

KARINA SCURUPA MACHADO

**ANTHROPOGENIC AND CLIMATE CHANGES OBSERVED BY  
BIOMARKERS IN PALEOGEOCHEMICAL STUDIES**

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Final version of the PhD thesis presented to the Posgraduated Program in Environmental Engineering and Water Resources, Federal University of Paraná, as a requirement to obtain the Water and Environmental Resources Engineering title, Concentration Area: Environmental Engineering.

Supervisor: Prof Dr Sandro Froehner.

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TERMO DE APROVAÇÃO

**Karina Scurupa Machado**

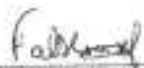
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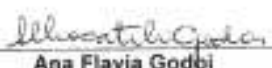
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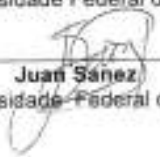
  
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"It is not in the nature of man to make a sudden and unexpected discovery; science advances step by step and every man depends on the work of his predecessors."

**Ernest Rutherford**

*I dedicate this thesis to people who have taught me the meaning of unconditional love, my mother Mrs Ruth and my husband Hamilton.*

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**ABSTRACT**

This study presents an assessment of the potential of biomarkers in the construction of past scenarios. Records of environmental changes in two distinct areas of study were obtained from the use of biomarkers from anthropogenic and climate changes in dated sediment cores. Changes that occurred between 840 to 1330 were studied in Lake Ohrid watershed (Southwest Macedonia), while more recent changes, in the last 400 years, were evaluated in the northern region of Curitiba and Almirante Tamandaré (Southern Brazil). The last millennium corresponds to the late Holocene climate in which important events in the history of the planet, such as the Dark Age, Medieval Warm Period-MWP, Little Ice Age-LIA, and the Modern Age occurred. In Lake Ohrid, changes in the amount and composition of organic matter inputted in the sediment were observed. The distribution of lipids showed a replacing of organic matter sources from plants by soil and a consequent increase in productivity in the lake. This change is related to increased deforestation in the area due to historical events, such as the foundation of the Saint Naum monastery and the elevation of Ohrid City to the capital of the Bulgarian Empire. Associated with human influence in the region is increased rainfall, characteristic of the transition from the Dark Age (colder and dry) for the MWP (warmer and humid). Another scenario was observed in southern Brazil, with more recent changes regarding the transition from LIA to the Modern Time. Evidence of changes in vegetation, climate and atmospheric composition were discussed, and in general, three periods were observed: 1600-1730, warmer and dry, predominance of herbaceous over woody plants; 1730-1800, colder and dry, predominance of woody plants and 1800-nowadays, the end of LIA, warmer and more humid. From the atmospheric compartment, changes in CO<sub>2</sub> levels appear to have occurred in the last 400 years. Based on the isotopic ratios from plant sources, two significant increases were observed between 1600-1880 and 1930-2012. Sources of CO<sub>2</sub> with global influence would be primarily responsible for the initial increase, while regional sources seem to be predominant in the second. Among the global sources, volcanism may have contributed to the change in atmospheric composition, especially between 1600 and 1880, since changes in soil pH were evident. More recently, the rise of atmospheric CO<sub>2</sub> has been attributed to anthropogenic sources such as biomass and fossil fuels burning. The anthropogenic impact on the region was assessed through the distribution of polycyclic aromatic hydrocarbons, whose sources have indicated the evolution of human activities in the region over the past 150 years, complementing the understanding of changes in atmospheric composition. Overall, this study contributes to the elucidation of environmental changes in significant periods of history, in regions where information of this approach are scarce. In times of strong discussion about climate change, the understanding of past events that may be repeated in a cycle and the real effects of human actions on the planet contribute to the construction of future scenarios and forecasts.

Keywords: Climate Changes, Anthropogenic Impact, Biomarkers, Holocene

## RESUMO

Este estudo apresenta uma avaliação do potencial de biomarcadores na construção de cenários passados. Registros de mudanças ambientais ocorridas em duas áreas de estudo distintas foram obtidos a partir do uso de biomarcadores de mudanças antropogênicas e climáticas em sedimentos datados. Mudanças ambientais ocorridas a mil anos atrás foram estudadas na bacia do Lago Ohrid (Sudoeste da Macedônia), enquanto alterações mais recentes, nos últimos 400 anos, foram avaliadas na região norte de Curitiba e Almirante Tamandaré (Sul do Brasil). O último milênio corresponde ao final do Holoceno, no qual ocorreram importantes eventos climáticos da história do planeta, tais como a Era Negra, Período Medieval Quente (PMQ), Pequena Idade de Gelo (PIG) e a Idade Moderna. No Lago Ohrid, alterações na quantidade e composição da matéria orgânica no sedimento foram observadas. A distribuição de lipídios mostrou uma substituição de fontes de matéria orgânica de plantas por solo e consequente aumento da produtividade no lago. Esta alteração se deve ao aumento do desmatamento na área devido a eventos históricos tais como a fundação do Monastério Saint Naum e a elevação da cidade de Ohrid a capital do Império Búlgaro. Associado a influência humana na região está o aumento das chuvas, característico da transição da Era Negra (frio e seco) para o PMQ (quente e úmido). Outro cenário foi observado no sul do Brasil, com mudanças mais recentes, referentes a transição da PIG para a Idade Moderna. Evidências de alterações na vegetação, clima e composição atmosférica foram discutidas e de maneira geral, três estágios foram observados: 1600-1730, mais quente e seco, predomínio de plantas herbáceas sobre lenhosas, 1730-1800, mais frio e seco, predomínio de plantas lenhosas e 1800-presente, final da PIG, mais quente e úmido. Do ponto de vista atmosférico, alterações nos níveis de CO<sub>2</sub> parecem ter ocorrido nos últimos 400 anos. Com base na razão isotópica de plantas, dois aumentos significativos foram observados entre 1600-1880 e 1930-2012. Fontes de CO<sub>2</sub> com influência global seriam as principais responsáveis pelo primeiro aumento, enquanto fontes regionais parecem ser predominantes no segundo. Dentre as fontes em nível global, o vulcanismo pode ter contribuído para a mudança na composição atmosférica, principalmente entre 1600 e 1880, uma vez que alterações no pH do solo são evidentes. No período mais recente, o aumento do CO<sub>2</sub> atmosférico foi atribuído a fontes antropogênicas, tais como queima de biomassa e combustíveis fósseis. O impacto antrópico na região foi avaliado através da distribuição de hidrocarbonetos poliaromáticos, cujas fontes indicaram a evolução das atividades humanas na região nos últimos 150 anos, de forma a complementar o entendimento da mudança na composição atmosférica. De maneira geral, o presente estudo contribui para a elucidação das alterações ambientais ocorridas em períodos significativos da história, em regiões onde informações desta natureza são escassas. Em tempos de discussão sobre mudanças climáticas, o entendimento de eventos do passado, que podem vir a se repetir em um ciclo, e dos reais efeitos da ação do homem no planeta, contribuem para a construção de cenários e previsões futuras.

Palavras-chave: Variações Climáticas, Impacto Antrópico, Biomarcadores, Holoceno

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**LIST OF SYMBOLS, ACRONYMS AND ABBREVIATIONS**

%: Percentage	<: less than
>: higher than	∑: Sum
°C: Celsius degrees	μA: microampere
μL: microliter	μm: micrometer
‰: Per mil	<sup>135</sup> Cs: Cesium
<sup>13</sup> C/ <sup>12</sup> C: isotopic ratio	<sup>210</sup> Pb: Lead
<sup>222</sup> Rn: Radon	<sup>226</sup> Ra: Radium
<sup>238</sup> U: Uranium	<sup>241</sup> Am: Amerindian
<sup>32</sup> Si: Silicon	<sup>55</sup> Fe: Iron
AC: After Christ	AEN: Acenathene
AEN: Acenathene	ANP: Acenaphthylene
ANT: Anthracene	ASE: Accelerated Solvent Extractor
BC: Before Christ	Bq: Bequerel
BaA: Benzo[a]anthracene	BaP: Benzo[a]pyrene
BbF: Benzo[b]fluoranthene	BgP: Benzo[ghi]perylene
BHPs: Bacteriophanoids	BkF: Benzo[k]fluoranthene
BIT: Branched and Isoprenoid Tetraether	C: Carbon
CaCO <sub>3</sub> : Calcium carbonate	Chol: Cholesterol
CAM: Crassulacean Acid Metabolism	CBT: Cyclization Branched Tetraethers
Chor: Cholesterol	CHR: Chrysene
cm: centimeters	CO <sub>2</sub> : Carbon dioxide
CIC: Const. Initial Conc. of unsupported <sup>210</sup> Pb	DA: Dark Age
CRS: Const. Rate of of unsupported <sup>210</sup> Pb	DIC: Dissolved Inorganic Carbon
DCM: Dichloromethane	d: deuterated
DFG: German Research Foundation	DhA: Dibenzo[a,h]anthracene
eV: volt electrons	FA: fatty acids
FLA: Fluoranthene	FLU: Fluorene
g: grams	GC: Gas Chromatography
GDGT: glycerol dialkyl glycerol tetraether	h: hour
HC: Hydrocarbons	HCL: Hydrochloric acid
HMW: High Molecular Weight	i.d: internal diameter
IcP: Indeno[1,2,3-cd]pyrene	ICP: Preferential Carbon Index
IP: Inorganic phosphorus	IRMS: Isotope Ratio Spectrometry Mass
Km <sup>2</sup> : square kilometres	LIA: Little Ice Age

## List of Symbols Acronyms and Abbreviations

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LMW: Low Molecular Weight	m/z: ratio mass load
m: meters	M: molecular weight
mA: miliampere	min: minute
mm: milimeters	MO: Organic matter
MAAT: Mean annual air temperature	MBT: Methylation Branched Tetraethers
MS: Mass spectrometry	MWP: Medieval Warm Period
N: north	NAP: Naphthalene
OH: Alcohol	OP: Organic phosphorus
pH: hydrogen potential	PHE: Phenanthrene
PVC: Polyvinyl chloride (plastic)	PYR: Pyrene
RE: Relative errors	rpm: Rotations per minute
RSD: Relative Standard Deviation	RTA: Terrestrial and Aquatic Ratio
SIM: Selected Ion Monitoring	spp.: species
TC: Total Carbon	TIC: Total Inorganic carbon
TLE: Total Lipids extract	TN: Total Nitrogen
TOC: Total Organic Carbon	TP: Total phosphorus
TS: Total Sulphur	V: volts
VPDB: Vienna Pee Belemite	$\alpha$ : alpha
$\beta$ : beta	$\omega$ : Hydroxy acids
$\delta^{13}$ : isotopic ratio	

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# **CHAPTER I**

## **INTRODUCTION**

## I.1 GENERAL INTRODUCTION

Throughout the history of science, biomarkers have gained importance due to their accuracy in reconstructing the past. Studies of environmental changes and their perturbations are assessed by the presence and distribution of biomarkers, providing reliable evidence about their sources and/or processes that are involved. Most of the current knowledge about the environmental ecosystems was obtained using biomarkers, which are a group of compounds able to represent environmental conditions, or inferences about the processes/agents that ecosystems have undergone. Such substances have natural or anthropogenic origin, low solubility, slow degradation, and in general, are present in all compartments (air, soil, sediments and water); thus, they can be considered powerful tools in the detection of anthropogenic pollution and environmental disturbances.

Due to high potential in stored biomarkers, sediments stand out among the environmental compartments and can be considered reliable archives, able to represent all natural and anthropogenic events and episodes occurring in the watershed. The application of chronological techniques in dating sediments makes possible the use of biomarkers not only in the study of pollution in a given locality, but also in evaluating these compounds' distribution over time, resulting in the reconstruction of past scenarios associated with environmental changes and detection of climatic events, occurring billions of years ago.

In the last few decades, the concern and debate regarding the magnitude of the anthropogenic impact on the Earth's climate and environment have been growing and have become the focus of several scientific discussions. Although the current global warming condition is evident, and the scientific community proves human's participation in this phenomenon, there are still many questions about the real potential of climate change by man and about climate change in the past and future. This subject is intriguing. Climate modeling does not entirely explain the variations in temperature as a function of greenhouse gas emissions. Moreover, the climatic conditions of the planet are conditioned by external factors (intensity of solar radiation) and internal (sea levels, arrangements of oceans, continents and atmospheric composition). Then, shifts in these parameters lead to an instability of the system and consequently to climate change. These changes have already occurred many times throughout the history of the planet, even without human interference, and may happen again in a global cycle of climate changes. Therefore, there is a meaningful scientific interest in the improvement of knowledge about climate change and its environmental

influences over time, in terms of global and local variations, beyond the distinction between natural and anthropogenic changes.

Although studies of periods of more than 100 thousand years ago have already been performed, the last 6,000 years are considerably important in understanding the climate system of Earth because the climate conditions during this period did not show significant fluctuations (compared to the large changes in glacial-interglacial eras). In this study, two periods of the late Holocene (the last 2,000 years) have major importance: the Medieval Warm Period - MWP (950 – 1400) and the Little Ice Age - LIA (> 1400). The scope and magnitude of these two climatic events become important for the detection of anthropogenic influences on global climate issues. For instance, the increase of mean air temperature in the 20th century can be a natural consequence of LIA. Although these climatic events had global effect, most of the studies are from the Northern Hemisphere. There is a lack of studies in the Southern Hemisphere and even fewer in Brazil where the studies are limited to evidence of changes in vegetation and sea level.

Considering the complexity of the past study, the use of biomarkers as paleoenvironmental proxies has been considered an efficient tool for understanding the planet history. In the selection of biomarkers for paleoenvironmental studies, the geographical, hydrological and geological characteristics of the area studied must to be considered in a historical context. Consistent paleoenvironmental studies rely on the use of representative, reliable and correlated biomarkers, with applicability already reported in the literature.

Thus, the present study aims to reconstruct past scenarios of climate and anthropogenic changes that occurred in the Late Holocene from the use of innovative and reliable biomarkers in dated sediments from two different regions: South Brazil (northern region of Curitiba and Almirante Tamandare) and the North Hemisphere (Ohrid - southwestern of Macedonia). The results contribute to a better understanding of environmental changes in transition periods between important climatic events in regions where the information about this approach are scarce.

## I.2 RESEARCH FOCUS AND OBJECTIVES

**Hypothesis:** “Environmental disturbances over time were triggered by anthropogenic and/or natural climate changes and can be evidenced by distribution of specific biomarkers in the sediment core.”

