all the studies about the climate of the past start from the knowledge of how natural phenomena act today. It works well for environmental processes acting in the past and that are still in action, but this is not always true. In order not to provide an inaccurate reconstruction of paleoclimate conditions, more than one proxy, as well as more than one calibration method, are used when possible.

Depending on the resolution provided by the method and the archive, we can have a numerical age or a relative age. The resolution is conditioned by two factors: the processes acting during the deposition and how the signal is recorded and protected from other disturbances [1]. For example, tree rings are usually annually resolved, provided that the tree was not cut, burnt or attacked by parasites causing a loss in the resolution and continuity of the information.

We can divide dating methods into four main categories: (i) radioisotopic methods, (ii) paleomagnetic methods, (iii) chemical methods and (iv) biological methods. A scheme that summarizes each method is shown at the end of this paragraph, whilst a thorough description is provided for the radioisotopic method, as it is the main technique used to date the speleothem archives.

DATING METHODS

- radioisotopic method
- paleomagnetic method
- chemical method
    - organic
    - inorganic
        - weathering rates
        - fingerprinting
- biological method

- growth rates
- growth layers

1.1.2 Radioisotopic method

The radioisotopic method relies on the radioactive decay of isotopes in the archives. An isotope is an atom of an element that differs in mass from other atoms of the same element. In fact, isotopes share the same number of protons, but vary in the number of neutrons. For this reason, they all react chemically in the same way, but they have a different weight. Nevertheless, mass-dependent differences occur in bond strength, and this is reflected in the amount of different isotopes that react, for example during evaporation of water molecules. In nature, we can find different percentages of isotopes of the same element. Usually, each element has a predominant and more stable isotope, which is found in higher percentages than the others. All the unstable isotopes decay spontaneously by losing nuclear α or β particles (radioactive decay) and are converted into a stable isotope of another element, e.g. ^{14}C decays to ^{14}N. The first isotope, the unstable one, is generally known as the parent isotope, while the transmuted one is called the daughter. The decay rate of the parent isotopes is invariant, and scientists take advantage of this knowledge for dating the archives. The rate of this decay is known as the decay constant (λ), but what is mostly used is the half-life $T_{1/2}$, i.e. the time taken for a radioactive material to decay to half of its original number of isotopes. The $T_{1/2}$ is related to λ through:

$$T_{1/2} = \frac{\ln 2}{\lambda}$$

In order to implement this method, we must know the initial concentration of the isotope and there must be a link between the event we want to date and the start of the decay process, an event that set the so called “clock”. In a nutshell, the archive must be a “closed system”, which means that the radioactive materials deposited cannot leave, and additional radioactive materials cannot enter. A good example is the basalt cooling: as this process happens very quickly, radioactive molecules are trapped inside and preserved. Therefore, the radioactive decay is not caused by external factors but occurs only internally.

Each parent isotope has a different half-life rate, ranging from long and slow decay, as uranium to lead, to short and fast decay, like potassium to argon. The decision about which one to choose depends on what we want to date and how far back in time. Rocks are usually dated with ^{238}U or ^{87}Rb as we want to track back the time of their formation (millions of years ago), whereas ^{14}C spans a shorter period (about 50,000 years) but can be referred to organic materials stored in fossils or remains [1].

Radioisotopic dating methods can be divided in three groups: (i) those which measure the amount of the isotopes present in the archive and consider them a fraction of the presumed initial level; (ii) those which use a comparison between the value of a local environmental process and a local radioactive process; (iii) finally, we have methods that study the equilibrium among members of a chain of radioactive decay, like the U/Th method. This last method is mainly used when the archives give information over the last few hundreds of thousand years, like ocean corals or stalactites and stalagmites in caves.

^{238}U decays in a series of several products, the main daughter isotope used for dating is ^{230}Th. The last product of the series is lead-206. The methodology is based on the incorporation of ^{234}U and ^{238}U in the archives, a substitute of calcium. U slowly decays and produces ^{230}Th, which in turn decays with a half-life of 75,000 years. The ratio between parent and daughter isotope will be constant, if a disturbance is not occurring. This highlights once more that the system must be closed, so any radioactive material unrelated to the event we want to date is left outside. Thanks to that, the daughter isotope produced by the parent can bring to the estimation of the amount of time elapsed [1, 2].

In order to use the U/Th method, the system has to be undisturbed and devoid of external ^{230}Th activity. As opposed to materials like shells, bones and corals, speleothems can be considered a closed system regarding U-series isotopes. In addition, ^{230}Th is relatively insoluble in surface waters, and so cave dripwaters can contribute, in some cases, extremely low abundances of Th to the speleothem [4]. The real issue is to measure accurately the initial 234U/238U ratio and to be sure that the sample was has not been contaminated by external ^{230}Th.

1.2 Speleothems

Speleothems, according to Moore [5] who introduced the term in 1952, are “secondary mineral deposits formed in a cave by a chemical reaction from a primary mineral in bedrock” [6]. Those deposits originate in caves made of limestone. They are known better as stalagmites and stalactites in their main depositional shapes. Stalagmites grow at the base of the caves; they are solid structures and increase their height in a continuous way at the drip site. Stalactites hang from the ceiling of caves and they often present an empty core, they grow around this central aperture and tend to be longer than larger, contrary to stalagmites [6].

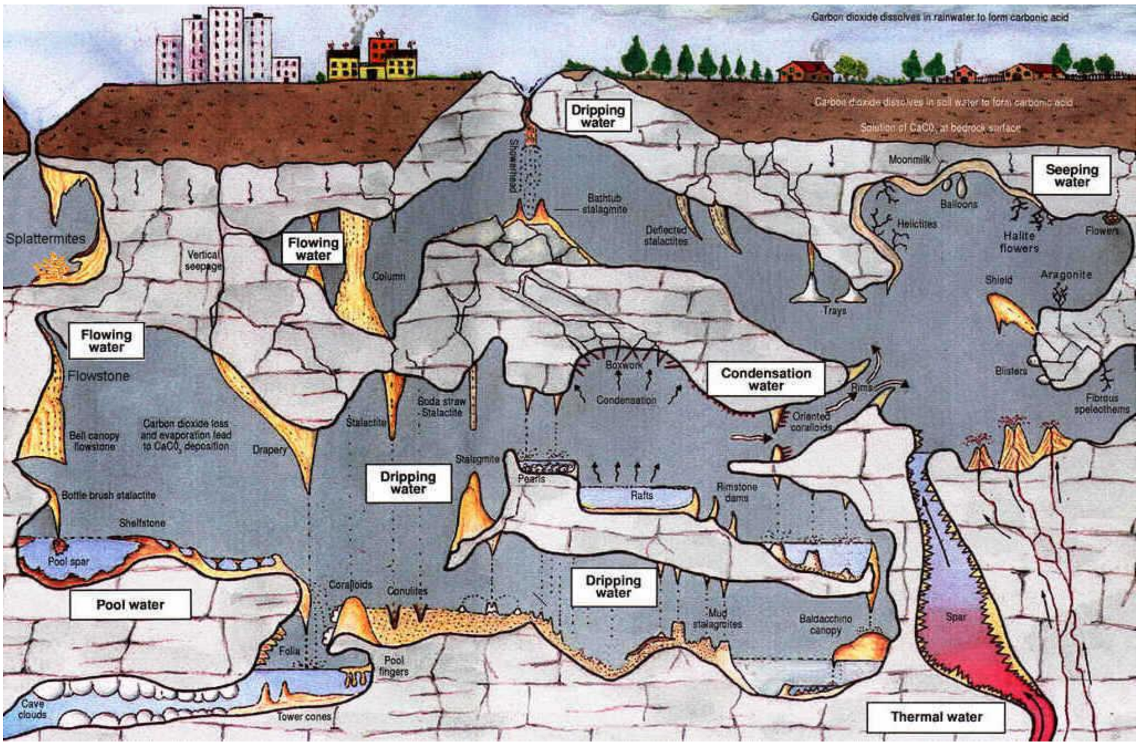


Figure 1.2 Idealized cave showing the main speleothems formation

Both are primarily made of calcium carbonate, in the form of calcite or aragonite. The elements that compose speleothems come from the groundwater, which percolates inside the carbonate host rock, dissolves it and then deposits in the cave. Speleothem growth occurs through two main phenomena: degassing of carbon dioxide from the water or evaporation. The degassing process is the most common because it can act in the entire cave, whereas evaporation takes place only only where significant air exchange allows for low humidity conditions to be maintained. The water that percolates through soil and the host rock tends to have a higher partial pressure of carbon dioxide than the cave atmosphere. This drives carbon dioxide to degas from water when it enters the cave, thus precipitating calcium carbonate (either calcite or aragonite) [2]. The deposition of calcium carbonate depends on many factors, e.g. chemical and geological, and a modification in any of these may cause the interruption of speleothem growth. Nonetheless, an undisturbed growth produces layers of calcium carbonate deposition. Major discontinuities can be easily identified from a change in the speleothem colors or from breaks and holes. Together with calcium carbonate, other materials can be found and can affect the speleothem color. One of them is uranium, generally present at 0.1-10 ppm in speleothem carbonate (the KNI stalagmites are composed of the mineral aragonite, which has much higher U abundances than calcite). As stated above, uranium is used for dating speleothems. In fact, uranium is soluble in shallow crustal waters and coprecipitates as uranyl carbonate with the calcite of speleothems. Moreover, cave dripwaters generally have very low ^{230}Th abundances, due mainly to the insolubility of the element in well-oxygenated waters [7, 8]. Dating the speleothems helps also to obtain information about the growth rate, which can vary from cave to cave [2, 9].

Figure 1.3 Example of stalagmites and stalactites in Cuevas del Drach, Mallorca (ES)

Before moving on to a detailed description of how speleothems are used for paleoclimatic purposes, a compendious outline of the environment where these archives grow and the phenomena who originate them is provided.

1.2.1 Caves and karst phenomena

The term cave encloses in its broadest sense every kind of underground void, both natural and artificial. The processes that contribute to the formation of these caverns are different, as well as the size, depth and conformations. If we focus on their speleogenesis, i.e. how these environments were formed, we can divide the caves in two main groups: primary caves and secondary caves. Primary caves are those whose formation occurs at the same time as the surrounding rocks: lava tubes, fractures in the rocks extended by gravitational forces are few examples of them.

Instead, secondary caves are formed mainly by the weathering of the rocks. Although it is true that this action can affect every kind of rock, there are some of them more likely to be eroded and therefore to generate caves, above all limestone [10]. These carbonate sedimentary rocks are made of CO_3^{2-}, and are water soluble, so the karst phenomena can take place. Through a cascade of equilibrium reactions, rainwater or groundwater, containing atmospheric CO_2, dissolves the calcium carbonate $CaCO_3$ that composes the limestone.

In its turn, $CaCO_3$ dissolves in bicarbonate and calcium, which is water-soluble. Organic acid can enhance the dissolution too. Therefore, more CO_2 can contribute to the more dissolution of $CaCO_3$. Usually, water is channeled in fractures, and the karst phenomena starts. Gradually, the fractures become larger and larger until the first void cave starts to grow. Sometimes caves can be flooded and water which invades the cave can bring sand and mud with it. While the cave is being formed, and the process can take millions of years, the above environment undergoes a change in the morphology, becoming a so called "karst" landscape, with typical formations like sinkholes and springs.

From an historical point of view, caves are part of our heritage, both in the past and in present-day. In fact, some of the most ancient evidence of human presence have been discovered in caves: the famous petroglyphs found inside the Cueva de las Manos or in the Lascaux caves come to mind when we think about prehistory. On the other hand, a lot of caves today attract many people for leisure activities and tourism, e.g. La Cuevas del Drach in Mallorca and Frasassi caves in Italy. Noteworthy, caves are also a habitat for many different animals, from roundworms and mollusks to mammals and amphibians [11].

Figure 1.4 Martel lake in Cuevas del Drach, is the biggest European underground lake

Figure 1.5 Cuevas de los Manos, Rìo Pinturas in Argentina

1.2.2 Speleothems as climate archives

In paleoclimate research, global and regional palaeotemperature records are very important. Nowadays, a conspicuous number of proxies have been discovered and tested, but the majority of them have been studied on ice-cores and oceanic sediments [12]. However, speleothems have some peculiar features, which allow them to be particularly appropriate for paleoenvironmental and climatic studies. Firstly, the environment where they naturally grow, the cave, is a specific environment where stalagmites and stalactites develop in a stable climatic situation. The archive is protected from weathering agents which tend to erode and consume rocks, e.g. wind. Usually light is totally absent in the deeper cave chambers and both humidity and temperature have very small variations through the year [8]. For this reason, speleothems growing in this zone are preferred for paleoclimatic studies.

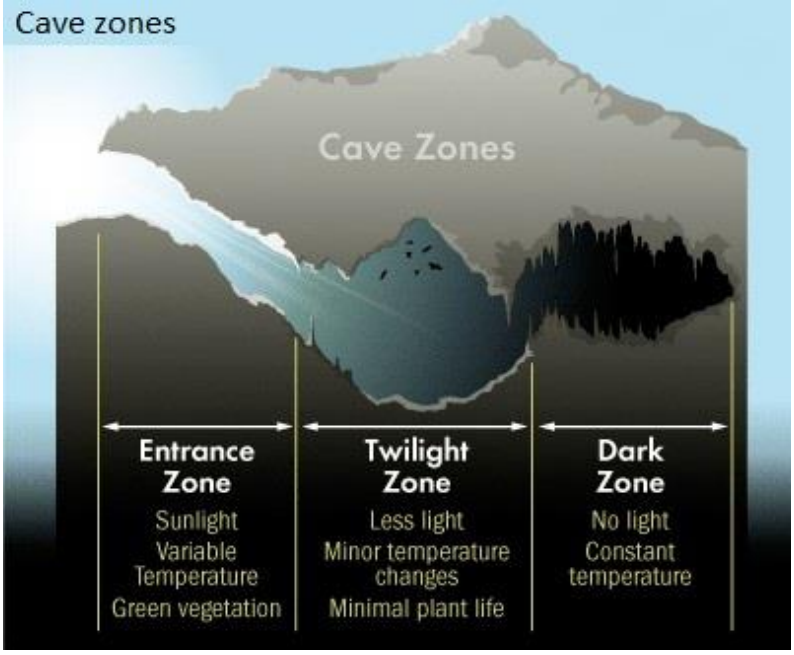


Figure 1.6 Cave zones: dark zone is the more climatic stable area

Secondly, once a speleothem is formed, it can be considered as a closed system itself, assuming it is not altered in its composition after deposition. The layer that is crystallizing will not interact with the previous layer, so what crystallizes with the calcium carbonate, including climatic proxies, is not affected by any other phenomena. For this reason, they do not face the common disturbances that other archives like sediments do, when a resuspension of particles occurs. With aragonite stalagmites, such as those used in this study, recrystallization to calcite can occur, but this is clearly evident under visual inspection.