**General Objective:** Reconstruct a past scenario of environmental changes that occurred in the two transitions periods of history: Dark Age to the Medieval Warm Period and the Little Ice Age to Modern Time, in two different areas, using biomarkers in dated sediments.

### **Specific Objectives**

- i) To identify in the literature, reliable and correlated biomarkers that are able to better represent environmental changes in the study areas;
- ii) To quantify and evaluate the temporal distribution of biomarkers in two sediment cores from Lake Ohrid and Barigui River;
- iii) To identify and understand the temporal trends of those biomarkers by their relationship with the geochronology and historical events of the studied areas;
- iv) To associate the temporal biomarker trends with anthropogenic and climate change to reconstruct scenarios of past environmental changes.

### I.3 THESIS STRUCTURE AND ORGANIZATION

The main contribution of this thesis is the reconstruction of past scenarios and their environmental changes for a better understanding about the evolution of climate and anthropogenic influences on it over time. The information obtained may contribute to answering questions about present and future climatic events. In this way, two areas with different characteristics were selected to evaluate two different transition periods of climate history: Dark Age (DA) to Medieval Warm Period (MWP) and Little Ice Age (LIA) to Modern Time (MT).

Thus, **Chapter I** provides an introduction and explains the objectives to clarify the focus of the thesis. Also, it provides an overview about biomarkers in sediments used to construct past scenarios, and geochronological techniques, all in a paleoenvironmental approach.

In the **Chapter II** is described the characteristics of the areas studied and the methodologies used.

**Chapter III** presents environmental changes in an earlier period of history (840-1330) during the transition between DA and MWP. Evidences of climatic and anthropogenic changes in Lake Ohrid watershed are obtained from shifts in organic matter composition in sediment core.

In the following three chapters (III, IV and V), most recent environmental changes are reported (1600 to present), during the transition between the LIA and MT. The upper portion of the Barigui River watershed was used for this goal. **Chapter IV** reports changes in vegetation in response to climate changes and variations in mean air temperature and precipitations, over time, while in **Chapter V**, changes in atmospheric composition over time are the main findings. Evidence of natural and anthropogenic sources, in local and global level, responsible for those changes is presented. The evolution of anthropogenic pollution sources in modern time are explained in **Chapter VI**, which is an extension of **Chapter V**. The increase in anthropogenic impact due to regional economic development is recorded by the presence of PAHs. Finally, since most of the results are from local sources **Chapter VI** tends to be regional. In contrast, the approaches presented in **Chapters IV and V** have a broader significance, in terms of space.

## I.4 LITERATURE REVIEW

### I.4.1 Sediment Transport

Sediments are composed of particles derived from rock fragmentation or soil segregation and are transported by a fluid, usually water or wind, from the origin to lakes and rivers (CARVALHO, 2008a). These particles aggregate tracer compounds or biological markers, typically termed “biomarkers,” and hence, sediments are considered true archives of information about chemical, physical and biological processes occurring in the watershed, in deposition layers, and temporally and sequentially accumulated (CAZOTTI *et al.*, 2006). The sediment formation by particles movement from the source to the water system basically, involves three processes: erosion, the movement of particles in the water column, and deposition (BLOESCH and BURNS, 1980). These three processes will be briefly explained.

Erosion is the initial particle movement process. Igneous, or sedimentary, rocks are degraded by weathering and are transported by erosion, or the particles of bare ground can be broken by the impact from raindrops (CARVALHO, 2008a). Such disaggregated soil particles are transported by runoff and are deposited in the lower parts of the basin (valleys and riverbeds/lakes). Among the environmental factors that may influence erosion, rainfall is the most important. The movement of the particles and the energy dissipated by raindrops compact the soil surface, reducing the infiltration rate, increasing runoff, and causing increased erosion (COLBY, 1963). Although the wind and groundwater can erode the soil, runoff is the more significant erosive agent.

Once inside the water system, the particles are separated by water activity into various sizes that define the transport process. Larger particles roll/glide by traction processes, and due to contact with the riverbed, suffer rounding while smaller particles dissolve or become suspended and are transported by water flow without undergoing any change (GARDNER, 1980). Regarding the particle composition of sediment, three main components are observed: organic matter (in different decomposition stages), particulate minerals (carbonates, clays, and silicates) and inorganic components from biogenic origin (CAZOTTI *et al.*, 2006.)

The flow and transport of sediment in river systems tend to be unique, with temporal and spatial variability (COLBY, 1963). The gravity force and shear stress are the main factors that influence fluvial dynamics. The shear stress is crucial to start the motion of small particles while the flow velocity has a greater influence on the loading

of larger particles (KLEINHANS and BRINKE, 2001). Sediment transported by a water channel (dissolved, suspended, or dragged on the riverbed) is a product of the interactions among the liquid mass in movement, the surface of the riverbed, and the different types of sediment transported (COLBY, 1963)

Deposition processes are related to the type of transport, channel flow conditions, composition and particle size. The particle suspension is subject to the action of the stream velocity in the horizontal direction and particle weight. When the water flow no longer has the energy to maintain particles in suspension, deposition occurs (BLOESCH and BURNS, 1980; COLBY, 1963). The sedimentation rate of a particle is measured by its resistance to transport. Fine particles are easily carried by natural forces and flow and tend to move over long distances while larger particles are transported over short distances or dragged on the riverbed (CARVALHO, 2008a).

In general, some characteristics of the basin directly affect the amount and composition of sediments. The main ones are precipitation level, geomorphology, and vegetation cover (GARDNER, 1980). Areas that are without vegetation and are physically degraded are more prone to erosion due to the impact from the direct action of raindrops, which are responsible for the release of particles, soil sealing, reducing infiltration and consequent increase in runoff (KLEINHANS and BRINKE, 2001). The vegetation cover and organic material in the soil can reduce up to 90% of the soil loss and up to 62% of the speed of runoff (CARVALHO, 2008a). The drainage runoff occurs when gravity forms a network of channels that increases the flow and, consequently, the laminar erosion. The efficiency of this channel network is defined by the watersheds geomorphism (BLOESCH and BURNS, 1980). Other factors such as riverbed composition, hydraulic and geometrical channel characteristics, and especially, the hydrological regime of the watershed are also directly involved in the process of sediment transport, so that any change in the natural dynamics of these factors affects the balance and nature of the sediment transported (COLBY, 1963).

#### **I.4.2 Biomarkers: An Environmental Approach**

Organic matter constitutes a minor, but important, fraction of sediments. It is made up of a complex mixture of lipids, carbohydrates, proteins, and other biochemicals contained in the tissues of living benthic microorganisms and from the detritus of organisms formerly living in the river and its watershed (KILLOPS and KILLOPS, 2005). The molecular composition of the lipid fraction of sediment organic matter provides particularly useful information about the sources and alterations of

organic matter. The biological heritage retained in lipid fraction is termed “biological marker,” commonly abbreviated to “biomarker”. This characteristic makes the lipid fraction especially important to geochemical studies even though it typically constitutes only a small percent of the total sediment organic matter (MEYER and ISHIVATARI, 1993)

Biomarkers are characteristic of human activities or natural processes and specific sources compounds. Since biomarkers are resistant to degradation, they are relatively persistent in the environment (NETTO *et al.*, 2000). Some of these substances are generally present in surface water, groundwater, and sediment, and due to their specificity, are highly likely to indicate their source, or origin. Sometimes these substances have origin in diagenetic processes of aquatic systems. Thus, due the potential to reflect environmental changes, biomarkers are considered as a source for obtaining information about climate change, pollution, or any other event with historical significance (GUIOT and CORONA, 2010).

In the biomarkers example, polycyclic aromatic hydrocarbons (PAHs) are consistently associated with contamination by petroleum products and biomass burning (FROEHNER *et al.*, 2010). These compounds are generally emitted by anthropogenic activities. Their formation occurs through three processes: pyrogenic, petrogenic, or biogenic processes. The petrogenic and pyrogenic processes are derived from human activities and are responsible for formation of PAHs in most environments (XU *et al.*, 2007). The biogenic process is natural and occurs through biochemical processes on a small scale. There is not much knowledge about the production of PAHs by such processes. Considering that anthropogenic contributions usually overtake the natural sources, human activities are most likely responsible for global emissions of these compounds in the last 100 years (LIU *et al.*, 2012). Given their physical and chemical properties, PAHs mainly with high molecular weight hardly suffer decomposition in short periods, constantly being found in sediment (BARRA *et al.*, 2009; McREADY *et al.*, 2000). The presence of PAHs can be safely associated with human activities and has been increasing since the industrial revolution, given the large amount of fossil fuels used for such development (GUO *et al.*, 2011; ITOH *et al.*, 2010; KANNAN *et al.*, 2005).

Although not all biomarkers have their origin in human activities, they are indirectly related, such as aliphatic hydrocarbons (HC). Such compounds are derived from a variety of allochthonous sources, mainly higher plants, and autochthonous sources, such as phytoplankton and bacteria. The characteristic of the molecular structure of HC usually distinguishes its possible sources (WAKEHAM, 1996). The presence of aliphatic hydrocarbons in sediments with a predominance of compounds

having odd carbon numbers and a maximum of C27, C29, or C31 is often used to indicate input of higher plants in the sediment, because its prevalence in waxes of such plants (LIN *et al.*, 2008). The phytoplankton contribution is more difficult to be recognized due to the low abundance of hydrocarbons in many algae. However, significant amounts of C15, C17, and C19 are commonly interpreted as indicators of algae supply (FICKEN *et al.*, 2000a). A nutrient supply can cause an increased concentration of n-aliphatic compounds, which can be attributed to climatic effects and vegetation removal, in recent and prehistoric times (PANCOST *et al.*, 2004). Due to the various sources and forms of interpreting the results of n-alkanes, some ratios have been developed to assess the distribution of these compounds, for example, CPI – Carbon Preference Index and TAR -Terrestrial and Aquatic Ratio (BOURBONNIERE and MEYERS, 1996; MADUREIRA *et al.*, 1995.).

Another remarkable group of compounds often used as biomarkers are the sterols. This group of compounds is found in the membranes of eukaryotic organisms (animals, plants, fungi, and microorganisms) (MUDGE *et al.*, 1999). Sterols and their derivatives are important geochemical biomarker compounds. The presence or absence of double bonds and methyl groups at various positions on the carbon framework, the length of the branched side chain at the C17 position, and the stereochemistry of the substituent bonds create a variety of geochemically useful compounds (MEYERS and ISHIWATARI, 1993). Some sterols are commonly found in sediments such as lanosterol, which is synthesized by fungi, while dinosterol and stigmaterol have origin in higher plants and algae (SUNDIN *et al.*, 1999). Sitosterol, campesterol, and sitostanol are examples of plant sterols, which can be used to obtain information about input of organic matter in surface water, as well as indicate the possible vegetation composition in this ecosystem (KILLOPS and KILLOPS, 2005). Moreover, certain compounds of the family of sterols can be used as markers of sewage. Coprostanol and epicoprostanol are typical markers of sewage because they originate in the intestines of animals. Thus, the presence of such compounds suggests the presence of sewage or of animal waste (EGLINGTON *et al.*, 1997). The difference between the sources is given by the ratios between the various sterols. Besides having specificity in relation to the source, the sterols are resistant to bacterial degradation, making it useful in identifying changes in the proportions of contributions on different sources (SUNDIN *et al.*, 1999).

Linear alcohols (alkanols) and fatty acids (alkanoic acids) are other example of markers of high environmental importance. They are commonly associated since they normally present the same sources although with substantial chemical differences and different diagenetic processes (ANDERSSON and MEYERS, 2012). With regard to

their origin, alcohols and fatty acids short chains (<C<sub>20</sub>) with lower molecular weight are produced by organisms and plants from marine and freshwater systems, such as phytoplankton, zooplankton, or bacteria. This instance is observed especially when the concentrations of alcohols and fatty acids C<sub>16</sub> and C<sub>18</sub> predominate (WAKEHAM *et al.*, 1980). On the other hand, alcohols and fatty acids from long chains (>C<sub>24</sub>) with high molecular weight are derived from the waxes of land plants (TREIGNIER *et al.*, 2006). Thus, the difference between the sum of the relative contributions of fatty acids and alcohols with short and long chains may be used as a tool to infer the origin of sedimentary organic matter retained in the aquatic system (MATSUDA and KOYAMA, 1997). Besides, the sum of alcohols and fatty acids, variations in the relative proportion among the chains also may be evidence of changes in the organic matter composition.

### **I.4.3 Diagenetic Processes**

The lipidic fraction, which comprises most biomarkers, is relatively resistant to degradation process in the environment. Studies of settling particles intercepted by sediment traps indicate that lipids can undergo substantial degradative alteration while they sink to the bottoms of rivers and lakes (MEYERS and ISHIWATARI, 1993). A variety of biochemical and geochemical processes may occur prior to the deposition and during the early stages of burial under conditions of relatively low temperatures and pressure (MOLDOWAN *et al.*, 1986). Moreover, benthic animals and microbes depend on organic matter, including lipids, for their nutrition. Bioturbation, or biological mixing, of surface sediments also prolongs the exposure of organic matter to oxidation and adds to the degradation due to the nutritional needs of benthic fauna. Once buried below the zone of bioturbation, organic matter is subject to alterations by anaerobic bacteria (MADUREIRA *et al.*, 1995). In organic geochemistry, the term applied to these processes is “diagenesis,” and biological agents (particularly heterotrophic microorganisms) are the main ones responsible for diagenetic transformations although some chemical transformations are also observed (MOLDOWAN *et al.*, 1986).

During diagenesis, biomarkers undergo the same main types of reactions as other biogenic organic compounds: defunctionalization, aromatization and isomerization. Oxygen-containing functional groups predominate among the lipids components at the start of diagenesis, and their loss involves reactions such as dehydration and decarboxylation. By the end of diagenesis, these defunctionalization processes lead to the formation of hydrocarbons, either saturated or aromatic. Non-aromatic, unsaturated hydrocarbons (alkenes) can be formed initially, such as sterenes from stenols, but they are reactive and do not survive in the longer term (KILLOPS and

KILLOPS, 2005). The interconversion of isomers (isomerization) among biomarkers also can occur in diagenesis, mainly in the migration of double bonds in unsaturated biomarkers, such as sterenes (MEYERS and ISHIWATARI, 1993).

The degradation rates of various compound classes differ. Aquatic lipid matter is preferentially degraded during the sinking of particulate material, probably because of the relative freshness of this material. In contrast, land-derived organic matter has been microbially reworked before arrival in the riverbed, and only its most resistant lipid components survive to become part of the sedimentary record (ALI and MUDGE, 2005). Hydrocarbons are generally not as sensitive to diagenetic alteration as are oxygen-containing lipids. Within the n-alkane fraction of hydrocarbons, longer-chain-length molecules are less degradable than are their shorter-chain-length counterparts and are common constituents in ancient sediment (FICKEN *et al.*, 2000a).

Fatty acids, on the other hand, are more sensitive to degradation and modification than most types of biogenic lipids. However, when labile compounds are associated with refractory materials, they are protected to a degree from the effects of diagenesis. This may account for the apparently greater stability of long-chain fatty acids (>C22) from higher plant material, which are abundant in waxy leaf cuticles, compared with the shorter-chain fatty acids (<C22) from microorganisms (MATSUDA and KOYAMA, 1997). Because of their low solubilities and presumably low susceptibilities to microbial utilization, the wax esters are resistant to diagenetic alterations and provide improved records of the original proportions of algal and watershed sources of sedimentary lipids (RIEDERER *et al.*, 1993). Unsaturated acyclic components, such as polyunsaturated fatty acids, are degraded relatively quickly, both microbially and chemically, during diagenesis. The more resistant saturated fatty acids,  $\omega$ -hydroxy acids, can survive in ancient sediments. Concentration of linear fatty acids and alcohols decrease relative to total organic carbon, faster than other biomarkers such as sterols (MATSUDA and KOYAMA, 1997).

Sterols may undergo important conversions during diagenesis. Stenols, the unsaturated biological form of most sterols, are converted into stanols, the hydrogenated analog. This conversion evidently begins in the water column with the consequence that both stenols and derived stanols are common in surficial riverine and lacustrine sediments. The rapid diagenetic alteration of biological sterols into their geochemical derivatives implies that microbes are responsible. Many of these compounds are intermediates in alteration pathways that will lead to either saturation or aromatization of the steroid carbon skeleton and, hence, improved geochemical stability (CORDEIRO *et al.*, 2008).

Concerning the polycyclic aromatic hydrocarbons (PAHs), these compounds are ubiquitous components of marine and riverine/lacustrine sediment, but in contrast to most biomarkers, they do not occur naturally in organisms. PAHs are produced by anthropogenic processes and insignificant amount by biosynthesis. High temperature is needed to reconstitute organic matter fragments into characteristic PAHs, and considering conditions of relatively low temperatures and pressure of sediment, PAH amounts from diagenetic processes in an environmental context are insignificant (BARRA *et al.*, 2009).

To assess the level of diagenetic processes in paleochemical studies, some proxies can be used. The Carbon Preference Index (CPI) is used to indicate the degree of diagenetic alteration of n-alkanes and can be modified for similar application to n-alkanoic acids. This index is essentially a numerical representation of how much of the original biological chain length specificity is preserved in geological lipids. In fresh lipid material, odd-numbered carbon chains dominate hydrocarbon compositions and even-numbered chains dominate compositions of fatty acids and alcohols. This specificity diminishes as diagenesis proceeds (FICKEN *et al.*, 2000a). Changes in proxies related to the primary productivity also can be used to make inferences about diagenetic processes such as C/N ratio (MEYERS and ISHIWATARI, 1993), or more specific ratios such as the Cholesterol/Cholestanol reported in literature in order to assess the level of diagenetic transformation in the sedimentary environment (WAKEHAM and CANUEL, 2006). Although assessment about the level of diagenetic processes in paleochemical studies is substantially important, Madureira *et al.*, (1995) reported that the concentration of various compounds within each class (n-alkanes, alcohols, fatty acids, alkenones, and alkyl alkenoates) associated with the same organic matrix have similar degradation and mixing rates. Therefore, biomarker indices remain largely unaltered by early diagenetic processes.

In order to obtain independent and complementary information about sources and sink of biomarkers in paleochemical studies, stable isotope ratios have been widely used recently as a technique for elucidation. Soil organic matter preserves this isotopic distinction with little or no isotopic fractionation ( $< 2\text{‰}$ ) (CERLING *et al.*, 1997). Plant biomass when it is decomposed, tends to keep its isotopic signature (STREET-PERROT *et al.*, 2004), and no significant carbon isotope fractionation of hydrocarbons has been reported for volatilization, photo-oxidation, thermal oxidation, or microbial degradation processes (BI *et al.*, 2005). Thus, measures of the carbon isotopic contents of individual biomarkers, especially n-alkanes, isolated from sediments, allows source and processes identifications to be more specific.

#### I.4.4 Isotopic Signature and Environmental Application

Stable isotopes of carbon, nitrogen, sulphur, hydrogen and oxygen are considered currently useful tools in the study of the matter and energy cycles in the environment (PEREIRA, 2007). In the last 20 years, this technique has been applied in aquatic environments and has proven to be very efficient and promising. Stable isotopes of hydrogen and oxygen are used in determining the composition of the water used by plants, whereas the carbon, nitrogen, and sulphur isotopes are used to elucidate photosynthetic pathways, physiological processes in plants, or to determine food sources in aquatic and terrestrial food chains. Stable isotopes are present in all ecosystems, and their natural distribution reflects the history of physical and metabolic processes in the environment (CARVALHO, 2008b). Thus, most of the physical, chemical and biological processes that occur in aquatic systems can be studied in the light of isotopic techniques. Lighter elements such as carbon, nitrogen, hydrogen, and oxygen are the most studied elements, due to their prominent role in biological processes (BOUTTON, 1996).

Carbon plays a central role in the structure and function of the system's earth-atmosphere-biosphere. Hence, considerable emphasis has been given to understanding the isotopic biogeochemistry of this element (LOPES and CECILIO, 2002). The carbon element has two isotopes, which occur naturally:  $^{12}\text{C}$  (98.89%) and  $^{13}\text{C}$  (1.11%). The ratio of these isotopes is conventionally compared in accordance with Equation 1 (LOPES and CECILIO, 2002), with an international standard that refers to belemnites cretaceous formation (inorganic carbonate), designated as VPDB (Vienna Pee Dee Belemnite) and expressed in per mille (‰).

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

In natural materials, the isotopic ratio relative to the standard VPDB ( $\delta^{13}\text{C}$ ) varies within a narrow range of values, approximately between about 0 to -110 ‰. This variation is the result of fractionation, or isotopic discrimination, during the physical, chemical, and biological processes (LOPES and CECILIO, 2002). The natural variation in ratio of carbon stable isotopes ( $\delta^{13}\text{C}$ ) in different components of the environment acts as a tracer of physical, chemical, and biological processes and determines the proportion among lighter and heavier isotopes in the study sample, based on the

premise that the isotopic ratios are conservative (JOHNSON, 1993). Moreover, the isotopic fractionation in important transfer processes carbon in nature are well known (WEISENBERG *et al.*, 2008a; MEAD *et al.*, 2005; BI *et al.*, 2005). Therefore, this natural variation is extremely useful to trace and quantify the sources, products and rates of biogeochemical fluxes in the carbon cycle since values of  $\delta^{13}\text{C}$  of several elements and environmental compartments are known and are reported in the literature, as shown in FIGURE I-1.

Among studies related to isotopic fractionation between different environmental compartments, the study of biomarkers, mainly alkanes from terrestrial plants is noteworthy. In photosynthesis, plants discriminate against atmospheric  $\text{CO}_2$  differently, due to different biochemical properties of enzymes, responsible by fixation of atmospheric  $\text{CO}_2$  (RuBisCo and PEPCase) and due some limitations of  $\text{CO}_2$  diffusion into leaves (LOPES and CECILIO, 2002).

Concerning the photosynthetic fixing  $\text{CO}_2$  process, plants are differentiated into three types: C3, C4, and CAM (Crassulacean Acid Metabolism). Due to these photosynthetic differences, the alkanes isotopic signature from plants differs widely. C3 plants are  $^{13}\text{C}$ -depleted, and their  $\delta^{13}\text{C}$  values typically range from -31‰ to -39‰ while C4 plants are  $^{13}\text{C}$ -enriched, with  $\delta^{13}\text{C}$  values ranging from -18‰ to -25‰. CAM plants can use both C3 and C4 carbon fixation pathways, so they have an intermediate  $\delta^{13}\text{C}$  range (-23‰ to -29‰) (BI *et al.*, 2005). These differences in isotopic composition of organic materials allow, for example, the tracing of the derived carbon of each photosynthetic cycle in the "pool" of organic matter in soil or sediment once there is an insignificant change in the value of  $\delta^{13}\text{C}$  (‰) of plant material when it is decomposed. Thus, the  $\delta^{13}\text{C}$  values in carbon from soil and sediment integrates the relative contribution of different photosynthetic cycle types in the "pool" of organic carbon (PEREIRA, 2007).

When analysis of isotopic composition of organic matter from plants is associated with geochronological data, it is possible to obtain information about vegetation change in past scenarios and practices and effects of changes in land use in the ecosystem structure. One can also quantify the rates and patterns of these changes in natural ecosystems (TIPPLE and PAGANI, 2010). Changes in the relative productivity of C3 and C4 plants in an ecosystem, over time, are often attributed to changes or disturbances in precipitation levels and climate since the vegetation is extremely sensitive to environmental factors (CERLING *et al.*, 1997). Therefore, carbon isotopic measurements in soil or sediment organic matter from biomass plants are also often used to substantiate and refine our understanding about climate-vegetation interactions (SUN *et al.*, 2011; BOUTTON, 1996).

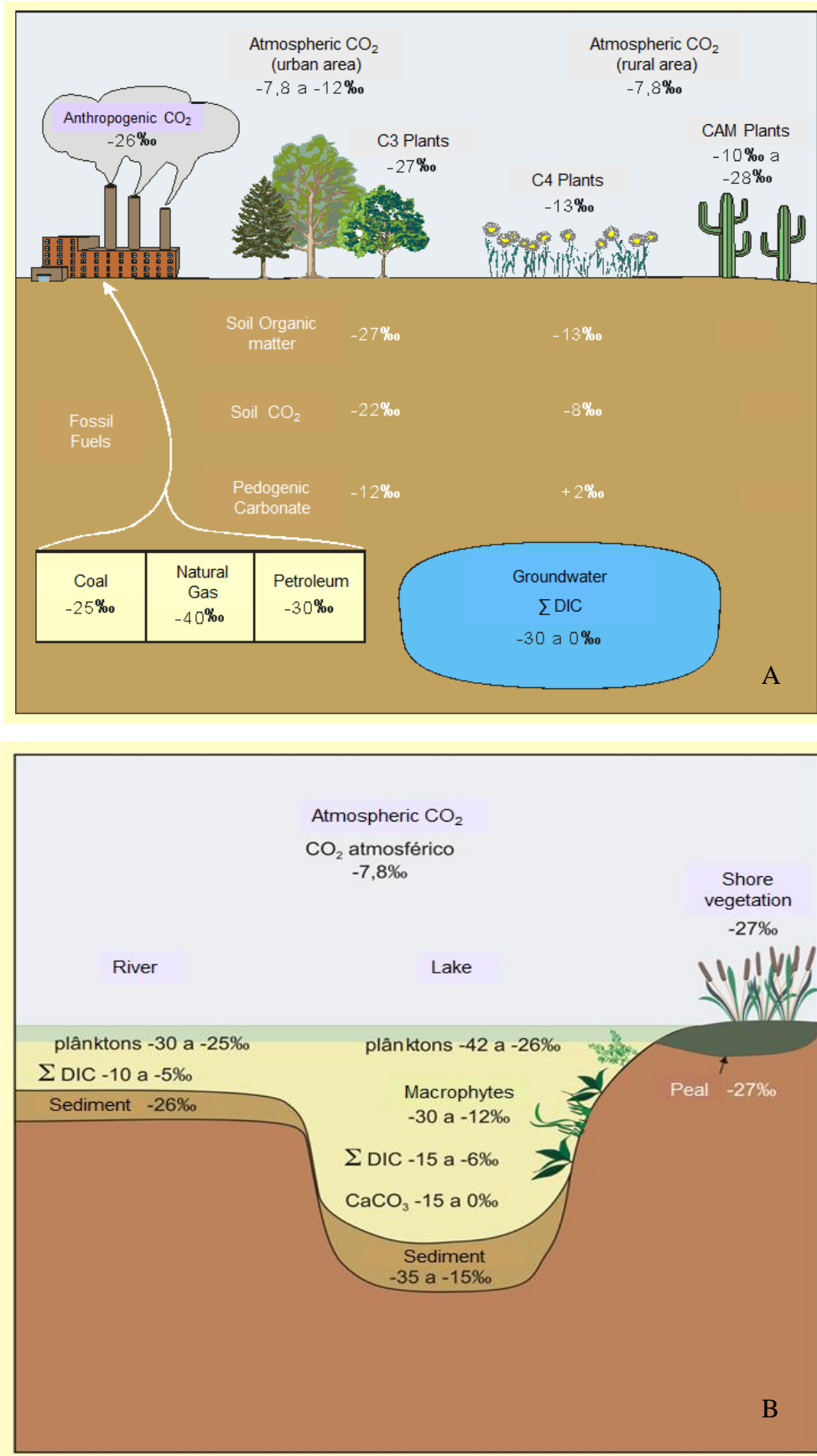


FIGURE I- 1.  $\delta^{13}\text{C}$  VALUES IN THE MAIN COMPONENTS OF TERRESTRIAL (A) AND FRESHWATER (B) ECOSYSTEMS. SOURCE: BOUTTON, 1996.

### **I.4.5 Climate Changes**

The current Earth's atmosphere provides air, water, food, and makes possible the physical processes and life on the planet. Thus, economic activities, architecture, and even garments are partially conditioned, , by climate. The influence of climate on man's life is reported throughout history. The geographical distribution of the human race has always had as its primary factor climate conditions, and from this, the development of eating habits, clothing, and agricultural practices that make up the culture of each nation (WANNER *et al.*, 2008).

Changes in the planet from the presence of humans, especially in the last 250 years, are evident. In the literature, the emission of greenhouse gases through the burning of fossil fuels and changes in land use are the main factors of change on the planet that may accelerate in thousands of years the natural processes of climate change and bring serious consequences to life on Earth (IPCC, 2007). However, despite the strong relationship between man and the environment, the understanding about the climatic system and their interactions is still partial and limited, since the atmospheric processes are variable in time and space, sensitive, and difficult to understand due to the complexity of the interactions among the sun, atmosphere, oceans, ice, vegetation, and living beings (MANN, 2002; CAMPBELL *et al.*, 1998). Therefore, there is a meaningful scientific interest in the improvement of knowledge about climate change, and its environmental influences over time, in terms of global and local variations, beyond the distinction between natural and anthropogenic changes.