As shown in fig. 1.7, the worldwide distribution of caves with the same formation and preservation features, together with the high resolution and the wide range of data obtainable by speleothems, may provide a powerful instrument to investigate not only local changes, but also global phenomena and climate oscillations [13]. This is well highlighted in literature, where some attempts can be found regarding the possibility to use speleothems for different geographical regions and environmental contexts [14].

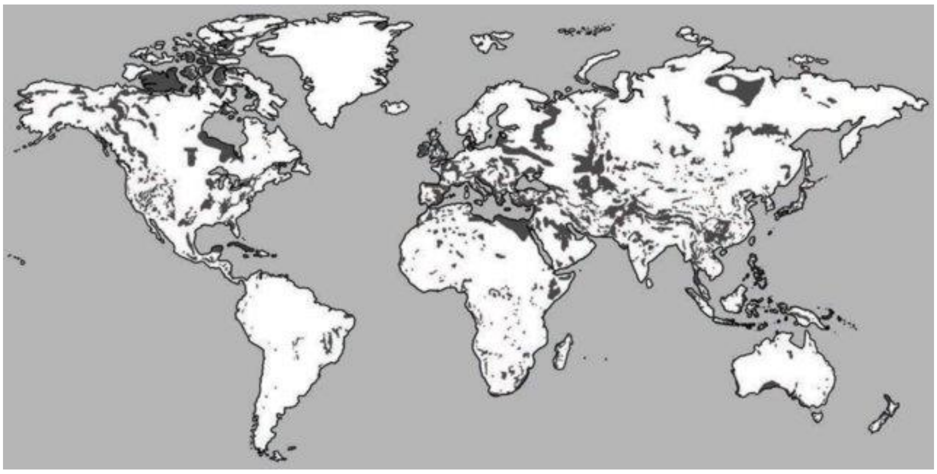


Figure 1.7 Karst regions distribution provided by "The Archaeology of Underwater Caves" by Peter B. Campbell.

Isotopic proxies

Focusing on the water which percolates through the limestone and then inside the cave, it is known that this water links the cave with the surface environment and carries proxies like oxygen, hydrogen and carbon inside the calcite or aragonite, which crystallize and retain the climatic information inside the speleothems [8]. Air and water inside the cave establish an equilibrium between the temperature of the bedrock and the temperature of the air, which corresponds to the mean annual surface temperature. By taking advantage of two well-known phenomena, i.e. the correlation between the oxygen isotopic composition in meteoric precipitation and the surface temperature, and the isotopic composition $\delta^{18}O$ temperature-dependent fractionation during the crystallization of the stalagmites or other speleothems, information about paleotemperature can be obtained. On the other hand, many other factors influence this equilibrium, such as evaporation, kinetic factors, isotopic composition in precipitation and changes in the seasonality like during the glacial/interglacial transitions [2, 7, 8]. In order to understand better what the $\delta^{18}O$ is and why it is important in our research, a more detailed description is provided.

Isotopic fractionation

The discovery that chemical and physical processes may cause isotopic fractionation can be dated back to 1925, when Briscoe and Robinson studied minerals collected from different places and discovered a variation in the atomic weight of the boron molecule [15]. Since that time, many investigations have been done to discover how natural processes can discriminate different isotopes of the same element. The first scientist who laid the foundation of paleotemperature studies using isotope fractionation was Harold Urey in 1947. In his work, Urey analyzed the ratio between ^{18}O and ^{16}O found in limestone and water of marine origin, together with the knowledge that temperature influences the oxygen isotope fractionation. If two molecules exist at the same temperature, their kinetic energies will equal, but their velocities (expressed as atom-atom interactions) will vary depending on their respective masses. These differences impact bond strength and thus drive mass-dependent isotopic fractionation. With these data, he recognized the possibility to reconstruct the ocean paleotemperatures from fossil shells [16].

An isotope fractionation can be described by studying the isotope composition or $\delta\ ^{0}/_{00}$. The formula to calculate it is the following:

$$\delta\ ^{0}/_{00} = \frac{R_{sample} - R_{standard}}{R_{standard}} 1000$$

Where R stands for isotope ratio and is calculated with the formula:

$$R = \frac{[Heavier\ isotope]}{[lighter\ isotope]}$$

An example of R is the $^{18}O/^{16}O$ in water. If we say that $\delta^{18}O$ is +5, it means that the sample is enriched by $5^{0}/_{00}$ in ^{18}O compared to the standard.

The natural isotope fractionation depends on isotopic exchange occurring during equilibrium reactions or on kinetic processes like evaporation.

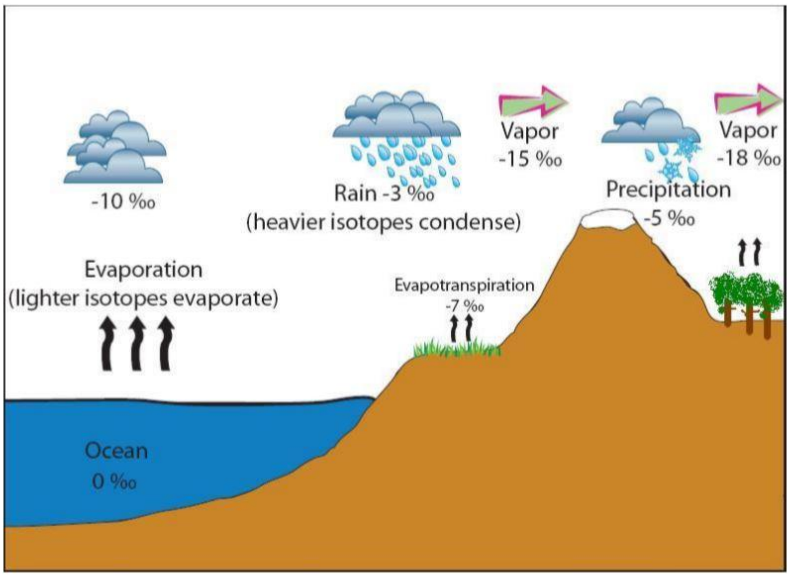


Figure 1.8 Water cycle and isotope fractionation

During evaporation, lighter isotopes tend to pass, thanks mainly to diffusion process, to vapor phase more than heavier isotopes, producing an impoverishment of heavier isotopes, like ^{18}O, in clouds. Temperature plays a key role in all these processes, so isotope fractionations are temperature dependent: this means that the difference of isotope composition between liquid and vapor phases will always be the same at the same temperature. $^{18}O/^{16}O$ in water vapor above the sea is significantly lower than the isotopic ratio in equilibrium. With the subsequent precipitation, heavier isotopes will condense more than lighter isotopes [17]. In 1964, Dansgaard found the relationship between $\delta^{18}O$ in precipitation and the mean annual temperature of some locations [18]. If we focus on rocks, ^{18}O in minerals are responsible for the isotopic fractionation. Crystal structure and type of chemical bond create a difference in concentrate the ^{18}O rather than the ^{16}O. Thanks to that, not only the temperature but also the water/rock interactions can be studied. Three mechanisms of isotopic exchange can take place: diffusion, chemical reaction, and precipitation from a solution [17]. $\delta^{18}O$ values in continental carbonate are used to estimate the $\delta^{18}O$ of the meteoric water from which they have precipitated. Carbonates can form at or near isotopic equilibrium with meteoric water. As the circulation of air in caves is poor, caves maintain a temperature that varies little and that approximates mean annual temperature. We already stated the two processes which contribute to the precipitation of calcite in a cave: if we focus on the outgassing of CO_2, studies proved that the carbonates precipitate in oxygen and carbon isotopic equilibrium and this permits to gain records of terrestrial climates [17].

Inorganic studies

Early research was focused on isotopes and paleotemperature reconstructions. While these first studies had difficulties in reconstructing climate [13], the improvement of techniques and instrumental equipment lead to meaningful results. Important results with the study of oxygen isotopes in speleothems provide a better comprehension of the link between insolation and paleomonsoons. Cave records can give information on a long-term record: one example is the study of the Hulu/Sanbao caves in China. This work has been important not only for the long-time interval that it covers (640,000 years) but also for the comparison with the Greenland and Antarctic ice cores, showing some similarities with these records. This confirms the possibility to use speleothem data in a wide range of applications, not only limited to the local area of study but also in different regions [19]. Studies conducted in different parts of the world, like Brazil [20, 21], China [22] and Australia [23], reflect the reliability and the repeatability of these researches, spanning from 2200 years study in the Australian cave to 640,000 years in the Chinese one. Data acquired by Denniston et al. [23] provide an interpretation of the influence of El Niño/Southern Oscillation, the primary driver of precipitation variability in northwestern

Australia, and the tropical cyclones variability, expanding the knowledge in the branch of paleoclimatology known as paleotempestology.

Regarding carbon isotopes, fewer studies have been conducted. Information is linked to the type and density of the vegetation present in the area surrounding the cave. In fact, the carbon isotopic ratios of CO_2 in soils reflect the variety of plants: isotopically lighter C_3 or heavier C_4 ones [24]. This is not always true with stalagmites [25]. As it is complicated to understand how the CO_2 signal varies in the system considered, due to a multitude of factors that influence the $\delta^{13}C$ changes in speleothems, the efforts have been focused on finding new $\delta^{13}C$ records in speleothems. For this reason, the study of Blyth et al. (2013) investigates this aspect, succeeding in finding complementary records to the CO_2, using organic compounds [26].

Together with carbon and oxygen isotope, other proxies less studied are deuterium in fluid inclusions [27], sulfur concentrations [28], and noble gases [29]. The studies of Gascoyne, M. (1983) [30] and Fernandez-Cortes et al. (2011) [31] investigate respectively the potential use of magnesium as a paleotemperature indicator and the correlation between manganese and rare earth elements found in stalactite layers with the dynamic hydrology of the cave.

Organic studies

Speleothems are a multi-proxy archive: the works of Fairchild et al. and Fleitmann et al. highlight this fact with a list of proxies extracted from stalagmites which provide reconstructions of temperature, rainfall, and other variations [32, 33]. In fact, not only inorganic compounds but also organic materials can be studied. Compounds like lipids, such as alkanes, fatty acids, and sterols have been used for describing vegetation change and microbiological activity, or PAHs as indicators of combustion and fires. Thus, multi-disciplinary studies can be conducted using the same stalagmite, ranging between different proxies and different environmental conditions. An example of that can be found in the work of Wynn et al. who compare speleothem record of atmospheric pollution studied with sulfur in stalagmites and tree rings [34].

The organic matter contained in the stalagmites was first considered only for describing the speleothem aspect, e.g. the color, and for inorganic analyses [35]. However, determination of organic compounds in speleothems is a pioneering field, and significant effort is put in identifying new potential molecular proxies.

Organic matter incorporated in speleothems comes from different sources, e.g. vegetation, soil that lies above the cave, fauna, and even bacteria. Organics enter the cave through different paths, for example transported by air or drip water, and thus are included or adsorbed in the crystal [36]. For this reason, stalagmites formed in deep cave environments are preferred for the study of organic matter because they are less exposed to potential contamination from the air. In this scenario, the organic compounds that reach the stalagmite come from plants and animal decomposition, or from the microorganisms living in the soil or at the surface of the stalagmite itself. It is extremely important to note that this organic matter transported by drip water is affected by the same chemical and physical processes as the soil above the cave [13, 37]. In general, understandings of these processes are insufficient to understand all the potential sources of organic matter in speleothems.

During recent decades, an increasing amount of studies have investigated organic compounds in stalagmites, in particular biomarkers [7, 25]. We can include in biomarkers compounds like sterols, alkanols, fatty acids, and alkanes. Biomarkers contain information not only about the climate but also about changes in the ecosystem and in microbiological activity [25]. The results from a study conducted on lipid biomarkers preserved in a north-west Scotland stalagmite show that some proxies, e.g. the chain length distribution in n-alkanes, can be compared among many different locations and applications (in the study Ethiopia and China), although being careful in interpretation as the resolution and the time scale play a key role in that [14].

Other relevant compounds that are under study are phenols, which give information about lignin precursors [38]. PAHs have been considered too, and shown to concentrate in stalagmites, although so far it was not clarified if they may represent a useful paleoenvironmental indicator [39]. All these analyses show that speleothems can have reasonable importance and potential in paleoenvironmental research. Studies have been carried out also for trying to avoid contaminants [36] or to enhance the possibility of finding trace elements [33]. This demonstrates that many other studies need to be implemented to face the difficulties in studying this peculiar archive.

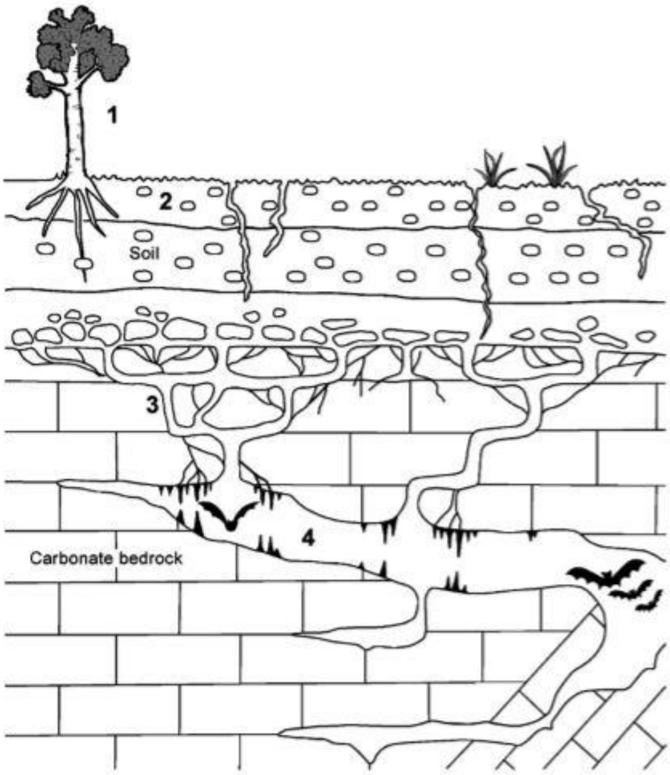


Figure 1.9 sources of organic compounds: 1 vegetation; 2 soil ecosystems;3 bacterial input; 4 cave fauna

Scientific studies applied to the organic material included in speleothems differ considerably from field to field [13]. An example could be the study of humic acids using luminescence. In fact, by running tests of luminescence spectrophotometry in stalagmites, the aromaticity and the molecular weight of humic acids can be determined, this is influenced in its turn by environmental factors like precipitations and vegetation [40]. The result shows a correlation between the soil and the speleothems. Evidence of a bond between trace metals and natural organic matter determine once more that there is an interrelationship that links the transport of organic compounds to the particles leached in the soil [41].