Over the 4.6 billion years of Earth's history, several episodes of cooling and warming have occurred. Since 1960, advances in the study of climatic events have occurred, and hence, it is possible to identify glacial advances especially in the last 2 million years (WHITE, 2014). However, about the last 6,000 years, there is a larger number of detailed records in the literature (WANNER *et al.*, 2008). The last 2,000 years (late Holocene) Garcia *et al.*, (2007) identified five climatic periods: Cold Period Sub-Atlantic (<150 BC), cold and arid; Warm Roman Period (150 BC to 270 AC), warm and humid; the Dark Ages - DA (270 AC to 950 AC), cold and dry; the Medieval Warm Period - MWP (950 AC to 1400 AC), warm and humid; and the Little Ice Age - LIA (> 1,400 AC), cold and dry. The Holocene itself encompasses the growth and impact of the human species, including all its written history and significant global transition to urban life in the present worldwide (MANN, 2002). In Modern Time, human impacts on the Earth and its ecosystems can be considered of global importance for the future

evolution of living species, including the data of the lithosphere or more recently evidence of human impacts on air (CAMPBELL *et al.*, 1998).

In this study, two periods of the late Holocene have major importance: the Medieval Warm Period and the Little Ice Age for representing changes in climate on a global scale and also for to be caused by internal and external factors to the climate system (MANN, 2002; CAMPBELL *et al.*, 1998). The scope and magnitude of these two climatic events become important for the detection of anthropogenic influences on global climate issues. For example, the increase of air temperature in the 20th century can be a natural improvement of the LIA (CLEAVELAND and STHALE, 1994).

The LIA period was characterized by changes in hydrological cycle and perturbation of ecosystem (WHITE, 2014; DEZILEAUE *et al.*, 2011; LEROY *et al.*, 2011). Even though there is a lot of evidence of LIA, the causes and mechanism are still under debate. Most studies have demonstrated this period based on data from the Northern Hemisphere (STANSEL *et al.*, 2013; LEROY *et al.*, 2011; DEZILEAU *et al.*, 2010). However, this cold period was also recognized in the Southern Hemisphere. In South America (GUTIERREZ *et al.*, 2009; MORALES *et al.*, 2009), advances in glacier were observed in the Andes (IRIONDO, 1999) and aridity in the lowlands of Argentina (CIOCCALE, 1999) and Venezuela (IRIONDO, 1999). There are few studies demonstrating such events in the South Hemisphere; nevertheless, evidence from mountain glaciers suggests increasing glaciation in Patagonia (GROVE, 2001). In Brazil, the studies are scarcer. Some studies have demonstrated changes in vegetal composition during the LIA period, as well as sea level regression in the northeastern and southeastern regions (OLIVEIRA *et al.*, 2014; PEREIRA *et al.*, 2009; COHEN *et al.*, 2005).

#### **1.4.6 Biomarkers of Climate Changes**

To correctly evaluate the climate data measured in recent times and estimate the impact of mankind relative to natural variability, reliable palaeoclimatic data (proxies) are needed. Among the reasons for the increasing advances in paleoenvironmental studies, the key issue is the improvement of high-resolution climate reconstruction using reliable, accurate, and well-calibrated proxies from both marine and terrestrial realms. Several geochemical proxies based on inorganic and organic fossil compounds remains have been applied in attempts to reconstruct continental/sea surface temperatures and atmosphere/biosphere changes in response to climatic events (BLAGA *et al.*, 2010; LIN *et al.*, 2008).

Sediments represent good and continuous archives of temporal and spatial variations in climate on the continent. Several proxies have been developed for reconstructing continental climate on the basis of such sediments, using preserved fossil parts of organisms, pollen (BEHLING *et al.*, 2004; EMPSON *et al.*, 2002), diatoms (KORHOLA and WECKSTROM, 2000), ostracods, and chironomids (RAMDANI *et al.*, 2001)—or using the TEX<sub>86</sub> proxy (BLAGA *et al.*, 2010). Although, accuracy is an important factor, some proxies are relatively robust, mainly proxies for early modern global cooling (such as tree rings and ice cores), and are often written under weather descriptions and observations of physical phenomena (such as glacial movements and river freezing) requiring additional confirmation (WHITE, 2014). In marine studies, the  $\delta^{18}\text{O}$  values and Mg/Ca ratios for foraminifera (ELDERFIELD and GANSSEN, 2000) are commonly used as inorganic sea water temperature proxies. Marine temperature reconstructions from organic fossil remains are generally based on the alkenone unsaturation index (ANDERSON and MEYERS, 2012), but more recently, also on the TEX<sub>86</sub> proxy (WEIJERS *et al.*, 2011; TIERNEY *et al.*, 2010). For terrestrial environments, few quantitative temperature proxies exist. For example, palaeoclimatic reconstructions from palaeosoils using fossil plant assemblages (UTESCHER and MOSBRUGGER, 2007) afford snapshots of past environments but are relatively scarce through the fossil record.

With more widespread biomarkers data and multi-proxy reconstructions of temperature change available today, the spatial and temporal character of these putative climate epochs can be reassessed. In the last decades, the magnitude of anthropogenic impact on the climate and environment was measured by biomarker distribution (ZOCATELI *et al.*, 2012; LEROY *et al.*, 2011; LIN *et al.*, 2008). However, biomarkers may present an even greater potential in providing information. Biomarkers that do not derive from the vegetation itself but from microorganisms living in the peat bog underneath could be a useful source of information. One group of such compounds consists of glycerol dialkyl glycerol tetraether (GDGT), which are lipids from cell membrane of archaea bacteria that live in soil (WEIJERS *et al.*, 2011).

GDGT is the polar part of basic constituents of the cell membrane, which is composed of the polar head group bonded to a non-polar structure of hydrocarbon (BLAGA *et al.*, 2010). The key attribute that makes GDGTs valuable as temperature proxies is their structural dependence on temperature. When temperature increases, cyclopentane moieties are formed within the isoprenoidal chain and the degree of methylation (MBT) and cyclization (CBT) of these compounds change (TIERNEY *et al.*, 2010).

According to Weijers *et al.* (2011), there are some environmental controls on the distribution of GDGTs. Thus, two indexes were suggested to quantify the degree of methylation (MBT) and cyclization (CBT) of these compounds and to calculate and reconstruct mean annual air temperature (MAAT) and pH. Branched GDGT compounds are fluvially transported and when became part of the sedimentary archive, their distribution, expressed in the methylation and cyclisation ratios of branched tetraethers (MBT–CBT), can be used to explain and assess soil pH and air temperature past, both in geological times, including the last glacial – interglacial transition (WEIJERS *et al.*, 2011; PETERSE *et al.*, 2009a; PETERSE *et al.*, 2009b).

#### **I.4.7 Chronology in Environmental Studies**

In general, the reconstruction of past scenarios by use of biomarkers in sediments is often associated with radio-analytical techniques. Radionuclides are elements with unstable atomic structures that emit several types of particles in order to become more stable (FORTE, 1996). These elements have chemical behaviors similar to their stable isotopes and are under the effects of the same natural processes found in all of Earth's matrixes, such as soil, oceans, and sediments. Hence, they can be used as tracers for physical and chemical natural processes (NEVES *et al.*, 2014). Thus, the discovery of analytical techniques able to measure the activity of these radionuclides became an important tool in environmental use, mainly in chronology of sediments and analysis of environmental changes over time.

Among chronological techniques, the dating of soils and sediments based on the determination of excessive  $^{210}\text{Pb}$  activity is a powerful technique to assess the geochronology of deposits and the processes of particulate matter in recent sedimentation (FORTE, 1996). It is an important tool when the aim is to obtain background values in periods prior to the man action; information related to anthropic impact with origin in industrial, agricultural or domestic processes; or to reconstruct past environmental scenarios (KOMARECK *et al.*, 2008; CAZOTTI, 2006). With the aid of geochronological dating profiles, it is possible to observe the temporal distribution profiles of chemical markers and correlate them with historical/climatic events and use/occupation of land (CAZOTTI, 2006; GATTI *et al.*, 1999; GALE *et al.*, 1995).

Some authors have evaluated the use  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ ,  $^{55}\text{Fe}$ , and  $^{32}\text{Si}$  for dating of sediments from lakes and rivers and for estimating recent sedimentation rate. This increase in use occurred thanks to remarkable technological advances, which allowed the development of proportional detectors, mass spectrometers, and liquid scintillation,

appropriated to accounting of several radioactive isotopes types in low concentrations (KOMARECK *et al.*, 2008,; EREL *et al.*, 1997).

The  $^{210}\text{Pb}$  radioisotope with a half-life of 22.26 years, is a natural radionuclide originating from the  $^{238}\text{U}$  decay series, which follows the  $^{226}\text{Ra}$ . The radioisotope  $^{226}\text{Ra}$  (half-life 1,622 years) decays to produce the inert gas  $^{222}\text{Rn}$  (half-life 3.83 days), following a series of nuclides with short half-lives to produce  $^{210}\text{Pb}$ . A fraction of  $^{222}\text{Rn}$  atoms, formed by the decay of  $^{226}\text{Ra}$  in soil, migrates for atmosphere where it decays to  $^{210}\text{Pb}$ , which reaches the surface of the soil, lakes, and oceans by "fallout" atmospheric (EREL *et al.*, 1997). The  $^{210}\text{Pb}$  that reaches the surface of the lakes is rapidly adsorbed by fine particulate matter ( $<0.45\ \mu\text{m}$ ) and deposited at the bottom of rivers, lakes and oceans (FORTE, 1996). Since sediments constitute a continuous deposition surface of organic and inorganic particulates, the maintenance of hydrosphere-atmosphere interaction ensures the introduction of trace elements, both stable and radioactive, in the sediments, either through runoff or the "fallout" atmospheric (KOMARECK *et al.*, 2008). In this decay process of  $^{238}\text{U}$  to  $^{210}\text{Pb}$ , the sedimentation rate can be calculated as a differential in chronological techniques for environmental studies.

Although in recent decades, there have been a large number of studies related to the use of  $^{210}\text{Pb}$  to determine man's impact on the environment over time (SCHEEL-YBERT, 1999; FAIRHALL and YOUNG, 1970), this technique is limited for dating of recent sediments due to Pb radioactive decay (FERREIRA, 2014). For dating of ancient sediment, other techniques are used. Among them, the  $^{14}\text{C}$  Carbon dating is most used in environmental studies (SCHEEL-YBERT, 1999; OLSSON and POSSNERT, 1992).

From 1950, there was an increase in the  $^{14}\text{C}$  dating method use. It became the main tool for chronological determination of the past episodes originating approximately 40,000-50,000 years ago (FAIRHALL and YOUNG, 1970). The method is based on the continuous production of radioactive carbon ( $^{14}\text{C}$ ) in the upper atmosphere by the reaction of cosmic neutrons with nitrogen atoms ( $^{14}\text{N}$ ).  $^{14}\text{C}$  is then oxidized to  $^{14}\text{CO}_2$  and enters the global carbon cycle. Plants assimilate  $^{14}\text{C}$  during photosynthesis, while animals ingest plants. Thus, all living beings maintain their  $^{14}\text{C}$  terrestrial input during their lives (OLSSON and POSSNERT, 1992). The  $^{14}\text{CO}_2$ , as well as  $\text{CO}_2$ , dissolves in the oceans and is available to the plankton, corals, mollusks and fish, so that all living beings are continuously  $^{14}\text{C}$  replenished. At the death of plants or animals,  $^{14}\text{C}$  input ceases, and the time of death can be established by determining the  $^{14}\text{C}$  residual once the half-life of  $^{14}\text{C}$  is 5,730 years (SCHEEL-YBERT, 1999).

The  $^{14}\text{C}$  activity of a sample is determined using the gas proportional counter, liquid scintillation spectrometers with low levels of background radiation, and/or technical AMS (Accelerator Mass Spectrometry). The last is used for samples with a carbon concentration lower than 0.1g. .

## **CHAPTER II**

### **STUDY AREAS AND METHODOLOGY**

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## II.1 STUDY AREAS AND SAMPLING

To study the climatic and environmental changes during two transition periods of important climatic events on a global level, two study areas with distinct characteristics were selected. For a more ancient period study, the Lake Ohrid, in Ohrid City, Southwestern of Macedonia, was chosen since its watershed has considerable potential to provide ancient sediment core. However, due to its great potential for ancient studies such as glacial and interglacial ages, more recent studies related to the anthropogenic and climatic changes in this area, such as of the last millennium (Late Holocene), are scarce.

For a more recent study of climatic and anthropogenic changes, the upper portion of the Barigui River watershed that comprises the northern region of Curitiba and Almirante Tamandare, South Brazil, was chosen. This area was selected from a study, in which were collected five sediment cores throughout the watershed. Such sediment cores were characterized according to chronology, pollution level, and representativeness. Among the five cores, one of them (labeled PT) corresponds to the deposition of approximately 400 years ago while the others between 150-200 years. So the PT core was selected for this study due to its temporal representation (approx. 400 years against 150-200 years in other cores) and the amount of pollutants, since slightly polluted areas may mask the human impact while highly polluted areas present difficulties with respect to analytical tools.

The characteristics of each study area are explained in sequence, which justifies complementarily the choice of such areas and collection procedures. For methodological reasons, the study areas were defined based on catchment area; however, since this study is to show evidences of climate changes, most of the results have regional significance.

### II.1.1 Lake Ohrid Watershed

Ohrid Lake is one of the oldest in the world. Its sediment preserves information about past events, including the ice age (LEZINE *et al.*, 2010). More than 200 endemic species ranging from phytoplankton and macrophytes to gastropods and cyprinid and predatory fish (CASTANEDA *et al.*, 2010) living in a water body covering “just” 360 km<sup>2</sup> make Lake Ohrid the most biodiverse lake in the world (ALBRECHT and WILKE, 2008). Assuming constant mutation rates for Lake Ohrid gastropod species, biologists estimated the age of the lake to be around 1.5 million years (VOGEL *et al.*, 2010), which matches the age estimate from the latest geophysical and geological

investigations. Lake Ohrid's watershed is one of three refuges of the Mediterranean, essential for the maintenance of important plants groups that survived the harsh climate during the Pleistocene ice ages (BREWER *et al.*, 2002).

Lake Ohrid is particularly suitable for studies of natural and anthropogenic environmental change due to its small catchment. As there is no contribution from larger rivers, the supply of allochthonous, organic matter depends entirely on the hydrology of the Ohrid watershed itself (CASTANEDA *et al.*, 2010; MATZINGER *et al.*, 2006). About half of the water inflow derives from seepage from neighboring mesotrophic Lake Prespa. However, despite calcium ions and nutrients such as phosphorous, these waters do not deliver any significant amount of organic matter (MATZINGER *et al.*, 2006). Thus, surface drainage of the immediate surroundings controls the inputs of plant and soil organic matter from the terrestrial biosphere.

Lake Ohrid ( $40^{\circ}54' - 41^{\circ}10' \text{ N}$ ,  $20^{\circ}38' - 20^{\circ}48' \text{ E}$ ) (FIGURE II-1) is located at 693 m above sea level, and it is shared between the Former Yugoslav Republics of Macedonia and Albania. The lake is 30 km long, 15 km wide, and covers an area of 360 km<sup>2</sup>. The basin morphology is a relatively simple tub shape with a maximum water depth of 286m (WAGNER *et al.*, 2008). From March to November, the water column of Lake Ohrid above 150 m depth is thermally stratified, being mixed in winter following the typical seasonality of temperate lakes. A Halocline is observed below 150 m depth. The salinity gradient is only overcome by complete mixing to the full depth of the lake roughly once per decade (MATZINGER *et al.*, 2006).

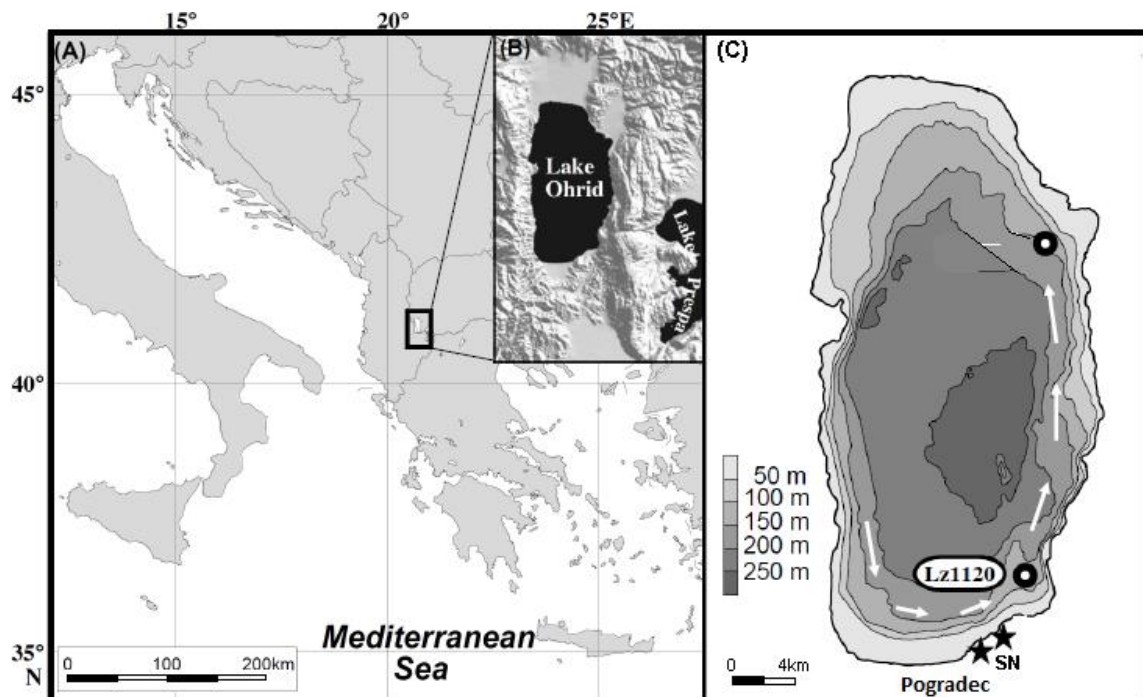


FIGURE II- 1. MAP WITH LOCATION OF Lz1120 COLLECTION AREA.  
SOURCE: HOLTVOETH *et al.*, 2010

The oligotrophic conditions in Lake Ohrid result from inflows, which are depleted in minerals and nutrients. Most of the inflow to the lake is supplied by groundwater from abundant karstic sources in the relatively small natural catchment area of 1,042 km<sup>2</sup>, which was artificially enlarged to 1487 km<sup>2</sup> by diversion of the Sateska River in 1962 (MATZINGER *et al.*, 2006).

Situated on the shores of Lake Ohrid, the town of Ohrid is one of the oldest human settlements in Europe. Around the middle of the 4th century B.C., King Philip II of Macedonia seized Lychnidos (as Ohrid was known) and the settlements on the shores of the Lake Ohrid. After conquering these regions in 148 B.C., the Romans built two other cities via Lychnidos, resulting in a development of the area. With the spread of Christianity, which began to penetrate these regions toward the end of the 3rd century, the classical shrines were gradually destroyed and replaced by monumental early Christian churches, fragments of which have been discovered in several areas in Ohrid and its surroundings (VAUCHEZ and LAPIDGE, 2000). Currently, most of the watershed is urbanized and is in an important touristic center. The main attractions at Ohrid include the ancient fortifications that surround the town and the ancient fortress of King Samuil that crowns the city (FIGURE II-2).



FIGURE II- 2.OHRID CITY AND FORTRESS OF KING SAMUIL (MACEDONIA).

One sediment core, labeled Lz1120, was taken in 2005 from a floating platform using a gravity corer for undisturbed surface sediment and a piston core for deeper sediments. The coring system is equipped with PVC liners of 6 cm in diameter. Composite sediment successions were obtained by overlapping 3 m core sections, resulting in contiguous sediment sequences of 11.51 m. Samples were freeze-dried and homogenised. Core Lz1120 was collected in the southeastern area of the lake at 105 m water depth. This area is influenced by the karst springs of Tushemisht and Sveti Naum, which, together with subaquatic karst springs in the area, are fed by seepage from neighbouring Lake Prespa and account for about 50% of the hydrological inflow of the lake (MATZINGER *et al.*, 2006). Near the Lz1120 site is the city Pogradec in Albania and the Saint Naum Monastery (FIGURE II-3) in Macedonia.



FIGURE II- 3. POGRADEC CITY (ALBANIA) AND SAINT NAUM MONASTERY (MACEDONIA).

### 2.1.2 Barigui River Watershed

The Barigui watershed (25°13'24" and 25°38'23" south and 49°15'00" and 49°22'29" West) is one of most studied watersheds in the Paraná state (DOMBROSKI *et al.*, 2012; FROEHNER *et al.*, 2009; FROEHNER and MARTINS, 2008; CHELLA *et al.*, 2005). It corresponds to 35% of the total area of municipality of Curitiba's watersheds and is a privileged area in terms of hydrometeorological observations (FROEHNER and MARTINS, 2008; CHELLA *et al.*, 2005; FILL *et al.*, 2005). The Barigui River is 67 km long, draining a watershed of 279 km<sup>2</sup>, with 120 km<sup>2</sup> of drainage located in the municipality of Almirante Tamandare, 144 km<sup>2</sup> in the municipality of Curitiba, and 15km<sup>2</sup> in the municipality of Araucaria. It flows from north to south through the city of Curitiba and has been strongly influenced by urbanization throughout time; hence, it has considerable potential to represent environmental shifts over time. The sampling station was located inside Tingui Park (FIGURE II-4) in latitude 25°23'55.81" and longitude 37°35'33.84", catchment is of this sampling station and comprises the northern region of Curitiba and Almirante Tamandaré. Previous monitoring studies define this area as reliable for collection and encompass the most significant data (DOMBROSKI *et al.*, 2012; FROEHNER and MARTINS, 2008).

Curitiba, the capital city of Paraná State, was founded in 1693, although historians report the first settlement in 1654 on the shores of Atuba River (northern region of the city). Its first settlements were European immigrants, mainly Portuguese, Spanish and local natives (WACHOWITCZ, 1995). The first economic activities were related to agriculture of subsistence and mineral extraction. Cultivation of yerba mate led to the increase of welfare in the city at the beginning of the 20th century when the urbanization of the city expanded (FERREIRA, 1996). From 1930, the settlement process was characterized by internal migration mainly from São Paulo, Minas Gerais and Rio Grande do Sul. Until 1960, demographic growth occurred as from the original nucleus of the city; after that, immigration occurs for adjacent neighborhoods and to the metropolitan area, including Almirante Tamandaré. This city began with gold mining in 1680; later, farmers arrived and settled the region, which grew slowly and became a village in 1890. Concomitantly with Curitiba's growth, the population of Almirante Tamandaré grew 79% in 1920, and 123% in 1980, due to the extension of Curitiba urban area (IPPUC, 2009).

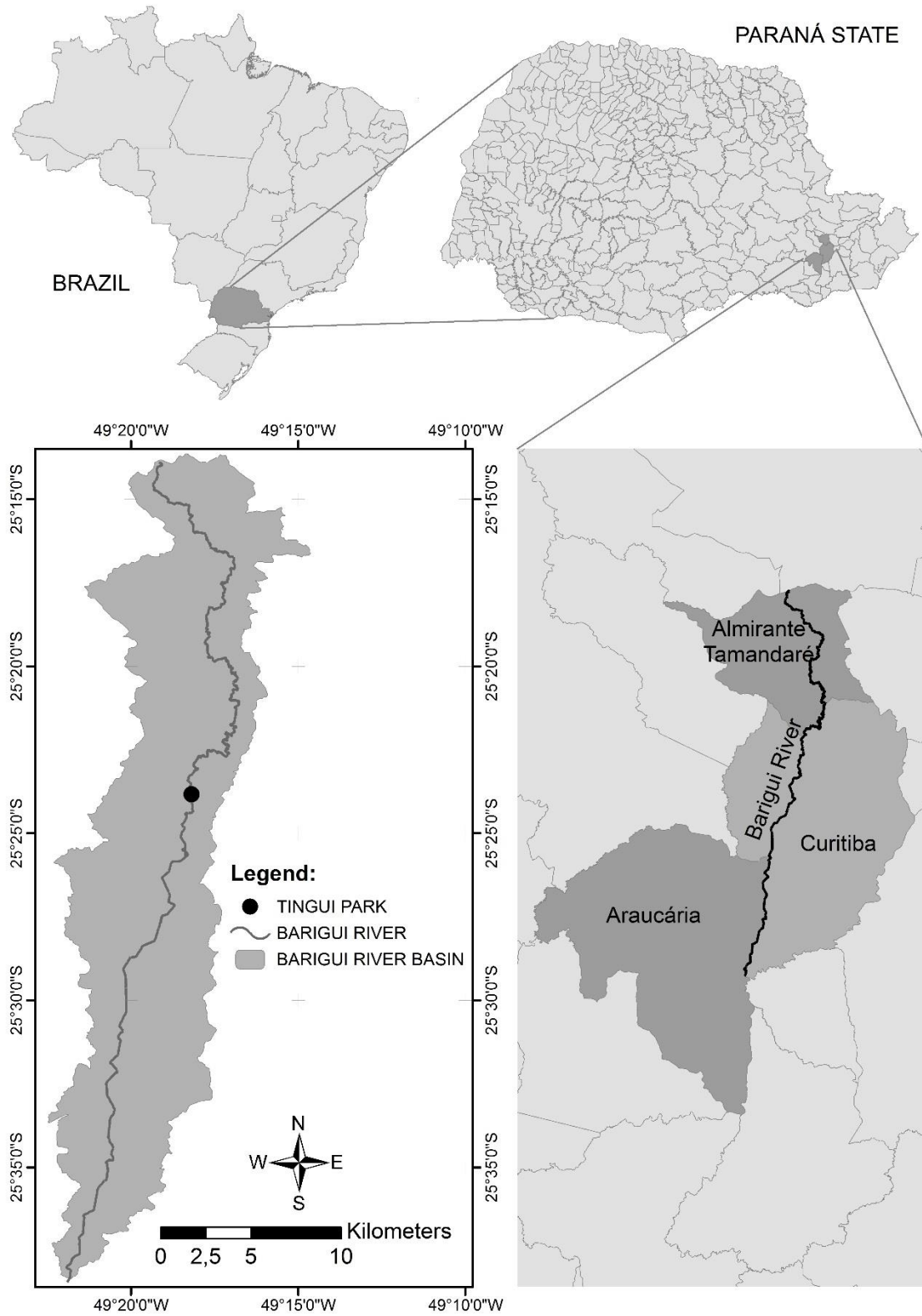


FIGURE II- 4. MAP OF THE BARIGUI RIVER WATERSHED AND SAMPLING SITE.

Currently, throughout the Barigui River watershed, areas with dense terrestrial vegetation appear mainly to the north of the watershed, while aquatic macrophytes are present throughout the river, once limnological conditions vary along its length. C3 plants are predominant, basically trees and some species of scrubs, while C4 plants play a minor role in the present vegetation and are summarized in herbaceous plants mainly from pteridophytes group (KOZERA *et al.*, 2006; CERLING *et al.*, 1997). The forest vegetation consists basically of caducifolia, subtropical trees with *Araucaria angustifolia*, *Bracatinga* (*Mimosa scabrella*) tropical and subtropical rainforest (FROEHNER and MARTINS, 2008) (FIGURE II-5). Tree species of Myrtaceae, Lauraceae, Aquifoliaceae and Flacourtiaceae are also found. Pteridophytes herbaceous in the area are predominant and consist in species of Poaceae, Cyperaceae and Dryopteridaceae, in addition to scrubs of Solanaceae and Asteraceae species (KOZERA *et al.*, 2006). Regarding aquatic plants, three families of macrophytes are generally found in south Brazil: Haloragaceae (*Myriophyllum aquaticum*), Onagraceae (*Nymphoides indica*) and Pontederiaceae (*Eichhornia azurea*) (MALTCHIK *et al.*, 2005).

In the northern watershed, belonging to the county of Almirante Tamandare, the predominant land use is rural. There are scattered urban areas, and among them the city of Almirante Tamandare. In the middle of the watershed, which contains part of the city of Curitiba, the occupation is preponderantly urban, with a predominantly residential use, trade, and services (FROEHNER *et al.*, 2009).