Another branch developed in the last decades is the lipid biomarkers analysis in speleothems: this class is composed of a wide range of molecular compounds, like sterols and alcohols, but also alkanes and polycyclic aromatic hydrocarbons (PAHs). Stalagmites, because of their characteristics, e.g. growth in a stable environment and link with the overlying soil, represent a good archive for this kind of studies, already implemented in other archives such as marine and terrestrial sediments and soils. According to Blyth et al. [13], a change in soil and cave fauna, as well as in the vegetation that lives in the soil above the cave can be recorded by lipids. Research can go further with specific molecular targets in order to identify distinct subgroups of microorganisms or plants, and they might also highlight the changes in the local environment, linked with the climate changes occurred in that region [13].

Amino acids are another type of molecular compound that can be used as proxies in speleothems, since they provide information regarding the date of carbonates produced by organisms. A research focuses on the racemization of the L amino acids in D amino acids, in order to track back the time of deposition. However, the interpretation can be rather ambiguous, due to the many sources of amino acids that can be found in the archive of interest [13].

What follows, before describing the specific area of study, is a brief summary of the proxies studied and the advantages and disadvantages of studying them in speleothems.

1.3 Proxies

Among organic compounds, the use of polycyclic aromatic hydrocarbons (PAHs) and n-alkanes as paleoenvironmental proxies in speleothems was the focus of this work. The aim of the present study was to test if the former (PAHs) can be used as a novel proxy in speleothems for paleo-fires, together with a reconstruction of the abundance and type of vegetation present provided by the latter (n-alkanes). This multi-proxy study will allow a more in-depth description and understanding of the fire-climate-environment dynamics and feedbacks at play during the last millennium in northwestern Australia, where the sampling site is located. As soon as a compound is originated - in our case by a fire event in the area or by deposition of plants remains - it is immediately subject to the action of several natural agents. Examples of these phenomena can be transportation, dissolution, degradation: any chemical, physical or even biological action can interfere with the structure of the molecule, and may transform it into another compound, thus causing the loss of the information carried by the original product and biases in the signal recorded [42]. Therefore, each biomarker needs to meet some particular features required to be suitable for paleoenvironmental research. In particular, two main criteria must be satisfied.

Firstly, a marker must be source-specific, i.e. the link between the source that has produced that molecule and the molecule itself has to be univocal, in order to correctly associate the entity of the signal to the intensity of the change recorded. Secondly, the molecule must be stable in the archive, therefore it has to be found intact and not subject to alteration during the time period under study. This principle comes under the name of conservative behavior [42].

1.3.1 Policyclic aromatic hyrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a family of organic compounds made of two or more aromatic rings fused together. They are generated as a mix of more than 100 different species from the incomplete burning of organic substances, e.g. tobacco, biomass, garbage, gas and coal. The main sources of PAHs today are volcanoes, fires, and exhaust from vehicles [43]. The chemical reactions that generate PAHs are the pyrosynthesis of fuel and the pyrolysis of the biomass in conditions of scarce oxygen availability [44]. They can be also created artificially in laboratories, for research purposes as isotope-marked compounds. The molecular weight of PAHs varies depending mainly on how many aromatic rings and substituents form the PAHs. Usually, they are divided into three categories: low, medium and high molecular weight PAHs. Studies have proved that the increase of the temperature during combustion stages generates higher molecular weight PAHs. Because of the tested toxicity and the increasing presence in the environment due to pollution and exhaust gas (the main anthropogenic source), 17 of these compounds are of high relevance for health and environmental studies [45].

PAHs are even used in today’s society to produce pesticides and plastics, and some of them are included in asphalted roads.

Virtually every environment on earth may contain traces of PAHs, as they tend to be adsorbed on atmospheric particulates and therefore can travel for long distances in the air, be deposited on water bodies and soil, and finally be accumulated in sediments and other archives as speleothems.

As markers of combustion, they are persistent and stable in the environment thanks to their chemical structure, giving scientists the opportunity to use them as markers of fires. As stated above, the higher the temperature of combustion, the more aromatic rings are linked together, thus knowing the type of PAHs and their relative concentration may inform on the temperature and the intensity of fires [46].

PAHs match one of the two principles of the ideal marker: they are very persistent in the environment, and PAHs deposited in archives older than the industrial revolution age most likely originate from biomass burning, and not from chimneys and exhausted gases or oil burn.

Most of the times PAHs are studied together with other markers and proxies, in order to aggregate the information acquired about fires and even climatic events such as seasonality and even human presence in older times [47].

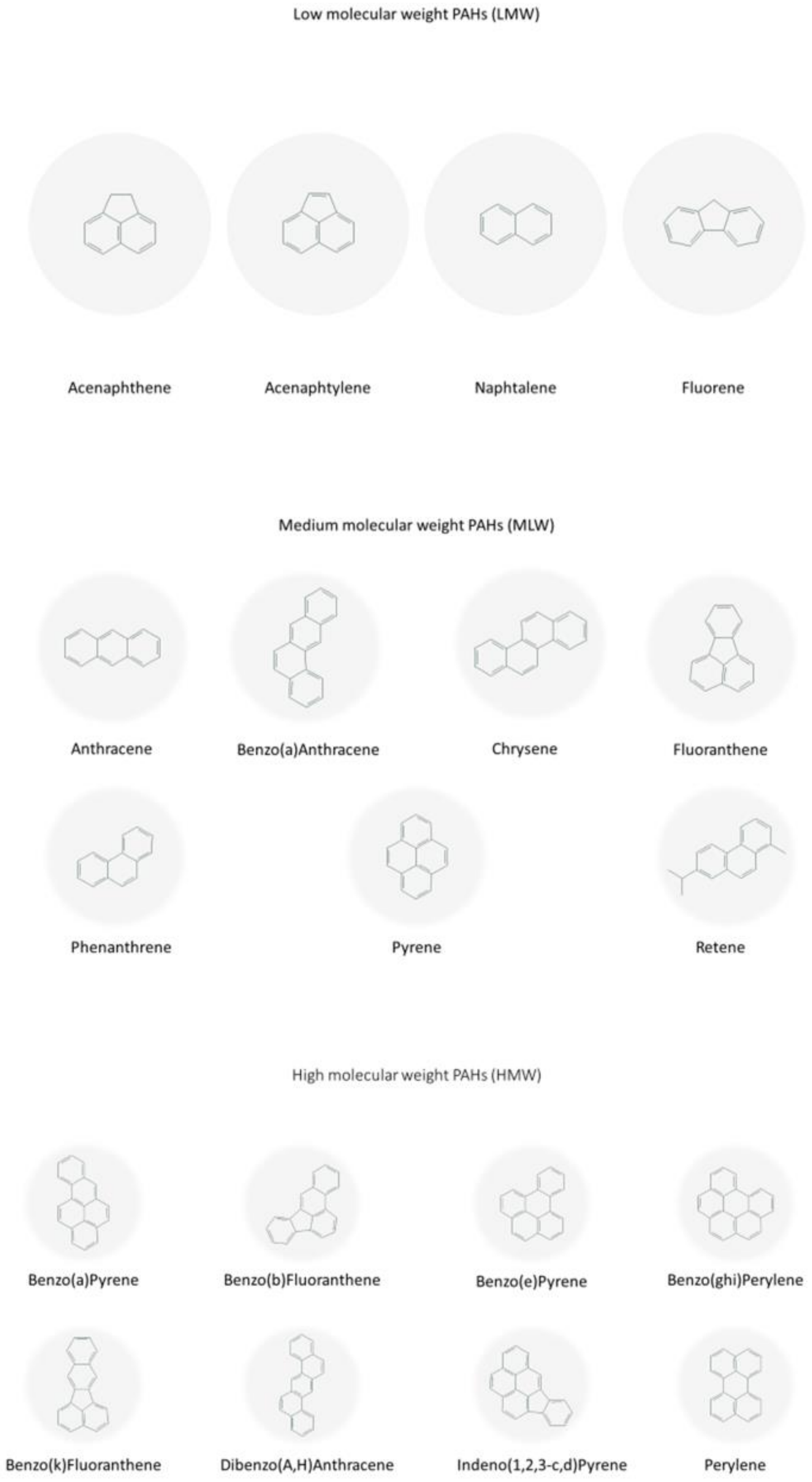


Figure 1.10 PAHs analyzed in this study, elaborated from https://pubchem.ncbi.nlm.nih.gov/

1.3.2 *n*-Alkanes

n-Alkanes are a class of the aliphatic hydrocarbons, made of carbon and hydrogen. All the bonds between carbon atoms are single, and for this reason they are called saturated hydrocarbons [47]. Alkanes differ in the number of carbon atoms linked together, from the simplest with only one carbon atom (methane) to even 50 (pentacontane). The term *n*-alkanes refers to straight-chain alkanes, where *n* stands for normal. The main chemical property of alkanes is their almost total absence of polarity, making them non-polar compounds. The boiling temperatures of alkanes are the lowest ones if compared to compounds of the same molecular weight, and all the alkanes have a lower density than water, making them float on it. Alkanes with one to four carbon atoms are gases at ambient temperature, liquid from five to seventeen carbon atoms. Alkanes having nine or more carbon atoms are called higher alkanes, and among this group are the waxes. Waxes are solid at ambient temperature and are produced naturally by plants to create the cuticle and epicuticular wax, a hydrophobic shield. Alkanes produced by plants are most of the times odd-numbered [48]. Regarding the reactivity of alkanes, they are inactive to almost all reagents. An exception is the combustion operated by oxygen, which explains their enormous use as combustibles [43]. In nature, some microorganisms know how to use alkanes as carbon and energy sources [49].

n-Alkanes are used as proxies for their capacity to well resist degradation during transport and deposition, reaching the archives almost unchanged. In speleothems, high molecular weight alkanes may indicate the presence of higher plants and leaf waxes [50], whereas low molecular weight alkanes represent fungi, algae and even bacteria presence, without excluding an alteration or degradation mediated by those microorganisms [51].

Anthropogenic land use can be seen from a change in the composition of high molecular weight *n*-alkanes, when surrounding areas start to be used as agricultural fields [25].

1.4 Wildfires

Humans have a special relation with fire: we link the use and control of fire introduced by first groups of humans with the enhancement of our civilizations, leading to settlements, technological development and more food availability, together with land management. However, natural fires were and still are a common presence on Earth, from the very first fires appearing on land surface at least 420 million years ago to the increase in the frequency of fire events due to oxygen increment in the atmosphere, caused in turn by the spread of vegetation on Earth. As plants are a good fuel, and natural phenomena like volcanic eruptions and lighting can initiate wildfires, it is not difficult to assume that wildfire events were very common in the past, even before humans learned how to harness fire [52]. Wildfires create also what it is known as fire ecology, i.e. plants are adapted and utilize the seasonality of fire events for their life cycle. Fire can pave the way to pioneer species, plants that are able to colonize environments barely unsuitable for life, like post-fire areas. The study of the reproductive cycle in obligate seeders as Proteaceae in Australia shows that some species have a higher ratio of reproductive outputs (confructescences or follicles) during short inter-fire intervals [53]. Fire plays also a key role in the ecological succession, creating the starting point of what is known as secondary ecological succession.

Sedimentary charcoal is a well-established proxy to reconstruct paleofires [54]. PAHs can be used as well, as they represent fire tracers of plants combustion and fossil fuel. If non alkylated PAHs are found in samples, they originated via plant combustion, where on the other hand fossil fuel combustion is more represented by alkylated PAHs [56].

1.5 Geographic and climatic framework

Australia is the sixth-largest country in the world (7,692,030 km^2), with a population of 25 million people, most of them living along the coast. Australia lies entirely in the Southern Hemisphere, between 10°41’S (Cape York) and 43°39’S (South East Cape, Tasmania) of latitude and between 113°09’E (Steep Point, Western Australia) and 153°39’ E (Cape Byron, New South Wales) [57]. For this reason, seasons are the opposite of the Northern Hemisphere. Its name came from the Latin words “terra australis incognita”, literally “unknown southern land”, where southern refers to south of the equator. This says something about the knowledge of this immense and vast area before the age of its exploration when the British explorer Thomas Cook (1770) colonized for the British crown the continent. Matthew Flinders first circumnavigated Australia between 1801 and 1802, making the name of this land become popular in the world. However, Australia was already settled since pre-historic times by the ancestors of what are today called Aborigines (ca. 45-50 ka) and who were able to use fire for a variety of reasons [58].

From a geographic point of view, Australia refers not only to the main island, called Mainland but also to a myriad of minor islands, all surrounded by the oceans. The most famous islands are Tasmania, Christmas Island, and Norfolk Island. Almost the entire continent was formed during the Precambrian Eon. The continent has been divided into three parts, and one of them, the Western Plateau, is the oldest part. It has existed for over 500 million years ago. The Kimberley region is located in this part [57, 59]. The Australian continent has been cut off from other continental masses for millions of years. Plant and animal life evolved in a unique variety of species, unlike any other continent. A glaciated Australia in the Permian (290 million years ago), followed by the melting and eruptions of volcanoes are the main contributors to the present topography. The territory is mostly flat, with the highest mountain located on the Main Range of the Snowy Mountains (Mount Kosciuszko, 2,228 meters) [57].

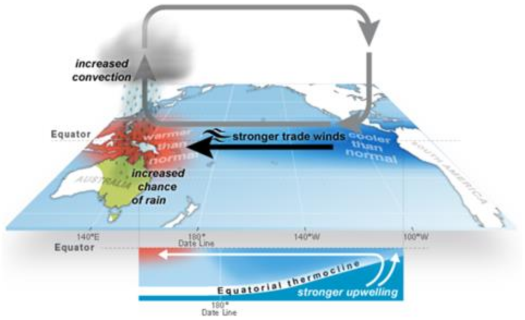
The climate of Australia varies significantly over the whole of Mainland. Because Australia covers a large range of climate zones, it has different types of climatic regions [57]. A climate zone is a region of the world that experience similar temperature and rainfall. Most of the continent is arid and desertic, and only south-east and south-west areas have a temperate climate. This is why most of the Australian population live in those regions. The temperature fluctuations can be large (from 50 °C to below zero), but thanks to the warm surrounding oceans and the lack of high mountains the minimum temperatures are somewhat mild compared to other continents [57]. Since the Tropic of Capricorn passes through the middle of the continent, it comes under the influence of the Trade Winds [57]. The south-west and the south-east parts of the continent are influenced by the Trade Winds in the summer and the Westerlies in the winter due to seasonal shifting of the pressure belts. In this part of the island, summers are hot and dry, winters are cold and wet. The direction of mountain ranges affects the climate too. The Eastern Highlands, running parallel to the east coast, act as a barrier to the moisture-laden Trade Winds, blowing from the south-east. Therefore, the east coast receives plenty of rainfall, whereas on the leeward side there is hardly any rain.