The area studied is geographically located in the south Capricorn tropic, where atmospheric intertropical and polar systems clash, resulting basically in a subtropical humid weather, mesothermal, with cool summers, severe frosts in the winter and no dry season (IAPAR, 2014; SILVA and GUETTER, 2003). Annual average temperature in the last decade was 17.8°C. Annual precipitation is well distributed along the year with an average of 1390mm (SIMEPAR, 2014). Currently, four streams control the climate in south Brazil: Tropical Atlantic, Atlantic Polar, Tropical Continental and Equatorial Continental, which are formed in action centers such as Intertropical Convergence Zone (ITCZ) migration and Southern Oscillation (SO) (HENDY *et al.*, 2002).

The sediment core sample was collected in January 2012 using a small gravity corer with a 6cm diameter core barrel. The core sample was transported to the riverside, extruded and sectioned at 2 cm intervals to a depth of 40 cm. Below 40 cm, the core was sectioned every 4 cm to 60 cm and then every 10 cm to 100 cm. The core sections were stored at 4°C during transport to the laboratory, where they were frozen (-20°C) and lyophilized prior to analysis.



FIGURE II- 5. SAMPLING STATION OF PT CORE INSIDE TINGUI PARK

## II.2 METHODOLOGY

Biomarkers that best represent the study areas were selected, according to a literature review, in order to identify those conventionally applied in studies of anthropogenic and climatic changes over time, with efficiency already reported in the literature.

Due to significant differences in chemical composition between cores PT and Lz1120, different analytical procedures were used. Samples from the Lz1120 core have a less complex chemical composition, and the application of improved procedures for cleanup is not required. In this case it was possible to determine total lipids. In contrast, samples from the PT core have a complex chemical composition, with a high amount of unknown compounds that may act as contaminants, and therefore, several clean up procedures, including fractionation on a silica column, were needed to quantify the analytes. Thus, the analytical methodology will be described separately in each core.

### II.2.1 Methodology applied in Lz1120 core (Lake Ohrid)

#### II.2.1.1 Geochronology

Radiocarbon dating of  $^{14}\text{C}$  was used to establish the chronology of the sediment sequence in the Lz1120 core. All samples were dated. Energy-dispersive-spectrometry (EDS) was performed using an EDAX-DX micro-analyser mounted on a Philips SEM 515 (operating conditions: 20kV acceleration voltage, 100 s live time counting, 10-9 A beam current, ZAF correction). Instrument calibration and performance are described in Marianelli and Sbrana (1998). Radiocarbon dating of core Lz1120 was conducted by accelerator mass spectrometry (AMS) at the Leibniz Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany. The radiocarbon ages were calibrated into calendar years using CALIB 5.0 (WAGNER *et al.*, 2009).

#### II.2.1.2 Determination of Lipid and inorganic compounds

The sediment samples were freeze-dried and homogenised. Measures of total carbon (TC), total nitrogen (TN) and total sulphur (TS) contents of Lz1120 core were carried out using a CE Instruments NC 2500 elemental analyser. Total Inorganic Carbon (TIC) was quantified from the difference between TC and total organic carbon

(TOC), after acid digestion with HCl (16% v/v). The weight percentages of calcium carbonate ( $\text{CaCO}_3$ ) were calculated according to Equation 1.

$$\text{CaCO}_3(\%) = \text{TIC} * \text{M}_{\text{CaCO}_3} / \text{M} \quad (\text{Equation 1})$$

where M = molecular weight.

Total Lipids were analysed and quantified according to the analytical procedure described by Holtvoeth *et al.*, (2010). Briefly, the samples were freeze-dried (1-2 g), then extracted by sonication (45 min.) using a mixture of dichloromethane (DCM) and methanol (9:1). The total lipid extract (TLE) was concentrated, and potentially remaining water was removed by passing through a column of anhydrous sodium sulphate. Free and bound acids were then trans-methylated by addition of a solution of acetyl chloride in methanol (1:30) and heating the samples to 45°C for 12 h. Acids in excess (acetic acid, hydrochloric acid) were removed by passing through a column of potassium carbonate in dichloromethane. In order to convert compounds containing hydroxyl groups into trimethylsilylethers, the TLE was derivatized with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, with 1% trimethylchlorosilane), at 65°C, for 30 min. A blank sample was included in every batch to ensure that no contamination was introduced to the samples during any of the steps. Molecular identification of total lipids was performed on a Trace 2000 Series gas chromatograph fitted with a J&W Scientific DB-5MS capillary column (60 m, 0.25 mm i.d.; 5% phenyl/95% methylpolysiloxane equivalent phase, 0.1  $\mu\text{m}$  film thickness; carrier gas: helium at 1.6  $\text{mL}/\text{min}^{-1}$ ; on-column injector). The oven temperature was programmed from 60°C to 170°C at 6°C  $\text{min}^{-1}$  after 1 min, then to 315°C at 2.5°C  $\text{min}^{-1}$  and held for 10 min. The column was fed directly into a Thermoquest Finnigan TSQ 7000 mass spectrometer (MS). Typical operating conditions were ionisation potential 70 eV; source temperature 215°C; trap current 300  $\mu\text{A}$ . Mass data were collected at a resolution of 600, cycling every second from 50–600 Thompsons.

The Mass spectra, relative retention times and authentic standards were used to identify the individual lipids. Compounds were quantified based on their peak area relative to peak area of the surrogateH)-cholestane that was added to the samples prior to extraction. The relative response factors of the analytes were determined individually using authentic standards for 35 representative fatty acids, alcohols, alkanes and sterols. Response factors for analytes in which standards were unavailable were assumed to be identical to those of available compounds of the same class.

### II.2.1.3 Isotopic Analysis

Stable carbon isotopic analysis were carried out on two samples from conjunction of several samples from specific periods to verify changes in isotope signatures of fatty acids and alcohols chains. The first sample consists of samples from the 910 to 955 period and represents the Dark Ages. The second sample consists of samples from 1160 to 1275 and represents the Medieval Warm Period. After extracting the total lipids (TLEs), fatty acids and alcohols were separated using silica columns and solvent mixes of increasing polarity. Methanol with 0.1% of hydrochloric acid was used to elute fatty acids and methanol (100%) to elute alcohols. After separation, fatty acids were trans-methylated using the same procedure as described for the TLE, while the alcohols were acetylated with 40  $\mu$ L of a solution of anhydrous pyridine and acetic anhydride (1:1 v/v; 40  $\mu$ L) at 60°C for 1 h (HOLTVOETH *et al.*, 2010).

Stable carbon isotopic compositions of individual fatty acids and alcohol chains were determined according to the procedures described in II.2.2.5, in sequence of this study.

## **II.2 2 Methodology applied in PT core (Barigui River)**

### II.2.2.1 Geochronology

All samples from PT core were dated.  $^{210}\text{Pb}$  and fallout  $^{137}\text{Cs}$  dating was performed on bulk sediment ground < 63 $\mu$ m using an EG&G ORTEC low-background gamma spectrometer (hyper-pure Ge, model GMX25190P) following the method described by Figueira (2007). Precision and accuracy of the methodology were evaluated using three certified reference materials: IAEA-326 (soil), IAEA-327 (soil) and IAEA-385 (marine sediment) to determine the radionuclides of interest. The activity concentrations obtained for the certified radionuclide were close to the reported values with mean deviations and errors not exceeding 6%.

Two models were used to calculate the sedimentation rate: initial concentration of unsupported  $^{210}\text{Pb}$  (CIC) and constant rate of supply of unsupported  $^{210}\text{Pb}$  (CRS) models according to Neves *et al.* (2014). Mass accumulation rates (MARs) were calculated based on bulk density measurements and  $^{210}\text{Pb}$  and fallout  $^{137}\text{Cs}$  data. The age of sample deposition (I) was established, considering the sample depth in the core (z) and the time of core collection ( $A_0$ ), and sedimentation rate (v), according to  $I = A_0 - (v/z)$ , and considering the time of core collection, year 2012.

The ages of the samples until 1760 present the accuracy of the method. The ages of samples before 1760 resulted from an extrapolation and were validated by historic events and previous features of the area reported by historians (SMITTEMBERG *et al.*, 2004).

### II.2.2.2 Granulometric composition

The sand, silt and clay content analyses were carried out at the Laboratory of Mineral and Rock Analysis (LAMIR-UFPR) using an integrated technique (sieving and laser diffraction). Briefly, samples were passed through a set of sieves with standardized mesh screens (mesh size 8, 14, 42, 80, 170, 250, 400, 500 and 635). The classification in sand, silt and clay was carried out according to the Wentworth granulometric scale (BESLER and RITTER, 2010). The fine grains were collected and analyzed with a laser analyzer that measures the grain size by detecting the diffraction angle from a bunch of light after it had passed through the sample in an optical cell (FROEHNER and MARTINS, 2008).

### II.2.2.3 Phosphorus, Nitrogen and Total Organic Carbon Analysis

A phosphorus analysis was carried out according to Mater *et al.*, (2004). For each sample, two subsamples of 0.5 g dried sediment were used for the phosphorus analyses. One subsample was initially placed in a furnace for 1 h at 500°C, after which both of the subsamples were shaken with 10 mL 1.0M HCl solution for 1 h. After centrifugation (6,000 rpm, 10 min), the supernatants were digested for 4 h with 1.6 mL potassium persulphate and 4.5 M sulphuric acid solution at 80°C. The extracted phosphorus from the calcinated fraction was considered total phosphorus (TP), while the extracted phosphorus without calcination was considered inorganic phosphorus (IP). After extraction, both the TP and IP contents were quantified as orthophosphate using the molybdate ascorbic acid method (KOROLEFF, 1983). The organic fraction contents of phosphorous (OP) were obtained by the difference between the TP and IP contents.

Total organic carbon (TOC) and total nitrogen (TN) contents were measured using a vario Micro cube. The TOC contents were determined after acid digestion of the carbonate fraction with hydrochloric acid 16% v/v (HOLTVOETH *et al.*, 2010).

### II.2.2.4 Extraction and Analysis of Alkanes, PAHs and Sterols

Molecular structures of polyaromatic hydrocarbons (PAHs) and sterols analysed are presented in FIGURE II-6.

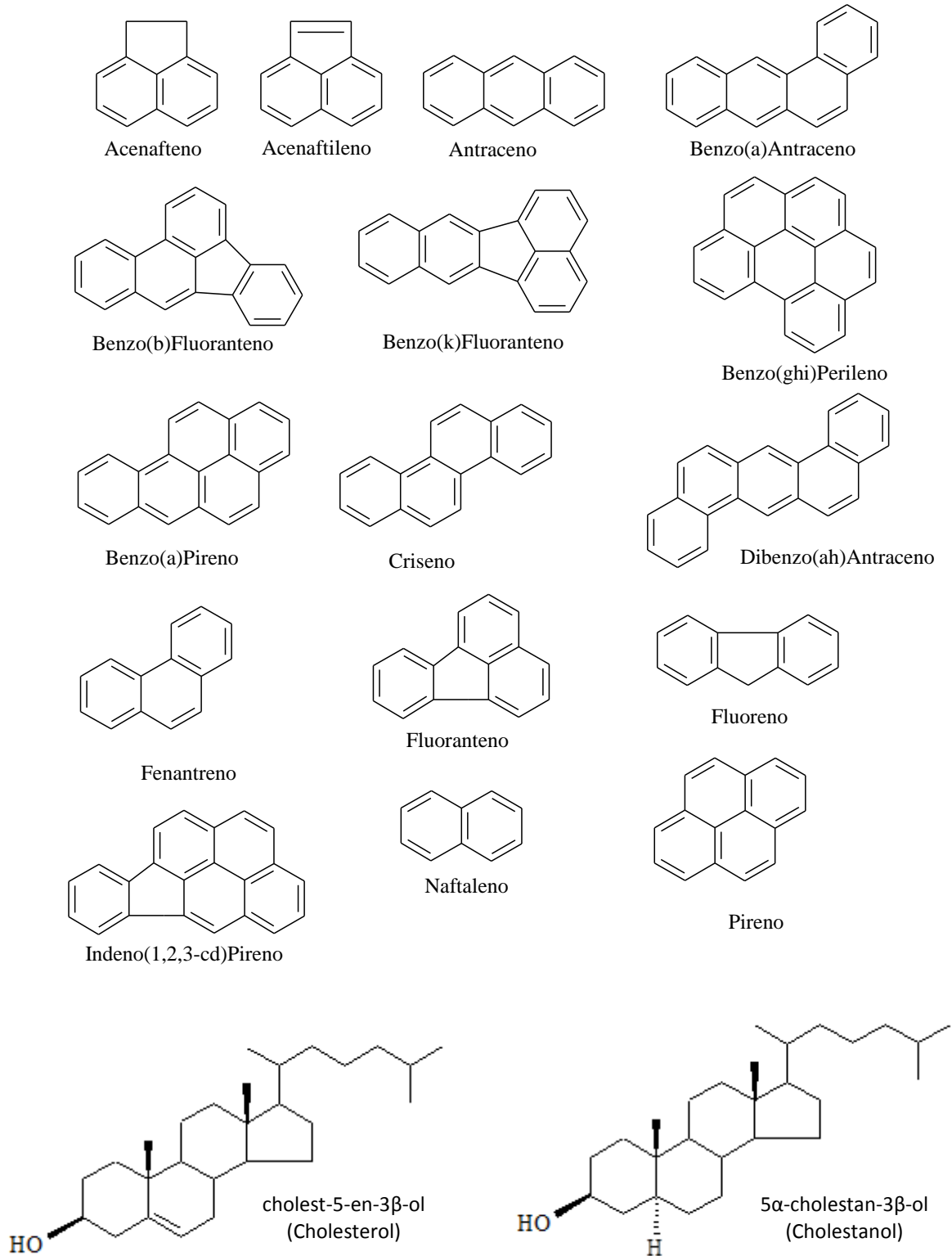


FIGURE II- 6. MOLECULAR STRUCTURE OF STEROLS (CHOLESTEROL AND CHOLESTANOL) AND PAHs ANALYZED.

The procedures described by Hofmann *et al.*, (2011) and Holtvoeth *et al.*, (2010) were followed with minor modifications. Bulk sediment (5 g) was extracted using an accelerated solvent extractor (ASE; Dionex) in 5-minute static cycles (100°C; 4MPa) with a mixture of dichloromethane (DCM) and acetone (1:1 v/v). Internal standards, 5 $\alpha$ (H)-cholestane and a deuterated PAH (acenaftilene-d8) were added prior to analysis. Sulfur was removed from the extracts using activated copper added directly after the extraction and contact time of 24h.