The northern part of Australia is closer to the equator and therefore hotter than the southern part. The northern territory presents a tropical climate, and the monsoons operate here. This area has alternate wet and dry seasons, as the tropical rain belt shifts south and north of the equator. Hot summers create low pressure over the land, attracting Monsoon Winds from the ocean. Summers are usually hot and wet, while winters are cold and dry. Rainfall is unreliable and varies from year to year. Tropical Deciduous Forests thrive in its climate. Trees like bamboo, palms, eucalyptus are commonly seen. South of the region with monsoon type of climate, summers are hot and moist, as it is the season for rains, while winters are cool and dry: the sub-tropical zone. In this region, the northern part has Savannah woodlands and as rain decreases, the woodlands give way to the Savannah grass, consisting mainly of eucalyptus. The whole of the Western Plateau is covered by the Tropical Desert. This is where the three major Australian deserts are ubicated: the Victoria Desert, the Gibson Desert, and the Sandy Desert. The region receives less than 25 cm of annual rainfall. Adapted to withstand long droughts, plants like spinifex or porcupine grass can be found here.

Western Australia: the monsoons and ENSO

Western Australia is a 2,529,880 km^2 area, with the Indian Ocean and Timor Sea around its coasts. The average altitude is 400 m above sea level, with the Kimberley Plateau in the far north, delimited by the two rivers Fitzroy and Ord [59]. The other rivers present in the area are active as torrents during the monsoon period, whereas they are dry for the rest of the year. The climate in this region is strongly influenced by the oceans around it and the atmosphere above it. During the winter period, from May to October, anticyclones coming from west territories move to the eastern territories. For this reason, western Australia experiences dry and cool winds; in the summer period (from November to April), the situation is reversed, with anticyclones moving from west to east. In this period of the year the hot rainy season occurs in the northern regions, due to the moist monsoonal air [57, 59]. Tropical cyclones are phenomena that can be seen in north coasts on an average of three cyclones per season, producing heavy rain and strong winds. The monsoons are referred to as a seasonal wind that generates conspicuous precipitations in a region. The Australian monsoons are a consequence of summertime higher heating of the land than the surrounding oceans, setting a sea breeze circulation. This attracts moisture from the oceans to the land, creating a monsoon trough [57, 60]. Between the monsoon bursts, isolated rainfall events may occur. Monsoon is the biggest contributor to the annual rainfall in northern Australia [60].

Another climate driver that influences the northern territory is the cycle of what is called El Niño-Southern Oscillation (ENSO). This oscillation impacts also on monsoonal variability [61]. ENSO shifts between phases, which sometimes can last for more than one year. On one hand, extreme years of drought conditions are called “El Niño” years, on the other hand, years of extremely wet conditions are called La Niña years. “Neutral” years lie in between, and do not suffer extreme climate [62]. This complex phenomenon and both El Niño and La Niña are caused by sea surface temperatures of the Pacific Ocean, deep and surface currents, atmospheric circulation of winds and clouds and the interaction between all these factors and the landmasses [57]. ENSO can produce extremes in the Australian climate, like floods or droughts. In a nutshell, during the La Niña phase, the temperature is usually lower, flood events are more widespread and more tropical cyclones may arrive. During the El Niño phase, the opposite conditions occur: higher temperatures lead to drought events and heatwaves [63]. The intensity of ENSO is measured with the so-called Southern Oscillation Index (SOI) [57]. The study and the attempts of forecasting ENSO are of vital importance in Australia, as it affects not only the daily life of Australians, e.g. rural productivity and economical devastating droughts but also environmental factors like wildfires [63].



El Niño–Southern Oscillation (ENSO): **La Niña**

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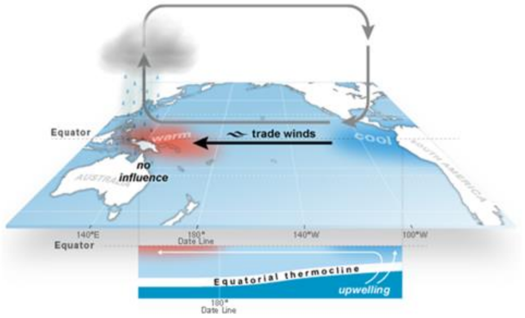

El Niño–Southern Oscillation (ENSO): **Neutral**

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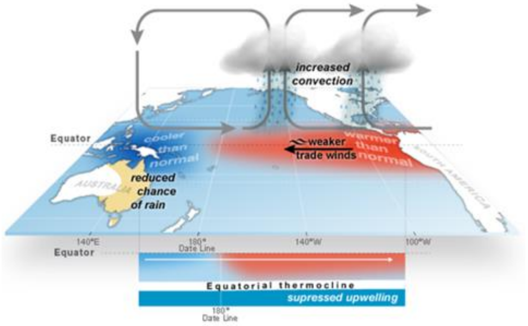

El Niño–Southern Oscillation (ENSO): **El Niño**

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Figure 1.11 ENSO phases; source: Australian Bureau of Meteorology

Figure 1.12 Distribution of fire seasons in Australia (source: Bureau of Meteorology)

As stated above, wildfires are common events in some parts of the Australian continent: the most affected district is the Kimberley region, where the cave KNI-51 under study is ubicated. One of the most tragic events in recent history is the Ash Wednesday bushfire, which caused the disappearence of 500,000 hectares in southeastern Australia (1983) [53]. The phenomenon is so common that some species of plants in Australia have adapted to these events, having a smoke-stimulated germination [64]. Some plants produce flammable litter and oils that increase the possibility of a wildfire, precisely because they need fires for their natural processes [65]. Studies highlight both the importance and the risk associated with wildfires. Murphy et al. (2010) demonstrate that too many fire events can be harmful to some plant communities [66], whereas a study conducted by Stephenson et al. (2013) shows that bushfires act as an environmental service [67]. These events, that can be rare in other parts of the world, are very well-known in Australia. The study of Dutta et al. (2016) demonstrates the increase in the frequency of bushfires in the last decade [68], and the Bureau of Meteorology of the Australian Government has implemented a fire weather service to inform the population who live close to a risky area about the possibility of these dangerous events to happen [59]. Even prehistorical Aborigines were aware of wildfires and with their practice called "fire-stick farming" and "patch mosaic burning" contributed also to the reduction of wildfires [69] and to the management of the region [65]. Weather conditions influence wildfires, and several reasons are connected to the higher possibility that wildfires take place in this region. Wildfires activity is different throughout the year, depending on the presence and on the intensity of the diverse "fire drivers". In the next paragraph, possible fire drivers are indicated.

Firstly, wildfires in the northwestern Australia appear mainly during the dry season (winter-spring period). Low humidity, hot temperatures in sunny and clear days, as well as drought conditions can increase the possibility for vegetation to burn. The Kimberley district is considered the hottest part of Australia, suffering heat waves during summer, although the driest conditions occur during winter (June-August) because of the absence of monsoons [58]. Even though wet periods decrease the likelihood of wildfires, it is true that the increase in vegetation abundance during the rainfall period can increase the risk of future bushfires, when the dry season arrives [70]. ENSO can stress these conditions and increase the possibility of fires, especially during the El Niño phase. Another important driver is wind: the high-pressure systems over South Australia makes winds blow from southeast to northeast [57]. Winds can enhance the fire in two ways: by spreading fire across the dry vegetation in the zone and by moving hot embers in the surrounding areas, creating new potential spots for wildfires [71]. Air movements can also modify the structure of existing fires. Lightning is an additional driver: the number of lightning strikes in the region caused by thunderstorms affects the possibility to ignite a fire. A poorly understood phenomenon that actually boosts the propagation of wildfires is the pyrocumulonimbus firestorm dynamic. This auto-created thunderstorm generated by large fires can change the behavior of bushfires and self-sustaining fire [72]. Finally, minor fire drivers are to be found in temperature inversions, smoke produced by a fire and the increasing presence of invasive species, as well as climate change [65, 70].

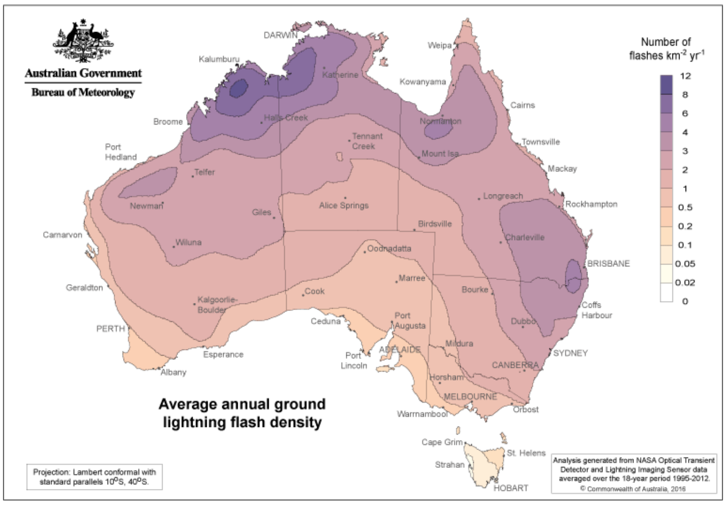


Figure 1.13 annual lighting flash density, Bureau of meteorology

1.5.1 Cave KNI-51

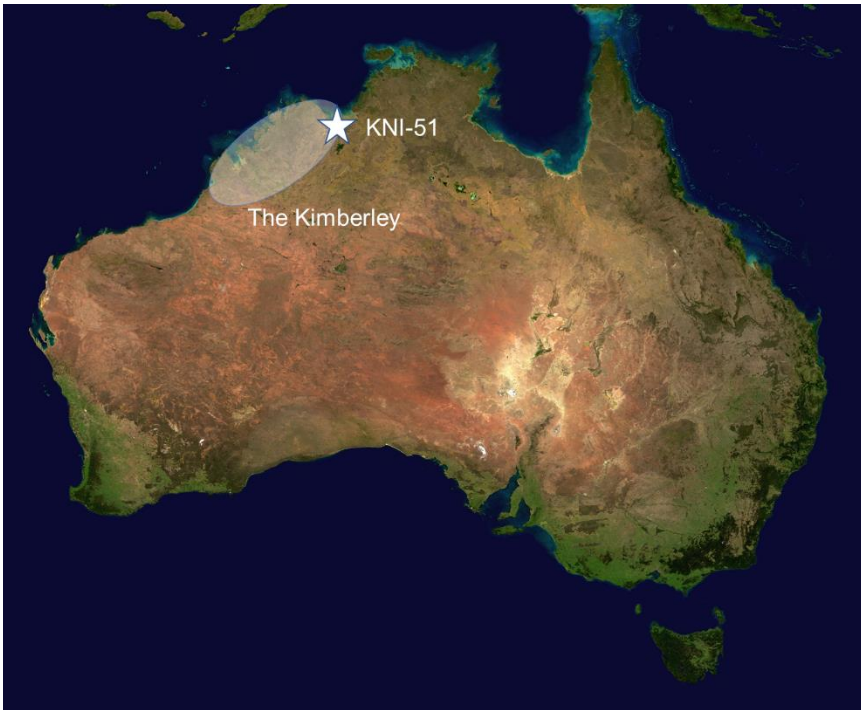


Figure 1.14 Location of the Kimberley region and of the Cave KNI 51; Source: Rhawn Denniston, personal communication

The stalagmite KNI-51 F comes from cave KNI-51, located in the Kimberley region, in northwestern Australia (15° 18’ S, 128° 37’ E), 100 meters above the sea level. The cave is not deep (from 10 to 15 meters) and is 600 m long [73]. There is one entrance and it is developed almost horizontally. It is composed of Devonian Ningbing Limestone, which allows the formation of speleothems inside it. The stalagmites growing in this cave are mainly composed of aragonite, but calcite is present in some contemporaneous stalagmites, too [74]. Stalagmite KNI-51 F was collected around 100 m from the far end of the passage. The average growth rate of this stalagmite is 1.2 mm yr^{-1}. Close to the cave (40 km south of it), the Kununurra Research Station provides the following information: mean annual maximum temperature: 35.1 °C; mean annual minimum temperature: 20.7°C. The highest amount of annual rainfall is 1564 mm, the lowest 424 mm, whereas the mean annual rainfall is 838 mm. [73].

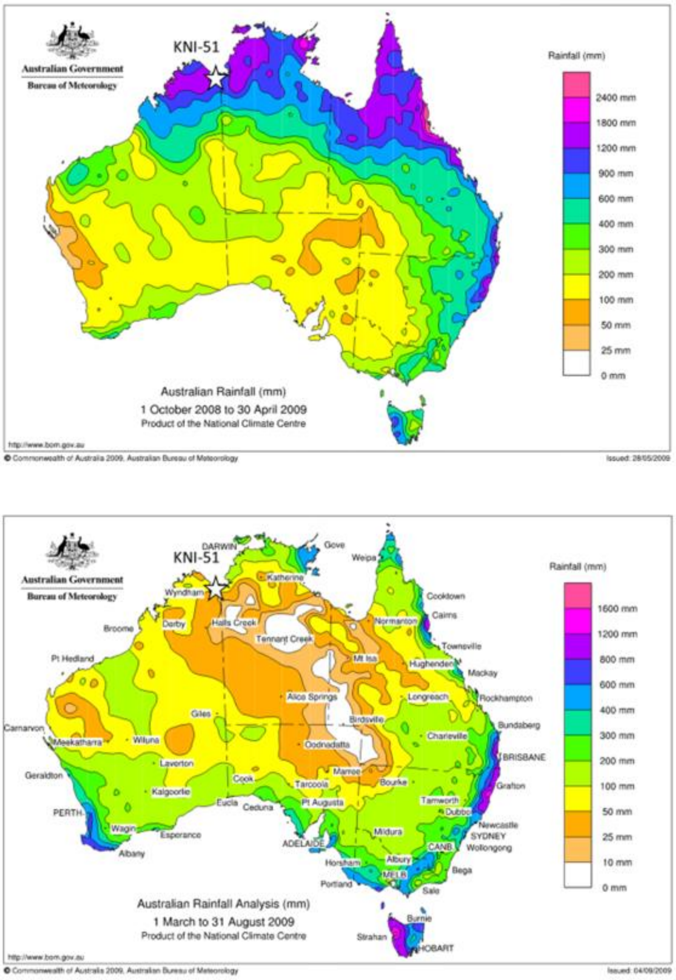
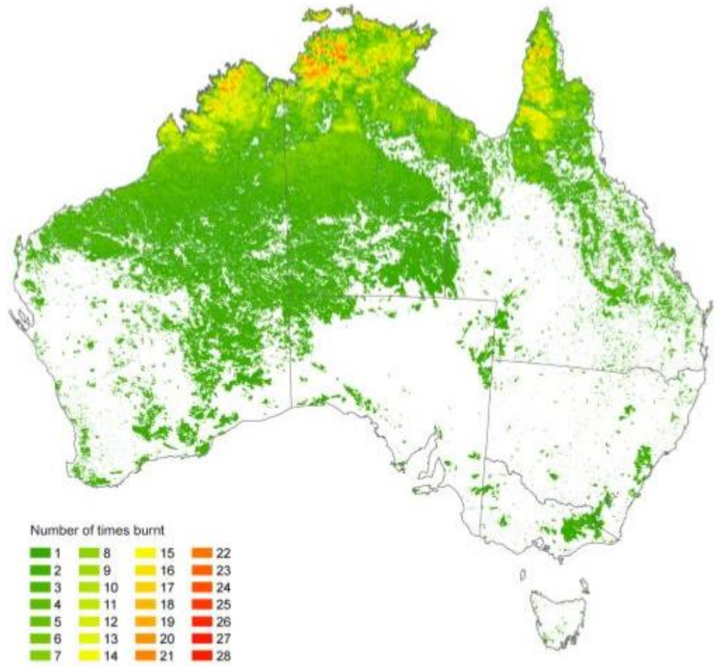


Figure 1.15 Australia Rainfall (mm) from 1 October 2008 to 30 April 2009, and from 1 March 2009 to 31 August 2009; Source: Rhawn Denniston, personal communication

KNI-51 is part of a cave system in the surrounding hills, all of them with the same characteristics, that is small entrances and not very deep caves [73]. One important feature for our study is the thickness of the above soil, which is very thin. This involves rainfall percolating in the soil for a small amount of time, and the organic compounds percolate too, depositing in the speleothems of the cave. It is estimated that the percolation time from the above bedrock is less than two months [73].

Figure 1.16 Interior room of the cave KNI 51; Source: Rhawn Denniston, personal communication

In the Kimberley region, the wooded savanna is composed mainly of spinifex and baobab trees. Eucalyptus and Acacia species can be found too. This area is subject to widespread fires during the dry season. These fires include natural ones, management practices and sometimes even incendiarism events caused by arsonists [59]. The vegetation around the cave is mainly composed by eucalyptus, with a savanna pattern [73].



Source: © Western Australian Land Information Authority (Landgate) 2016, used under CC BY 3.0

Figure LAN4 **National fire return frequency for Australia, 1988–2015**

Figure 1.17 Frequencies of burn events, Western Australian Land Information Authority

2. MATERIALS AND METHODS

2.1 Reagents and standards

The following products described in this section were used through the entire work procedures. Pesticide grade solvents SpS (*n*-hexane, dichloromethane, dichloromethane Ups and 2,2,4, trimethylpentane) were from Romil Ltd., (Cambridge, UK). Other solvents employed for decontamination or as reagents were SpA Hydrochloric acid 34-37%, acetone, methanol 205 from Romil Ltd, anhydrous ACS Na2So4, ≥99% was from Sigma Aldrich (Saint Louis, USA). Na2So4 was oven-dried at 150 ^{o}C for 24 h, washed with both DCM and n-hexane and stored with n-hexane.

Internal standard solutions adopted in this study were isotope-labeled compounds ($^{13}C_6$-acenaphtylene, $^{13}C_6$-phenanthrene, $^{13}C_4$-benzo(a)pyrene from Cambridge Isotope Laboratories and hexatriacontane from Sigma Aldrich).

Native compounds were obtained from Dr. Ehrenstorfer (Augsburg, DE) for the PAH Mix-9, while the solution of n-alkanes C10-C35 and hexatriacontane were acquired from Sigma Aldrich.

2.2 Instrumental equipment

2.2.1 Cleanroom

All the pre-analytical phases, except for the sub-sampling, were conducted in a class 10,000 organic cleanroom laboratory (ISO 7, <10,000 particles/m^3) at the Department of

Environmental Sciences, Informatics and Statistics of Ca’ Foscari University. The cleanroom is a delimited environment where contamination, temperature, and pressure are controlled, entirely built-in stainless steel to avoid any source of contamination from organic-based materials. It is therefore built and used in order to minimize the introduction, the production and the accumulation of atmospheric aerosol through a high-performance air filtration system. Air follows a vertical path from the ceiling to the floor, in a laminar flow. Air vortexes and streams are thus avoided, in order to prevent sample contamination.

Notwithstanding this, the users of the cleanroom must follow operating procedures and protocols in order not to compromise the cleanliness of the laboratory. For example, the use of a special lab coat and clean footwear is mandatory in order to operate inside a cleanroom.

Figure 2.1 Clean room used for the analysis

2.2.2 Turbovap II

Turbovap II (Caliper Life Science, Hopkinton, CA, USA) was used inside the cleanroom to reduce the volume of sample extracts. Samples (up to six) are put into glass tubes and held at constant temperature in a thermostatic bath. A gentle stream of nitrogen gas flows inside the glass tube, creating a helical flow: this trajectory produces a vortex, which enhances the homogeneity of the solvent evaporation. Optical sensors allow the system to stop the concentration at a final volume of 500 µL.

2.2.3 Gas chromatography – mass spectrometry (GC-MS and GC-MS/MS)

The analyses of *n*-alkanes and PAHs were performed by gas chromatography coupled to single quadrupole and triple quadrupole mass spectrometry, respectively.

The first instrument, used for the analysis of *n*-alkanes, is an Agilent Technologies 7890A GC system coupled to an Agilent Technologies 5975C single-quadrupole mass spectrometer, while the second, employed for PAHs, is a Thermo Fisher Scientific TRACE 1310 gas chromatography coupled to a Thermo Fisher Scientific TSQ 9000 Triple Quadrupole mass spectrometer. The combination of gas chromatographic separation and mass spectrometric analysis allows the identification of compounds with low detection limits with good quantitative performances. Compounds are volatilized in the GC injector and enter a capillary column coated with a polymeric stationary phase, where they are separated according to the temperature, which increases gradually following a specific program (ramp). Depending on their retention time, compounds enter the mass analyzer at different times and are fragmented by an electron impact source. Ions, positively charged, are forced through the quadrupole, which allows detection and minimizes interferences by selecting characteristic mass/charge ratios. The signal is then amplified by an electron multiplier and converted in a chromatogram, where each compound results in a peak, univocally identified by its retention time and m/z [75].

In the triple quadrupole mass spectrometer two mass analyzers are present, the first and the third quadrupole, separated by a quadrupole in total transmission filled with an inert gas (argon): the collision cell. Fragments selected in the first quadrupole are fragmented again and can be analyzed by the third quadrupole, resulting in a better qualitative analysis and good quantitative detection. This enhancement is achieved with a double identification for the selected analytes, taking advantage of the major specificity that fragments produced in the collision cell have with the compound studied.

Figure 2.2 Left: Agilent Technologies 7890A GC system coupled to an Agilent Technologies 5975C single-quadrupole mass spectrometer; Right: Thermo Fisher Scientific TRACE 1310 gas chromatography coupled to a Thermo Fisher Scientific TSQ 9000 Triple Quadrupole mass spectrometer.

2.3 Stalagmite samples

Stalagmite KNI-51-F, the object of this study, was bisected vertically with a water-cooled rock saw, producing different slices. Uranium-thorium dating was carried out at Radiogenic Isotope Laboratory at the University of New Mexico, and the subsequent calibration provides us with a high-resolution age model for the stalagmite. In addition, stable isotopic analyses took place in the same laboratory. This contributes to create a paleomonsoon reconstruction with a resolution of ~0.5-5 years [23].

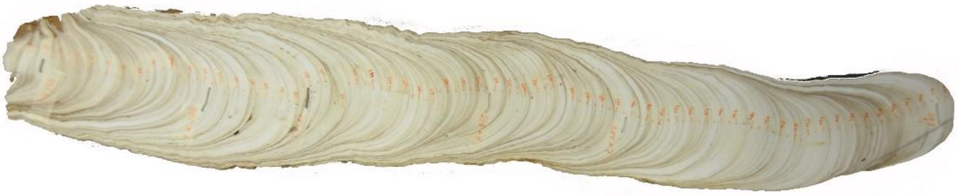


Figure 2.3 The section of the stalagmite KNI 51 F

2.4 Cleaning and decontamination

All the following procedures have been carried out at the DAIS (Department of Environmental Sciences, Informatics and Statistics) laboratories at the Ca' Foscari University of Venice.

A precise and accurate determination of trace and ultra-trace compounds is rather difficult to achieve. One of the most important issues to deal with is the sample contamination during all the analytical phases. In fact, sources of contamination are almost ubiquitous: the laboratory atmosphere, the reagents used, the sample containers, even the instrument used for the analysis and the workers themselves may contaminate the samples. Contaminants can affect the signal by causing an increase in the background noise or by directly interfering with the analytes. The lower the abundance of the analytes in the samples, the higher the risk of contamination.

In consideration of this, each step in sample treatment was optimized in order to minimize the contamination. Therefore, all glassware and tools used were prewashed with an aqueous Contrad® solution (5%), rinsed, dried and washed three times with clean *n*-hexane and dichloromethane, respectively. Where possible, glassware was also muffled at 400 °C for a minimum of 4 hours. To avoid sources of contamination, no plastic tools or containers were employed.

The subsampling (drilling) was conducted under a fume hood, previously cleaned with solvent and covered in aluminum foil. All the other pre-analytical operations were conducted in the cleanroom (ISO 7).

2.5 Sample preparation

The following procedure was applied to each sample in this work, employing an analytical method that was developed, optimized and tested in previous work at Ca’ Foscari University [76]. A total of 230 samples was processed, together with 1 blank every 5 samples prepared.

2.5.1 Sub sampling (drilling)

Powdered samples were obtained from the stalagmite by drilling with a hand-held TACKLIFE Cordless rotary tool. Before drilling each sample, the stalagmite surface was washed with *n*-hexane and dichloromethane to prevent external and cross-contamination and the drill tips were sonicated with a mixture of *n*-hexane and dichloromethane. In order to obtain approximately 1 g of material from each sample, layers of 2-3 mm were drilled. The samples were then collected in sealed 60 mL vials, closed with a pierceable cap and stored in the cleanroom until processing.

Figure 2.4 Left: stalagmite before drilling operation; Right: measured stalagmite with drilled parts

2.5.2 Extraction

Using an SGE™ eVol™ Handheld Automated analytical syringe, each sample was spiked with 50 µL of $^{13}C_6$-acenaphtylene, $^{13}C_6$-phenanthrene, $^{13}C_4$-benzo(a)pyrene (1 ng μL^{-1}) and 50 µL of hexatriacontane (50 ng μL^{-1}) as internal standards. 10 mL of UpS DCM were added to each sample. With a disposable syringe, 2 mL of 34-37% HCl were initially added to dissolve the aragonite matrix. The injection was made through the vial septum, in order to avoid the evaporation of volatile compounds. For the same reason, the DCM was added before the acid and samples were put in an ice bath. To enhance the dissolution and transfer of analytes to the organic phase, samples were vortex mixed repeatedly through the extraction process [39].

If necessary, 2 to 3 mL of HCl were added to dissolve the matrix completely. Once the dissolution was completed, 10 mL of n-hexane were added. The extract was transferred to another vial. Subsequently, the extraction was repeated twice with 5 mL of DCM and 5 mL of n-hexane, and the three extracts were merged. Before volume reduction, ~2 g of anhydrous Na_2SO_4 was added to each sample for anhydrification and pH buffering.

2.5.3 Volume reduction

The extracts were concentrated with a volume reduction operated by the Turbovap II at 23 °C. Once the solvent was evaporated to the volume of ~ 0.5 mL, 100 µL isooctane was added to prevent evaporation and thus allowing sample conservation.

Further concentration to ~100 µL was performed and 20 µL of a 100 pg μL^{-1} $^{13}C_6$-chrysene solution was added to serve as recovery standard. Samples were finally transferred to a GC vial and stored refrigerated until instrumental analysis.

2.6 GC-MS analysis

n-Alkanes were analyzed with a 7890A GC system coupled to a 5975C single-quadrupole mass spectrometer, while a Trace 1310 GC-TSQ 9000 GC-MS/MS was used for PAHs. The capillary column used on both instruments to separate the compounds is an HP-5ms column (60 m, 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies), with a stationary phase made of (5%-phenyl)-methylpolysiloxane. The GC separation was performed as follows:

- For PAHs:
    - Temperature of injection: 300 °C
    - Temperature of transfer-line: 300 °C
    - Oven temperature program:
        1. 70 °C for 1 min
        2. 20 °C $\min^{-1}$ to 200 °C
        3. 10 °C $\min^{-1}$ to 250 °C for 5 min
        4. 15 °C $\min^{-1}$ to 280 °C for 5 min
        5. 10 °C $\min^{-1}$ to 310°C for 30 min
    - Carrier-gas: Helium, with a rate of 1 mL $\min^{-1}$
    - Injection mode: splitless (split valve open 1.5 min after the injection at 50 mL $\min^{-1}$)
- For *n*-alkanes:
    - Temperature of injection: 300 °C
    - Temperature of transfer-line: 300 °C
    - Oven-temperature program:
        1. 50 °C for 5 min
        2. 18 °C $\min^{-1}$ to 315 °C for 16 min
        3. Post-run of 315 °C for 30 min
    - Carrier-gas: Helium, with a rate of 1.2 mL $\min^{-1}$
    - Injection mode: splitless (split valve open 1 min after the injection at 50 mL $\min^{-1}$)

The single quadrupole mass spectrometer was operated in the SIM mode (Selected Ion Monitoring), with an electron-impact source set at 70 eV and 230 ^{0}C. The temperature of the quadrupole was 150 ^{0}C. The chromatograms obtained were processed by Agilent MSD Chemstation software. The analytes were quantified through the isotope-dilution technique and were corrected by the response factor, obtained by repeated injections of a solution containing all native and labeled compounds at known concentrations. The triple quadrupole analyzer was used for tandem mass spectrometry, operating the first quadrupole in SIM mode, the collision cell to fragment the selected ion, and the third quadrupole in SIM mode. This results in the so-called MRM analysis (multiple reaction monitoring), which improves the quantification of the analytes.