The fractions containing the n-alkane, PAHs and sterols were isolated using columns (7 mm i.d.) filled with sodium sulphate (approx. 0.2 g) and activated silica gel (approximately 1.3 g). n-Alkanes were eluted with 3 x 4 mL of hexane (Fraction 1); PAHs were eluted with DCM (3 x 4 mL, Fraction 2) while sterols were eluted with methanol (3 x 4 mL, Fraction 3). The fractions 1, 2 and 3 were completely dried under gentle nitrogen flow prior to dissolution in 80  $\mu$ L of hexane in F1 and 100  $\mu$ L of DCM in F2 for analysis. Immediately before analysis, 2.21 Dimethyldocosane (DMD), a deuterated PAHs (fluorine-d10) and 5 $\alpha$ (H)-cholestane were added to fractions 1, 2 and 3, respectively, as internal standards. The fraction 3 before analysis was derivatised using (N,O-bis-(trimethylsilyl)-trifluoroacetamide (+1% trimethylchlorosilane) at 65 °C for 30 min. After derivatisation, it was dried and dissolved in 80  $\mu$ L of DCM for analysis. Extraction efficiency of all compounds was assessed before analysis by adding of 200 $\mu$ L of standard solution (1mg/L) in samples free of analysed compounds. Extraction procedure followed as mentioned above and recovery tests were greater than 70% for most analytes. All solvents were HPLC grade; silica gel was extracted with DCM (4h) and activated in an oven (400°C; 4h). FIGURE II-7 presents a brief outline of the extraction process.

Standard solutions with 35 n-alkanes (AccuStandard Alkanes Mix DRH-008S-R2), 16 PAHs USEPA priority pollutants (AccuStandard PAH Mix Z-014 G) and individual sterols (Cholesterol Sigma Aldric-C8667, Cholestanol Supelco-47129) were used to identify the peaks.

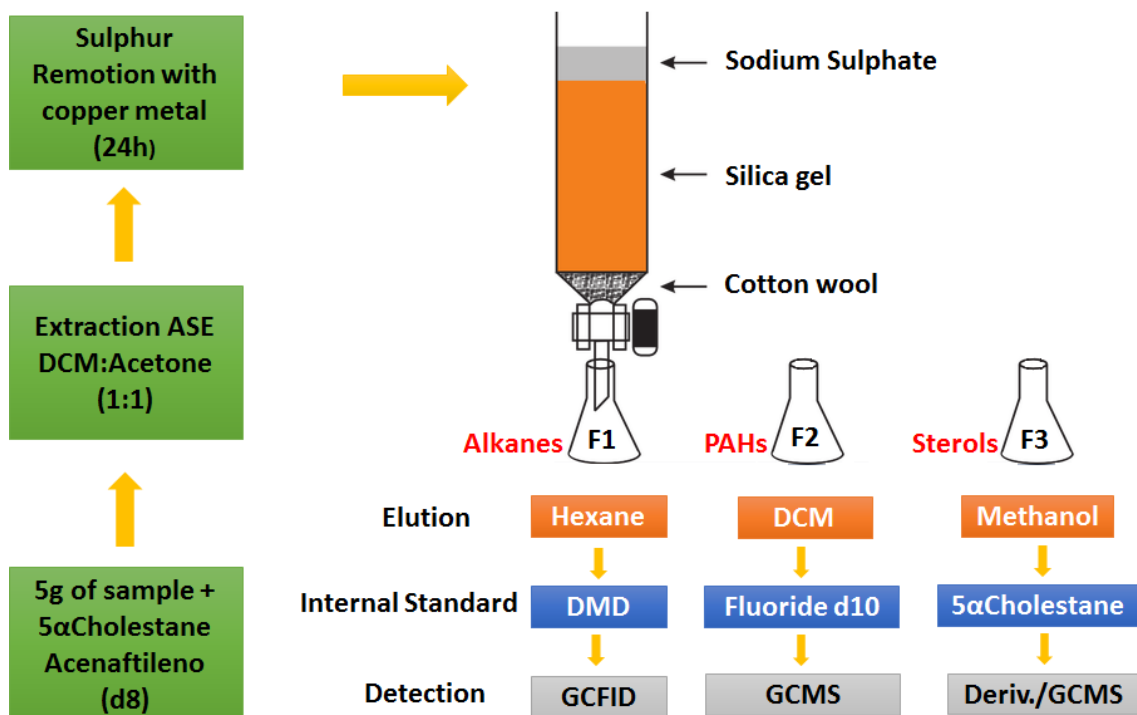


FIGURE II- 7. SCHEME OF EXTRACTION PROCESS OF ALKANES, PAHs AND STEROLS.

n-Alkanes were identified and quantified using an Agilent 6890 Series Gas chromatographic with flame ionization (GC/FID) fitted with a CP Sil 5CB capillary column (60 m, 0.32 mm i.d.; 5% phenyl/95% methylpolysiloxane, 1  $\mu\text{m}$  film thickness). The carrier gas was helium with a flow rate of 2  $\text{mL}\cdot\text{min}^{-1}$ . The temperature programmed was settled as 50°C to 90°C at 15°C $\cdot\text{min}^{-1}$ , and 90°C to 310°C at 5°C $\cdot\text{min}^{-1}$  and held for 10 min. PAHs were identified and quantified by GC-MS using a Varian 450 gas chromatograph (GC) coupled to a Varian 320 Quadrupole mass spectrometer with electron-impact ionization. The GC was equipped with a Factor Four VF 5MS fused silica capillary column (60 m, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) and helium as a carrier gas with a constant flow of 1.2  $\text{mL}\cdot\text{min}^{-1}$ . The GC oven was programmed with an initial hold of 1 min at 40°C, from 40°C to 120°C (at 30°C  $\text{min}^{-1}$ ), held for 1 min and from 120°C to 280°C (at 10°C  $\text{min}^{-1}$ ), held for 15 min. Both full scan and selected ion monitoring (SIM) modes were applied.

Gas chromatographic and mass spectrometric analyses (GC/MS) of the derivatised sterols were performed using a Trace 2000 Series gas chromatograph (GC) fitted with a J&W Scientific D-5MS capillary column (60 m, 0.25 mm i.d.; 5% phenyl/95% methylpolysiloxane, 0.1  $\mu\text{m}$  film thickness). The carrier gas was helium at 1.6  $\text{mL}\cdot\text{min}^{-1}$ . The oven temperature was programmed from 60°C to 170°C at 6°C $\cdot\text{min}^{-1}$ , and after 1 min the temperature increased to 315°C at 2.5°C $\cdot\text{min}^{-1}$  and held for 10 min. The column was fed directly into a Thermo Quest Finnigan TSQ 7000 mass

spectrometer (MS). The operating conditions were ionisation potential 70eV, source temperature 215°C, and trap current 300  $\mu$ A. The mass data were collected at a resolution of 600, cycling every second from 50 – 600 Thompsons.

The alkanes were identified by their relative retention times compared to authentic standards, while PAHs and sterols were additionally identified by their mass spectra. All compounds were quantified based on their peak area relative to peak area of the internal standard. The relative response factors of the analytes were determined individually using authentic standards.

#### II.2.2.5 Stable Carbon isotopic Analysis

After quantification of n-Alkanes, the fraction 1 obtained in II.2.2.2 was used to stable carbon isotope analysis. Before analysis in IR-GCMS, n-Alkanes were separated of branched and cyclic hydrocarbon of fraction 1 via urea adduction according to the procedure proposed by Kristy *et al.* (2002) with minor modifications. First, urea was cleaned with DCM for 24 h. Then, it was dissolved in methanol until the solution was saturated. Methanolic solution of urea (1 mL) was then added to the aliphatic fraction previously dissolved in 4 mL of hexane:DCM (1:1) solution, leading to the immediate formation of a white crystalline precipitate. After centrifuging (3000 rpm, 10 min), the solvent was removed and 4 mL of hexane:DCM (1:1) solution was added again. Urea adduct crystals were then dried under gentle nitrogen flow. In order to remove remaining contaminants, 4mL of hexane was added to the urea adduct crystals, centrifuged and the sobrenadant completely removed. This procedure was repeated.

The non-adduct fraction was obtained by adding 5 mL of water (previously cleaned with DCM) to the crystals, breaking up the urea adduct crystals and releasing n-Alkanes. The liquid-liquid extraction method was used to separate n-Alkanes from water by adding 4 mL of hexane two times. Finally, in order to remove possible remaining water, the organic fraction with n-Alkanes was passed through a sodium sulphate column and completely dried under nitrogen flow for analysis in IR-GCMS.

Stable carbon isotopic compositions of individual n-alkanes were determined using a Delta V Advantage (Thermo Fisher, Bremen) linked to a Trace Ultra with a ConFlo IV interface. Samples in hexane/ethyl acetate were loaded on a TriPlus auto sampler and 1 $\mu$ l injected in splitless mode on a DB5 fused silica column (30 m, 0.25 mm id, 0.25  $\mu$ m film thickness, J&W Scientific). The GC oven temperature was programmed from 45°C (held for 1 min) to 295°C, at 6°C min<sup>-1</sup> and held for 15 min. The injector temperature was 280 °C; ultra-high pure grade helium was used as the carrier gas at a constant flow of 1.4 mL min<sup>-1</sup>. CO<sub>2</sub> reference gas (externally calibrated relative

to Vienna Pee Dee Belemnite (VPDB)) was automatically introduced into the isotope ratio mass spectrometer (IRMS) in a series of pulses and its  $^{13}\text{C}/^{12}\text{C}$  ratio measured. The accelerating voltage was 3 KV, and the trap and box currents were set at 0.84 mA and 0.66V, respectively; the electron energy was set at 124V. Isodat 3 software automatically computed the  $^{13}\text{C}/^{12}\text{C}$  of each sample peak, referenced to the standard  $\text{CO}_2$  gas and its known  $^{13}\text{C}/^{12}\text{C}$  content. Carbon isotopic compositions represent averaged values of duplicate or triplicate analyses. The results are reported in per mil (‰) relative to the VPDB international standard. Standards from Arndt Schimmelmann, Indiana University, Bloomington, (USA) were used to determine instrument precision (<0.3‰) and accuracy (<0.5‰).

#### II.2.2.6 GDGTs Analysis

GDGTs contents and distribution in PT core were used to calculate Methylation index of Branched Tetraethers (MBT), Cyclization ratio of the Branched Tetraethers (CBT) and Branched and Isoprenoid Tetraether (BIT) indexes. GDGT structures are presented in FIGURE II-8. All extraction and analysis were conducted according to procedures described by McClaymont *et al.* (2012) and Blaga *et al.* (2010). Briefly, 5g of bulk sediment was extracted using an accelerated solvent extractor (ASE; Dionex) in 5-minute static cycles (90°C, 4MPa) with a mixture (9:1 v/v) of DCM and methanol. N-Tetracontane (C46) (>98%, Sigma 87096) was added as internal standard. The extract was concentrated and separated by flash columns filled with activated silica (7 mm i.d., 1.3 g approximately) in two fractions: non-polar and polar. A mixture of hexane:ethyl acetate (1:1 v/v) was used as eluent for non-polar fraction (includes GDGTs), while polar fraction was eluted with methanol. The non-polar fraction was dried and redissolved in 2 mL of 99:1 v/v hexane:isopropanol mixture using sonication. Before injection, the solution was filtered through a 0.45  $\mu\text{m}$  PTFE filter.

GDGTs were determined using an Agilent 1200 HPLC coupled to an Agilent 6130 quadrupole MS, normal-phase LC-MS and a Grace Prevail Cyano HPLC column (30cm x 150mm x 2.1mm i.d.) and a guard column of the same material. Separation was achieved at 30°C with a flow rate of 0.2 mL  $\text{min}^{-1}$  with the following gradient profile: 1% isopropanol (IPA) in hexane (0–5 min); 1.8% IPA in hexane (at 25min); 10% IPA in hexane at 30 min (hold 10 min). Analysis was performed in single ion monitoring (SIM) mode via the  $[\text{M}+\text{H}]^+$  ions of Crenarchaeol and branched GDGTs (1022-I, 1020-II, 1018-III, 1050-IV, 1036-V, 1034-VI, 1032-VII, 1292-VIII). After evaluation of the mass chromatograms, the indexes were calculated using only peaks that were at least one order of magnitude higher than the background noise.