What follows is a summary of the mass-charge ratios (m/z) of the molecular ions detected with the GC-MS, both for the qualitative and the quantitative analysis of PAHs. For *n*-alkanes (C_{11}-C_{36}) all the molecules have been determined in SIM mode with the detection of molecular ions with a mass-charge ratio of 71, 99, 85 and 113. For the quantitative analysis only mass charge ratio of 71 has been used.

Table 1 mass-charge ratios of PAHs molecular ions for the qualitative and quantitative analysis

Qualitative Analysis			
Name	**m/z**	**Product Mass**	**Collision Energy**
Naphthalene	128	77,7	20
Acenaphthylene	152	150	28
Acenaphthene	154	152,4	24
Fluorene	165	163	30
Phenanthrene	178	151,6	22
Phenanthrene C13	184	157	28
Anthracene	176	149,9	22
Fluoranthene	203	201,1	32
Pyrene	203	201	36
Retene	234	202,9	30
Benzo(a)anthracene	226	224,1	34
Chrysene	226	200	28
Benzo(b)fluoranthene	250	248,3	34
Benzo(k)fluoranthene	250	248	32
Benzo(a)pyrene	253	251,1	34
Benzo(e)pyrene	253	251,1	34
Perylene	253	251,1	34
Benzo(a)pyrene C13	257	255	34
Indeno(1,2,3-cd)pyrene	138	124,6	15
Dibenzo(a,h)anthracene	276	274	38
Benzo(g,h,i)perylene	138	136,8	16

Quantitative analysis			
Name	**m/z**	**Product Mass**	**Collision Energy**
Naphthalene	128	102	18
Acenaphthylene	152	125,8	24
Acenaphthylene C13	158	156	28
Acenaphthene	154	153,1	16
Fluorene	166	165,1	16
Phentanthrene	178	150,9	28
Phenanthrene C13	184	158	22
Anthracene	178	151	32
Fluoranthene	202	200	32
Pyrene	202	200	36
Retene	234	219,1	10
Benzo(a)anthracene	228	226	28
Chrysene	226	223,9	32
Chrysene C13	232	230	32
Benzo(b)fluoranthene	252	250,1	32
Benzo(k)fluoranthene	252	250	34
Benzo(a)pyrene	252	250	34
Benzo(e)pyrene	252	250	34
Perylene	252	250	34
Benzo(a)pyrene C13	256	254	34
Indeno(1,2,3-cd)pyrene	276	274	40
Dibenzo(a,h)anthracene	278	276	34
Benzo(g,h,i)perylene	276	274,1	38

3 RESULTS AND DISCUSSION

Age model and $\delta^{18}O$

Before showing the results acquired within this study, data acquired by Denniston et al. (2015) [23] are presented in this paragraph. Their results provide the age model for the stalagmite KNI-51 F (fig. 3.1), together with a precise record of $\delta^{18}O$ recorded in the stalagmite (fig. 3.2). The data have been here used to provide a time framework for our results and support the interpretation.

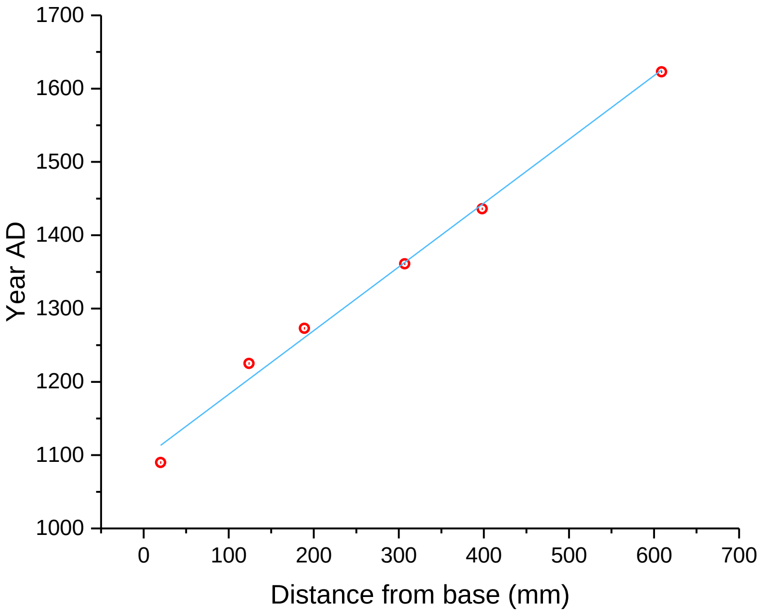


Figure 3.1 U/Th dates and age model for stalagmite KNI 51 F [23]

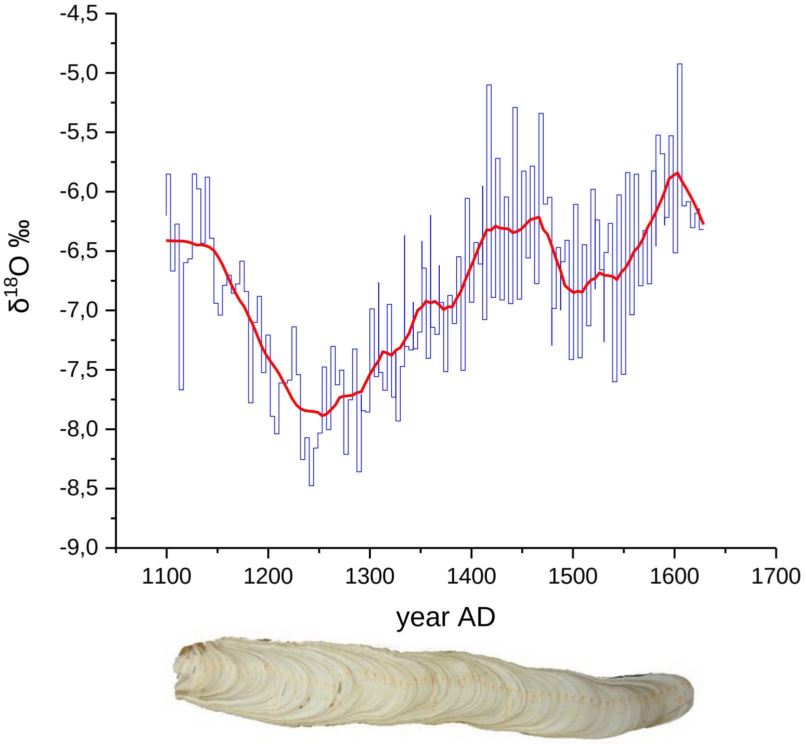


Figure 3.2 $\delta^{18}O$ profile of stalagmite KNI-51 F (smoothing lowess with 17 points)

The stalagmite was dated with the U/Th methods and with a precision of two standard deviations (± 1.3 years) [76]. From fig. 3.2, the trend in the oscillation of $\delta^{18}O$ can be appreciated. Most of the time, the isotopic composition of percolating water corresponds to the average isotopic composition of rainfall, and if it is accumulated and stored, e.g. in stalagmites, it can give information about the isotopic composition of past rainfall [17]. However, we must keep in mind that the interpretation of the stable isotope information preserved in speleothems is very complex, as it involves an overabundance of factors that can influence the $\delta^{18}O$ in cave drip waters [77]. Nonetheless, lower d18O values in KNI-51 stalagmites have been interpreted to reflect increased monsoon rainfall totals.

3.1 Polycyclic aromatic Hydrocarbons

The analysis of PAHs is a proxy of the intensity and the frequency of fires in the area. PAHs can be formed from incomplete combustion of organic materials, e.g. wood. As underlined by Lee et al (1977), the combustion of wood produces mostly non-alkylated PAHs [78], which are the focus of this work. Figure 3.3 represents the total concentration of PAHs, given in ng g-1 (powdered material), found in the stalagmite under study, whereas figures 3.4, 3.5 and 3.6 show the total concentration of low (LMW), medium (MMW) and high molecular weight (HMW) PAHs, respectively.

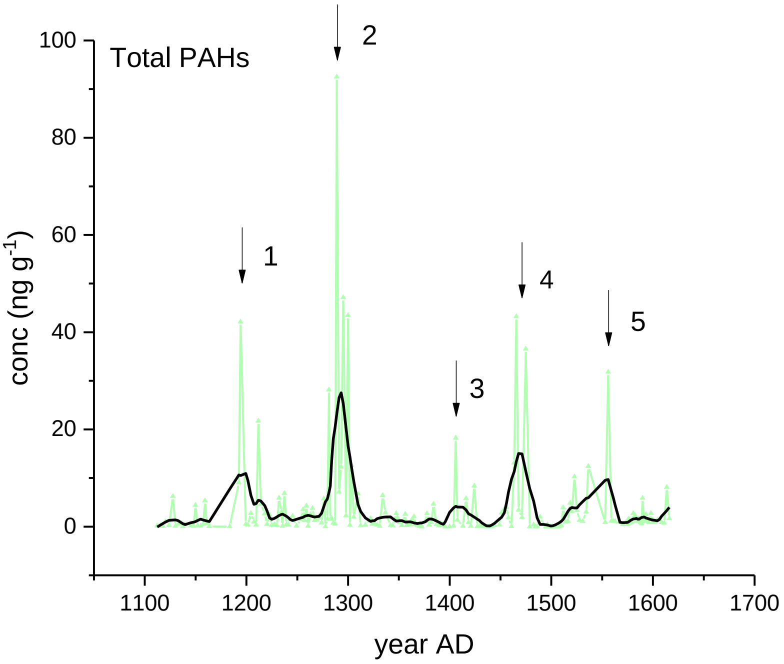


Figure 3.3 Total amount of PAHs (ng g^{-1}); smoothing lowess with 11 points

In figure 3.3, a total of 5 distinct paleofire events can be observed (numbers and arrows in the graph). The most intense events, number 2 and 4, fall between the 13th and the

14th century (ca. 1270 – 1320; 201.5 to 261 mm from base) and during the second half of the 16th century (ca. 1444 – 1492; 404 to 460 mm from base). Results show a marked difference between the low and medium molecular weight compounds and the high molecular weight compounds in these two events. In event 2, as can be seen in figures 3.4 and 3.5, LMW PAHs and MMW PAHs are more abundant than HMW PAHs, which are instead more abundant in event 4. LMW and MMW PAHs are considered as markers of low/medium intensity fires, with a range of temperature that fluctuates between 400 °C and 500 °C [79]. Moreover, these PAHs indicate a higher frequency in fire occurrence. For this reason, we can assume that during event 2, fire events had low-intensity and high-frequency features. On the other hand, event 4 could be interpreted as a period of high intensity (600- 650 °C) but low-frequency fire events [79].

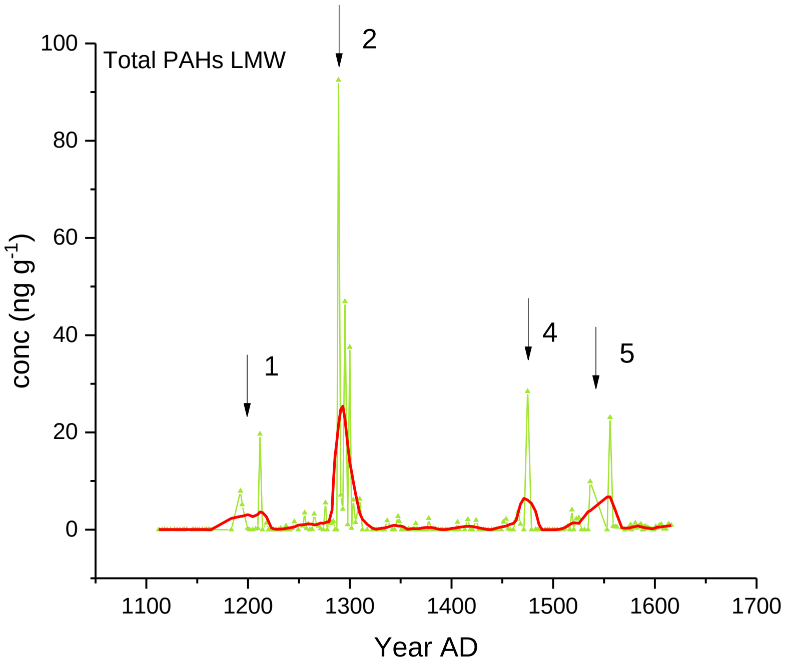


Figure 3.4 Total amount of Low Molecular Weight PAHs (ng g^{-1}); smoothing lowess of 11 points

Among LMW PAHs, acenaphthene and fluorene are the more abundant (both in 46 samples out of 230), with a maximum concentration of 22 ng g^{-1} for acenaphthene and 28 ng g^{-1} for fluorene. On the other hand, acenaphthylene is the less frequent (12 out of 230), with a maximum concentration of 11 ng g^{-1}.

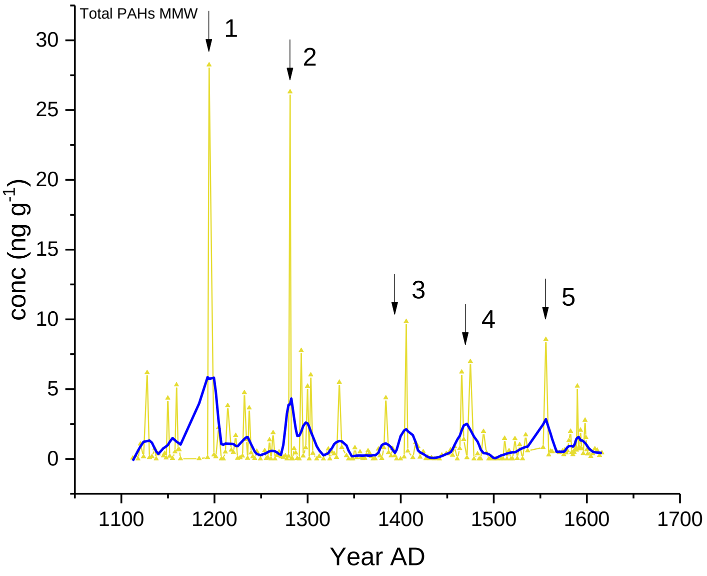


Figura 3.5 Total amount of Medium Molecular Weight PAHs (ng g^{-1}); smooting lowess of 11 points

Retene is the major compound found among MMW PAHs (90 out of 230), with a maximum concentration of 7 ng g^{-1}, while Benzo(a)Anthracene appears in 42 samples out of 230, and a maximum concentration of 1 ng g^{-1}.

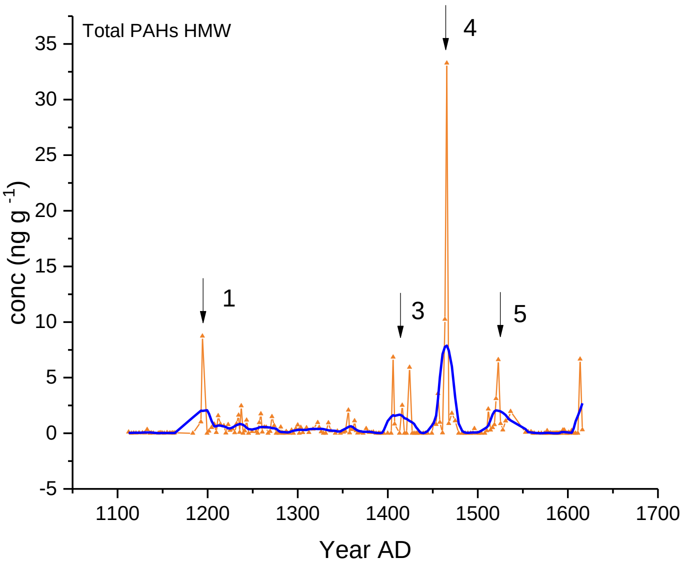


Figura 3.6 Total amount of High Molecular Weight PAHs (ng g^{-1}); smooting lowess of 11 points

Benzo(a)Pyrene has been found in 88 samples, almost four times the presence of Benzo(b)Fluoranthene (20 out of 230), the less abundant among HMW PAHs. Their respective maximum concentrations are 3 ng g^{-1} and 7 ng g^{-1}.

Figure 3.7 represents the distribution of each PAH compounds with a boxplot. The more abundant compounds are very well underlined.

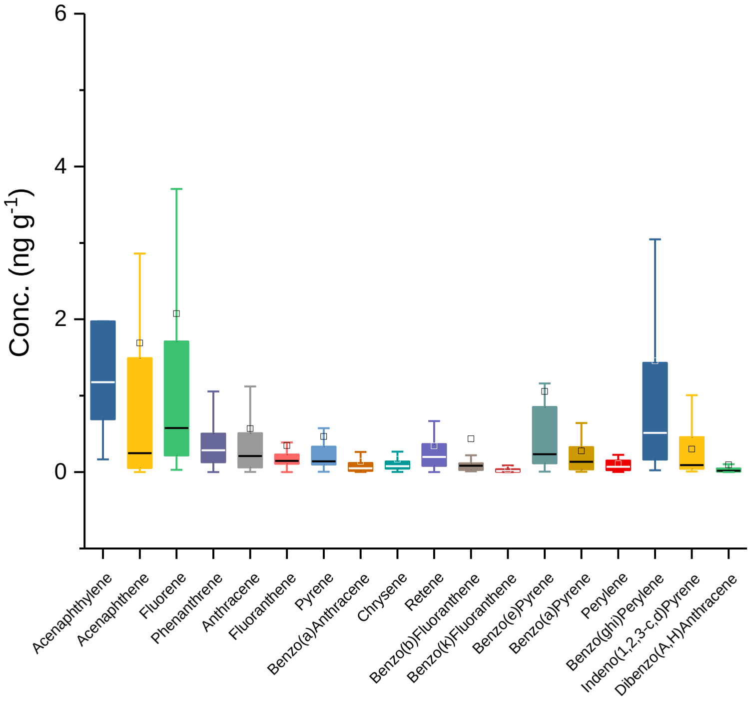


Figure 3.7 Distribution of single PAH compounds in the KNI-51 F record.

3.2 *n*-Alkanes

n-Alkanes in paleo archives are markers of the type and abundance of the vegetation present during the period analyzed [50]. For this reason, they can describe the area surrounding the cave, and using some indexes they can provide more specific information about the climate and the conditions in that area. Before describing them, figure 3.8 shows the total amount of *n*-alkanes found in stalagmite KNI-51 F.

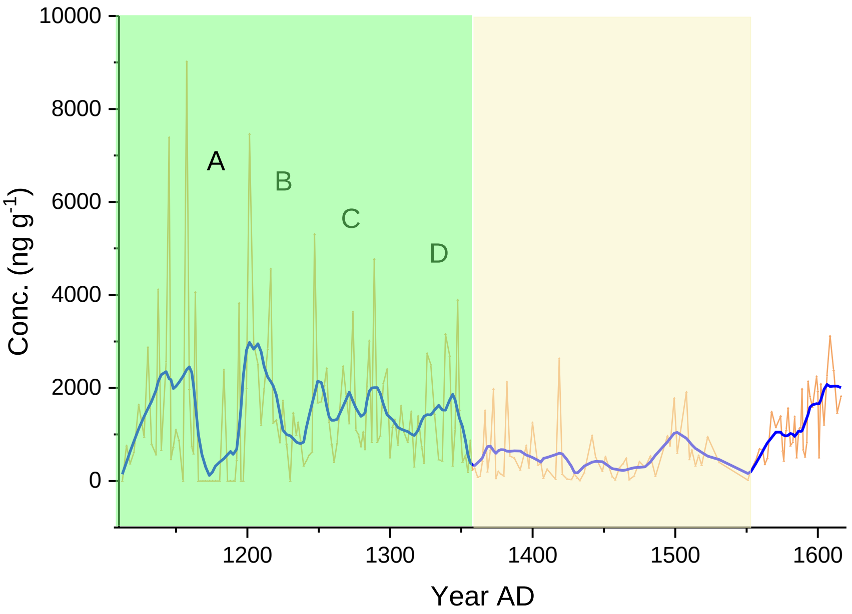


Figure 3.8 Total amount of n-alkanes in stalagmite KNI 51 F; smooting lowess of 11 points

As can be seen from figure 3.8, the period of time considered can be divided in two parts: a first section where the average amount of alkanes is higher than in the second section. The events marked with the letters represent the periods of time when the n-alkanes were more abundant: the two major events are from 1115 to 1173 (A) and from 1188 to 1234 (B). Events C and D range from 1237 to 1303 (C), and from 1319 to 1356 (D), with a lower abundance of n-alkanes but for a longer period. *n*-Alkanes may increase due to several reasons, but we can assume that two main features have intensified the total amount of n-alkanes. On one hand, we can have a climate period characterized by drier seasons (El Niño-like periods): this may lead to a thickening of the cuticles of the plants, in order to protect themselves from water loss [80]. Therefore, a higher presence of *n*-alkanes may be interpreted not as a higher abundance of individuals but as an increment in the relative mass of *n*-alkanes per single plant. On the other hand, periods of intense rainfall events and warm-wet conditions (La Niña-like periods) may correspond to the development of a larger plant community thanks to the increased availability of water resources, thus the total amount of n-alkanes may increase. The main species found in northern Australia and around the cave KNI-51 are Eucalyptus and Acacia. Both of them thicken their cuticle, but with a different typology of *n*-alkanes when exposed to drought conditions. In paleoenvironmental archives, n-alkanes come from different plant sources, so it is not possible to distinguish between species, even if our data lies in the range of concentration presented in the work of Hoffmann et al. (2013) for both Eucalyptus and Acacia [81]. The same article reports that both the species increase their *n*-alkanes concentration in leaves when the aridity increases.

Average chain length (ACL)

The Average Chain Length, or ACL, is an index calculated from higher plant n-alkanes, and it is a weighted average of the number of carbon atoms per molecule [82]. Because of their structure, i.e. straight chain of hydrocarbons and without functional groups, they are very stable; if any external or during depositional factors do not interfere, once they are stored in the archive, they can be preserved in it for millions of years without being altered [83]. The ACL for this study was calculated as follows:

$$ACL = \frac{\sum_{n=10}^{35}(C_n \times n)}{\sum(C_n)}$$

Where n stands for the number of carbon atoms present in the n-alkane compounds.

In figure 3.9 the graph of ACL calculated from samples of stalagmite KNI-51 F can be observed.

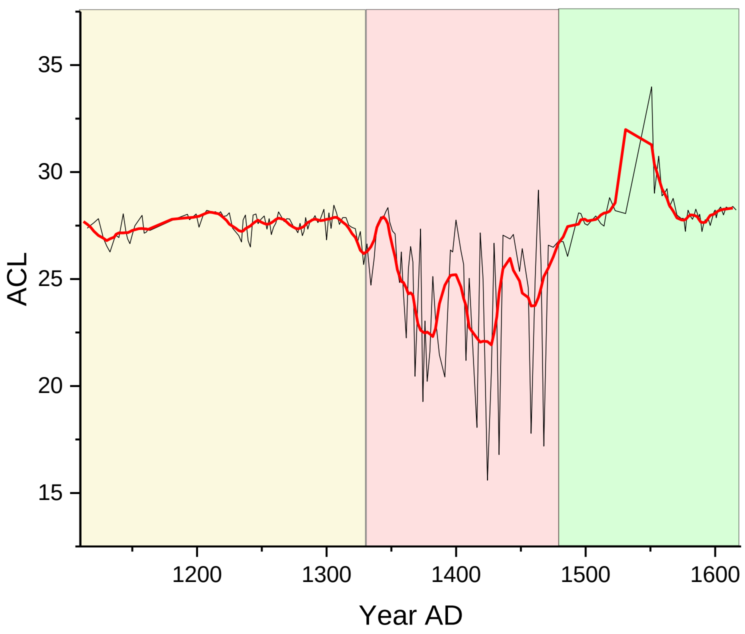


Figura 3.9 ACL calculated with data from stalagmite KNI 51 F, smoothing lowess of 10 points

The ACL trend can be ideally divided in three main moments: a first stable period where the ACL does not decrease or increase (ca. 1112- 1331; yellow section), with an ACL oscillating between 27 and 28, followed by a period of substantial decrease (ca. 1331-1478; red section), with more fluctuations and an average ACL of 25. Finally, an increase in the ACL (ca. 1478-1621; green section), with an ACL mean value of 28, but with peaks at 32-33. Simoneit et al. (1991) propose that plants which live in warmer climates produce long chains of n-alkanes [84]. The study of Oros et al. (1999) instead shows a correlation between the decreasing length of n-alkane chains and the higher humidity conditions in the region studied [85]. However, it is true that as alkanes are site-specific, the results of these works may not be valid in other contexts. We must underline that leaf wax *n*-alkanes length is influenced also by other factors, e.g. the species considered and how the same species react if subjected to different climatic conditions [86]. The article by Hoffmann et al. (2013) documents that the ACL of these two species in northern Australia ranges between 26.83 and 29.34 for Eucalyptus and between 29.11 and 32.10 for Acacia. It is worth mentioning that Hoffmann et al. (2013) show a decreasing trend in Eucalyptus ACL from the moist region (north) to the arid region (south). The trend is the opposite for the Acacia ACL, and the reason for this opposite trend may be attributed to biochemical differences. For this reason, the interpretation of the ACL results cannot be used as a robust proxy if it is not accompanied by additional proxy data [81]. This underlines the importance of having a multi-proxy approach in the description of a past environmental changes.

Reworked alkanes

Terrestrial plants build up chains of hydrocarbons with 25-35 carbon atoms, longer than what aquatic plants or bacteria do [82]. Many studies prove that in nature a strong odd-over-even predominance is expected from plant-derived *n*-alkanes and discovered in river or marine sediments [81, 82, 87]. Instead, in marine organisms, the predominance can be absent or occur in rare cases [88]. A lack in this odd/even predominance or, on the contrary, an even/odd predominance, in higher plant-derived *n*-alkanes can be caused by different factors. One of them is the microbial community activity, both reworking the odd *n*-alkanes or with their own inputs [89]. A study conducted by Villanueva et al. (1997) of *n*-alkanes in the North Atlantic Ocean proposed an index to calculate the number of reworked *n*-alkanes in their samples [90]. As in our data odd/even predominance was not present (see figure 3.10), we use the same index in order to assess if *n*-alkanes in KNI-51-F were modified by bacteria. However, even if proves of bacteria activity on speleothem are evident [13], this index was never applied to stalagmite samples before.

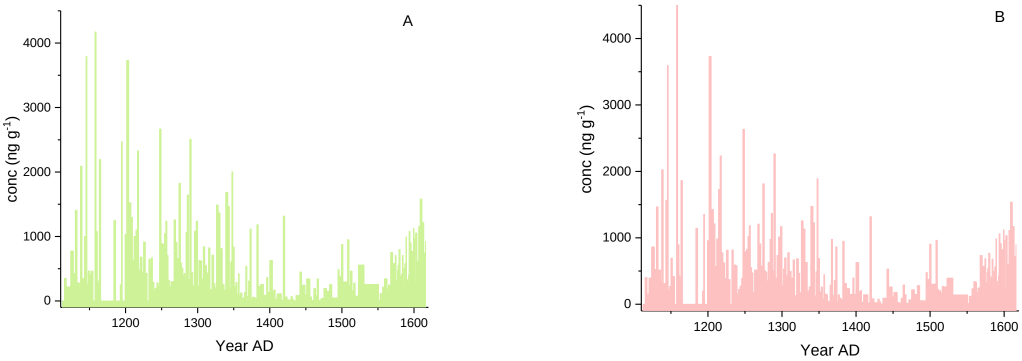


Figure 3.10 Even and odd n-alkanes pattern in stalagmite KNI-51-F

The index of reworked n-alkanes (Ar) was determined as follows:

$$A_r = C_{nr} - A_0$$

In order to calculate C_{nr} (non-reworked alkanes) and A_o, the following formula have been used:

- For A_0:
  - First $A_{(21+2i)}$ has been calculated with the formula:

$$A_{(21+2i)} = C_{(21+2i)} - \frac{C_{(22+2i)} + C_{(20+2i)}}{2} \quad with\ i = 1 - 6$$

  - A_0 calculated with:

$$A_0 = \sum_{i=1}^{6} A_{(21+2i)}$$

- For: C_{nr}

$$C_{nr} = \sum_{i=20}^{33} [C_i]$$

Figure 3.12 shows a box plot with the distribution of each *n*-alkane compound. The absence of the odd-over-even predominance can be appreciated.

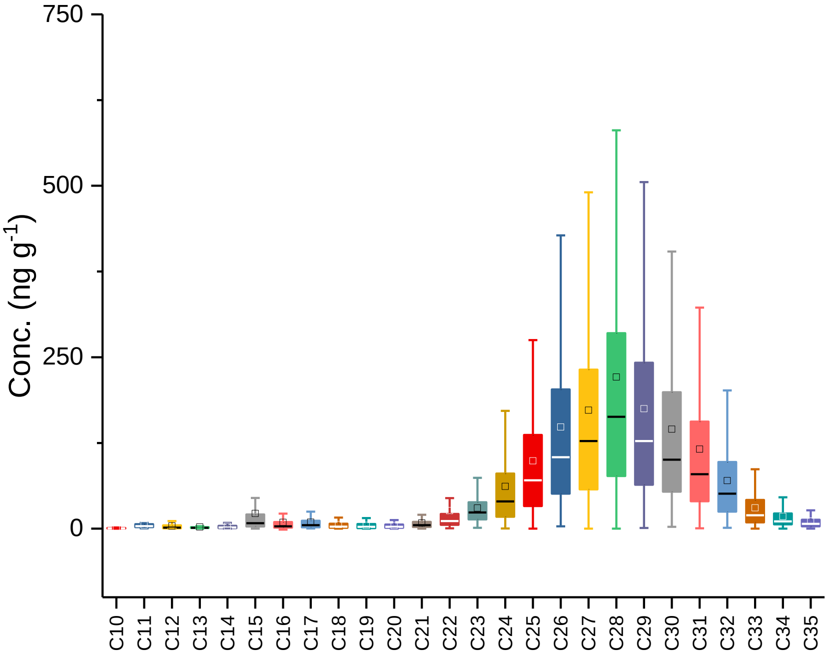


Figura 3.12 Distribution of single n-alkanes compounds in the KNI-51 F record

The index Ar shows that almost the totality of *n*-alkanes has been reworked. This means that a strong microbial activity is likely to be present. Further researches can contribute to investigate whether this activity is more present on the surface of the stalagmite or in the above soil. Figure 3.13 shows the amount of reworked and non-reworked *n*-alkanes. Negative values indicate a microbial input to the system.

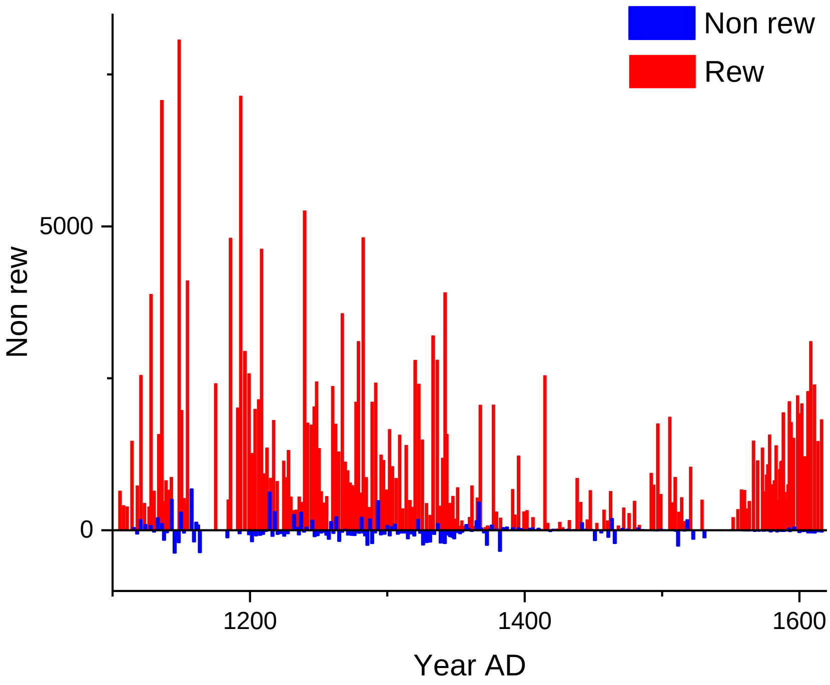


Figura 3.13 Reworked and non-reworked n-alkanes in stalagmite KNI 51 F.

Mud layers

The stalagmite KNI 51 F presents a continuous alternation between mud layers and aragonite layers. One of the questions this study aimed to answer was: does the presence of mud in the stalagmite influence the accumulation rate of different types of PAHs differently from pure aragonite? Do the deposition and the organic content of samples affect the distribution of PAHs in the stalagmite? Figure 3.14 shows an overlap between the pattern of mud/aragonite layers (brown lines represent mud layers) and the LMW, MMW and HMW PAHs. Mud layers data were acquired from National Centers for Environmental Information, NESDIS, NOAA, U.S. Department of Commerce and provided by Denniston et al. (2009).

After a preliminary analysis, it seems that PAHs are not affected by an accumulation in mud layers rather than aragonite layers. As shown in fig. 3.14, in fact, peaks in PAH compounds do not coincide with periods of intensified flood frequency. The concentrations detected for the three weight classes (LMW, MMW, HMW) appear not the be related to the distribution of flood layers, which seem not to influence the deposition of organic compounds in stalagmites. Therefore, it is unlikely that PAHs display a preferential distribution in mud layers, possibly influenced by the higher organic matter content. However, given the number of factors at play, further research is certainly needed to test if PAHs increase or decrease with an increase or decrease in the flood events frequency.

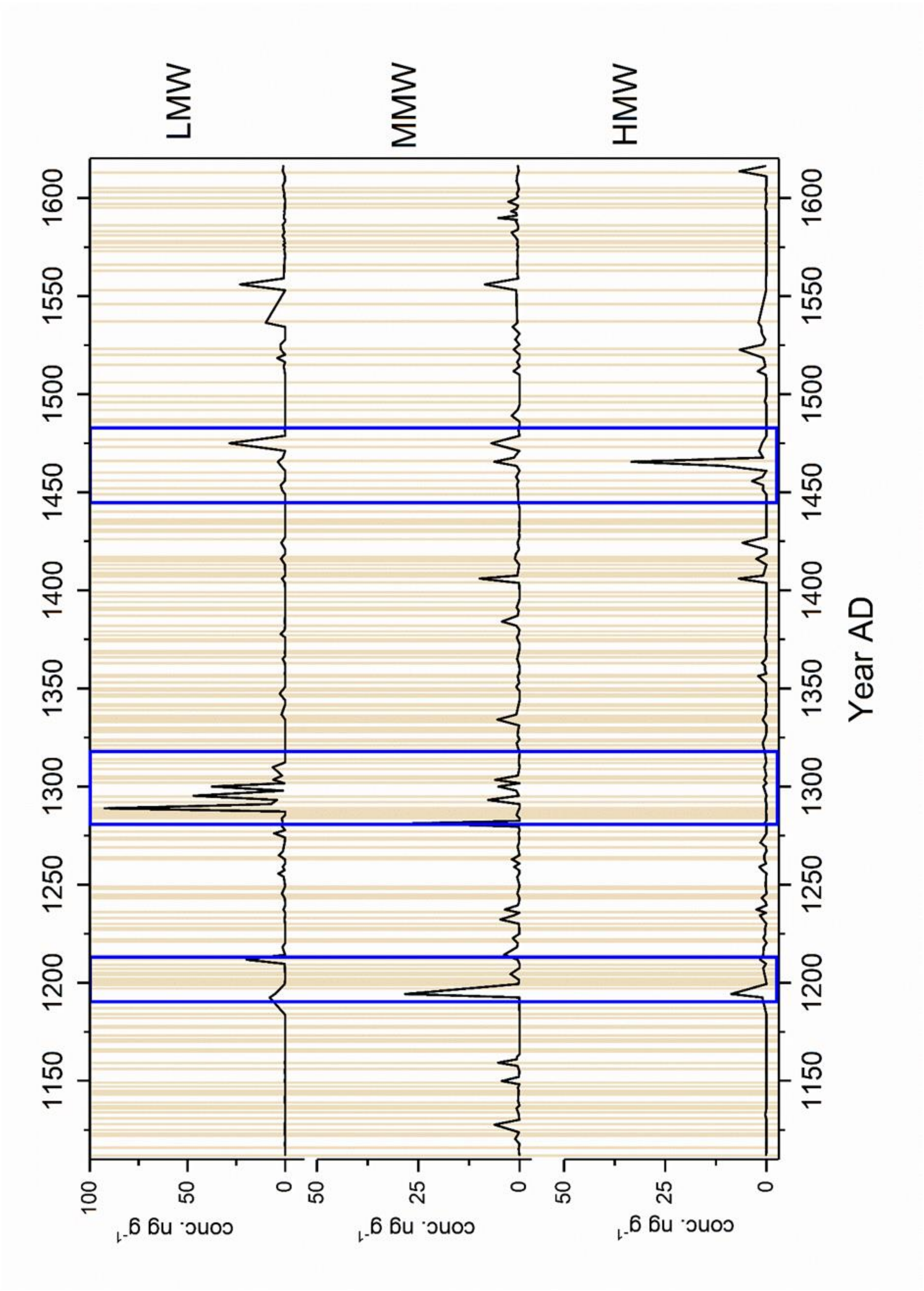


Figure 3.14 Overlap of mud/aragonite layers (brown/white bands) and PAHs concentrations

3.3 Multi proxy interpretation

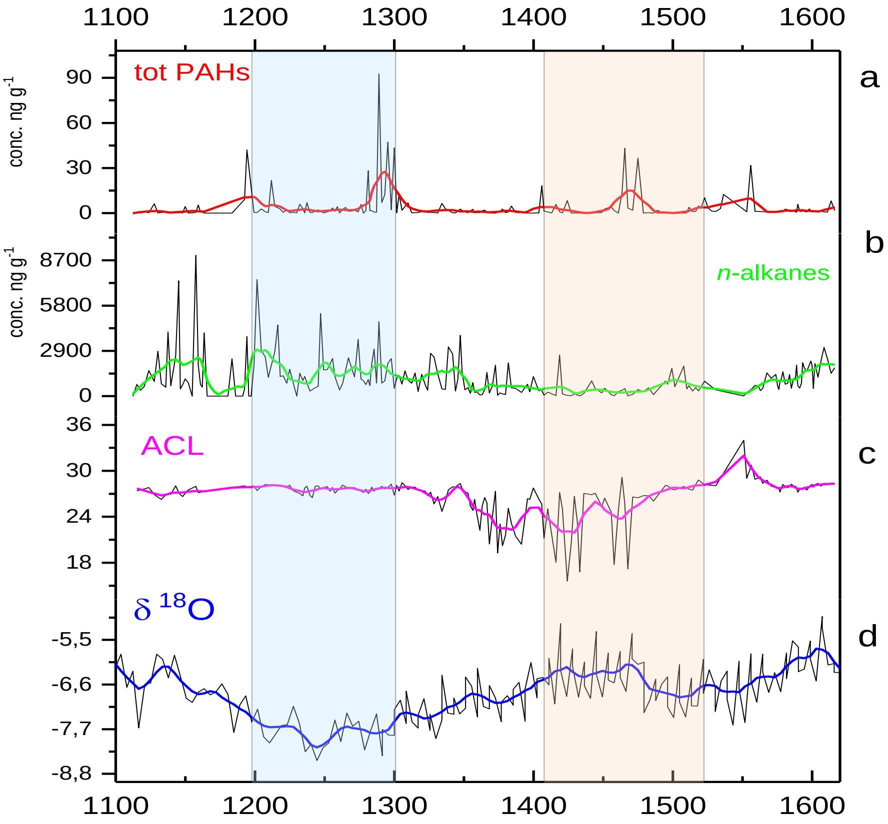


Figura 3.15 a) Total amount of PAHs; b) total amount of n-alkanes; c) ACL index; d) δ18O. Lowess smoothing of 10 points.

Figure 3.15 provides an overview of the main results obtained from this study. Two major events can be observed and described from the multi proxy analysis. The first period (light blue section), ca. 1200-1300 AD, is more humid and therefore characterized by lower values in $\delta^{18}O$. During these years, humidity resulted in biomass build-up, that can be observed from the total amount of alkanes, the highest in the record. This observation is supported by the work of Denniston et al. (2016) [91]. In fact, their study confirms a period of extreme monsoon activity and rainfall during the Medieval Climate Anomaly, particularly intense at the end of this period, together with an expansion of the Indo-Pacific Tropical Rain Belt. ACL confirms the relative wet period that characterized the 13th century. Species like Eucalyptus, largely abundant in the Kimberley region, tend to have a higher ACL index when the environment is more humid [81]. The prevalence of LMW and MMW PAHs indicates higher frequency of paleofires but with a lower intensity. With these conditions (La Niña-like), the total amount of *n*-alkanes may indicate also the possibility of a frequent regrowth of shrubs and grasses caused by bushfires. Shrubs and grasses are in fact species that can grow rapidly in an area where the secondary ecological succession (e.g. after a wildfire) occurs, thus increasing the biomass present in the area.

Focusing on the second period (light-orange area), the situation is different. $\delta^{18}O$ drifts towards less negative values, indicating drier conditions (El Niño-like). ACL decreases as well, indicating a likely drought period if we look at the results showed in Hoffmann et al. (2013) [81]. This can be strengthened by the total amount of *n*-alkanes, which is relatively lower. A possible explanation may be found in the minor abundance of biomass, caused by drought conditions. If we look at the PAHs profile, the fire events are marked even in this period, and as stated above, they are characterized by a major presence of HMW PAHs. This indicates high intensity fires but with a lower frequency.

4 CONCLUSIONS

The purpose of this study was to test the possibility to determine trace polycyclic aromatic hydrocarbons in speleothems, and especially in the stalagmite KNI-51 F. In doing so, this work can be seen as an exploratory survey in this not so well-developed field of speleothem research. In fact, PAHs analysis in speleothems are sporadic and with low resolution results. With our data we try to shed light on the possibility to use PAHs preserved in stalagmites as a novel proxy for paleo-fires reconstruction. In order to do this, a new analytical method to extract organic compounds from an inorganic matrix has been applied. Difficulties in this approach consist mainly in the low concentrations of the analytes, together with a very high risk of contamination of the sample. This is reflected in all the pre-analytical procedures aimed at avoiding contamination and to maximize the extraction of organic compounds. Together with PAHs, the study of *n*-alkanes, organic compounds synthesized in leaves which act as regulators for avoiding water loss in plants, contributes to elucidate and corroborate the interpretation of the results. Together with the record of $\delta^{18}O$, this multi proxy approach appears to be a reasonable attempt in validating the use of PAHs as a proxy for paleofires. Reconstructing past conditions and events is of key importance for the future management of areas affected regularly by these episodes.

Results shows periods of low intensity fires, but more common, followed by high intensity fire events, more infrequent. $\delta^{18}O$ underlines a more humid period in the first part of the timeline recorded, replaced by an interval of time with drier conditions. This could mainly be attributed to more La Niña-like conditions (wet circumstances) or El Niño-like conditions (dry circumstances), phenomena that affect the area under study.

Furthermore, odd-over-even prevalence in *n*-alkanes, which is usually common for these kinds of explorations, was not detected. We attributed this finding to a possible reworking action operated by bacteria involved in calcite deposition.

It is essential to highlight the importance of further investigations in different aspects of this field, from depositions and percolating mechanism to bacteria actions. A better understanding of the mechanism and the reconstructed records may enhance the awareness about the informative power that speleothems contain, and thus open new fields of exploration.

Figure 4.1 Fire panel in Australia; Bureau of Meteorology

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