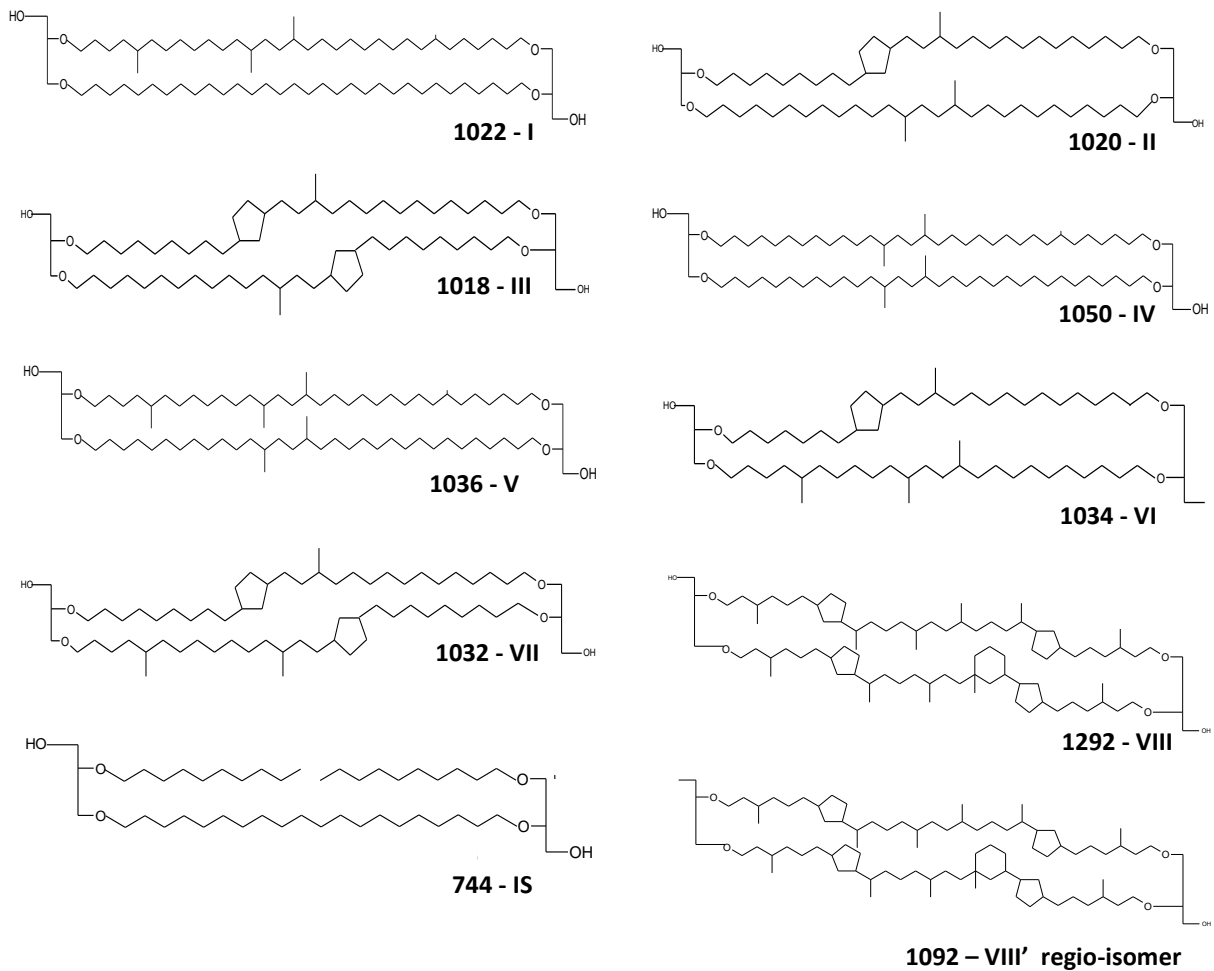


FIGURE II- 8. GDGT STRUCTURE INCLUDING BRANCHED GDGT (I-VII), CRENARCHAEOL AND ITS REGION-ISOMER (VIII AND VIII') AND C46 INTERNAL STANDARD (IS).

MBT, CBT and BIT indexes were calculated using the equations 2, 3 and 4 respectively: (ZHU *et al.*, 2011; BLAGA *et al.*, 2010).

$$CBT = \frac{\log^{(II+VI)}}{I + V} \quad (\text{Equation 2})$$

$$MBT = \frac{(I + II + III)}{(IV + V + VI + I + II + III)} \quad (\text{Equation 3})$$

$$BIT = \frac{(IV + V + I)}{(IV + IV + V + I)} \quad (\text{Equation 4})$$

The annual mean air temperature (MAAT) and pH reconstructed profile were obtained according the equations 5 and 6, proposed by Tierney *et al.* (2010).

$$MAAT = [0.81 - (5.67 \times CBT)] + (31 \times MBT) \quad (\text{Equation 5})$$

$$pH = [7.9 - (1.97 \times CBT)] \quad (\text{Equation 6})$$

## **CHAPTER III**

# **ANTHROPOGENIC AND CLIMATE CHANGES IN LAKE OHRID BASIN IN TRANSITION FROM DARK AGE TO MEDIEVAL WARM PERIOD**

### III.1 CHAPTER INTRODUCTION

This chapter discusses the results of the inorganic analysis and total lipids in the Lz1120 core. From the biomarkers were evaluated major climate and environmental changes during the transition period between the Dark Ages and the Medieval Warm Period (840 to 1330) that occurred in the Lake Ohrid watershed and how these changes may affect the organic matter flux. First, we describe the results obtained and their variation over time. Concomitantly, a temporal variability description of biomarkers is presented in a brief overview from the literature and is used in interpreting the results from each class of compounds. After the description of the results, a general discussion is made to reconstruct a past scenario of the Lake Ohrid watershed in terms of environmental changes based on the temporal variability of biomarkers and changes in the composition of organic matter delivered toward the lake. Besides the paleogeochemical record, an additional finding of this study is the survey of specific biomarkers for the Lake Ohrid watershed.

### III.2 BIOMARKERS AND INORGANIC ANALYSIS RESULTS

#### III.2.1 Inorganicanalysis

In order to assess the variability in productivity and the sources of organic matter (OM),  $\text{CaCO}_3$ , TOC, TS and the ratio of total organic carbon over total nitrogen (TOC/TN) were determined (FIGURE III-1). TOC values ranged from 0.5 to 1.8%,  $\text{CaCO}_3$  from 1.7% to 54%, TS from 0.02 to 0.14% and TOC/TN from 6.0 to 9.5. All proxies ( $\text{CaCO}_3$ , TOC, TOC/TN) show a similar variability over time. Lesser values are observed at 950 with an abrupt increase between 995 and 1020. Before 950 the data are roughly constant, whereas after 1020  $\text{CaCO}_3$  and TOC/TN continued to increase slightly.

TOC represents the amount of organic matter buried in sediments, after initial and subsequent degradation of the sedimentary organic matter. In lakes, sedimentary OM derives from multiple sources such as aquatic, bacterial and terrestrial biomass (MEYERS *et al.*, 2003). TOC values are often used to assess bulk productivity in lake settings (HAHN *et al.*, 2013). The values of TOC determined in this study match those of Wagner *et al.*, (2008), also in Lake Ohrid. Low TOC and TN values suggest low primary productivity typical of an oligotrophic lake (HAHN *et al.*, 2013).

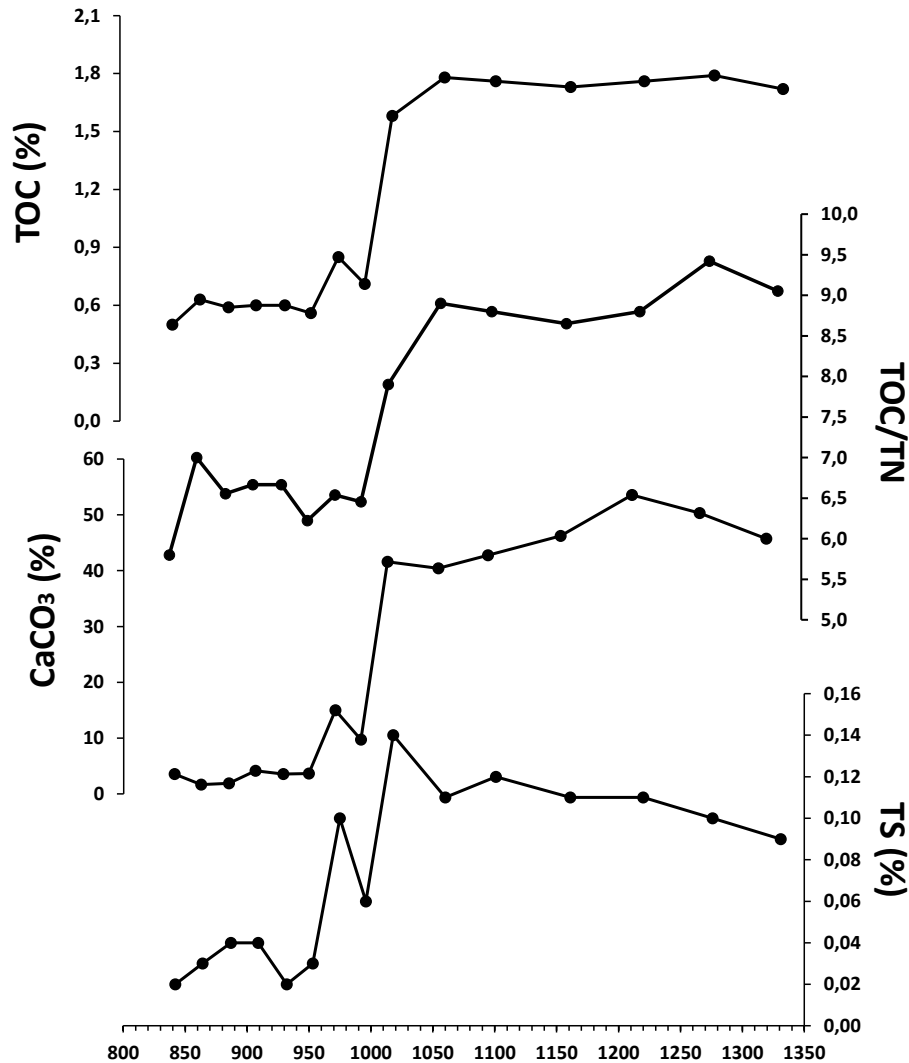


FIGURE III- 1. VARIATION OF INORGANIC DATA OVER TIME IN PERCENTAGE OF TOC, CaCO<sub>3</sub>, TS AND RATIO TOC/TN.

Climate controlled environmental changes affect terrestrial and, via nutrient supply, aquatic productivity. Therefore, variation in TOC/TN can reveal the main source of OM in sediments (HAHN *et al.*, 2013). The low (5-10) TOC/TN ratios in Lake Ohrid drive to phytoplankton, mainly algae and cyanobacteria, also soil input cannot be discarded (MAYER *et al.*, 2009; MEYERS AND ISHIWATARI, 1993). Low TOC/TN values were found in lakes sediments from Baikal (WATANABE *et al.*, 2004), Laguna Potrok Aike (HAHN *et al.*, 2013) and previous study in lake Ohrid (WAGNER *et al.*, 2008). However, such low TOC/TN values have also been attributed to the presence of inorganic nitrogen in sediments with low organic matter contents (LEHMANN *et al.*, 2002). In order to estimate the inorganic nitrogen content it was tested the correlation between TOC and TN contents. The high linear correlation coefficient ( $R^2=0.97$ ,  $p<0.002$ ) indicates that the higher fraction of nitrogen is organically bound. In FIGURE III-2, the regression line intercepts the y-axis at 0.08% TN. This suggests that the

percentage of not organically bound nitrogen is small, with 0.08% TN corresponding to the maximum amount of inorganic nitrogen to be expected in these sediments (VERES *et al.*, 2008).

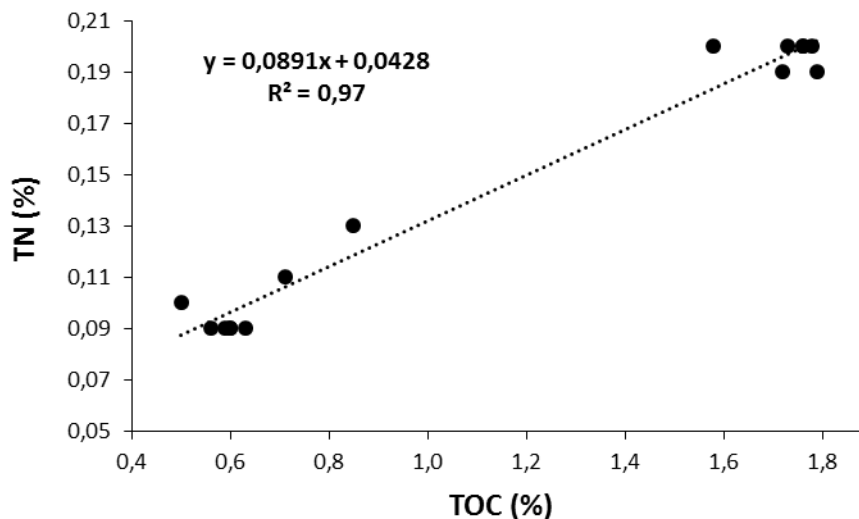


FIGURE III- 2. CORRELATION BETWEEN CONVENTIONALLY MEASURED TOC AND TN CONTENTS INDICATES THAT THE NITROGEN IN THE SEDIMENT IS MAINLY ORGANIC.

The sudden increase in the TS values after 995 could be indicative of changes in the redox conditions of the sediments, which could imply changes in organic matter accumulation. Sulphur generation is related constantly to degradation of OM under low oxygen conditions by H<sub>2</sub>S production, or development of phototrophic sulphur bacteria (BERNER, 1964).

The TIC contents vary from 0.2 to 7%. Assuming that CaCO<sub>3</sub> accounts for most of the TIC. Recent study in Lake Ohrid by Lezine *et al.*, (2010) have demonstrated that CaCO<sub>3</sub> is absent or occurs in trace amounts in alluvial and molasse sediments that outcrop in the adjacent watershed. Such values of TIC content indicate that in this study terrigenous contribution of CaCO<sub>3</sub> is not the predominantly sources. Based on the low amount of calcareous fossils, mineralogy and morphology lacustrine, previous studies in Lake Ohrid consider the sources of CaCO<sub>3</sub> in sediment to be autochthonous, more likely its formed authigenetically by precipitation (LEZINE *et al.*, 2010; WAGNER *et al.*, 2008).

### III.2.2 Lipid Biomarkers

Normal, branched, mono-unsaturated and hydroxy fatty acids, alcohols, alkanes, sterols and some miscellaneous compounds including  $\beta$ -amyirin, 17 $\beta$ ,21 $\beta$ -

bishomohopanol and 17 $\beta$ ,21 $\beta$ -bishomohopanoic acid were identified and quantified in the Ohrid sediments (TABLE III-1).

With exception of sample from 865, the amounts of extracted lipids in samples pre dating 995 were significantly lower (1.06 - 2.53ng<sub>lipids</sub>·g<sub>sed</sub>) than those after 995 (3.74 - 14.52ng<sub>lipids</sub>·g<sub>sed</sub>) reflecting the differences in TOC contents (TABLE III-1). The difference between these two periods may be related to the qualitative and quantitative changes in OM delivery to the lake. These changes could include variation in the proportion of non extractable organic carbon such as lignin or cellulose from terrestrial plant tissues or black carbon (HOLTVOETH *et al.*, 2010), alteration in the preservation potential during diagenesis (MATSUDA AND KOYAMA, 1997; MEYERS AND ISHIVATARI, 1993) or variation in the rate of sedimentation induced by anthropogenic changes land use surrounding of the lake (VOGEL *et al.*, 2010).

The variation in the composition of total lipid over time was evaluated and showed in FIGURE III-3. The major compound classes of the total lipids were fatty acids and alcohols, accounting for 60% (975) to 80% (840) of the total lipids. The total lipid composition is not constant over time. A subtle increase of the fatty acids can be observed, in contrast with a slightly decrease of the alkanes and sterols as from 995. Despite there is no a significant pattern on the variation of the total lipid composition, the more important changes were observed in the distribution of single compounds within the compounds classes (e.g., long vs. short and mid-chain normal acids and alcohols).

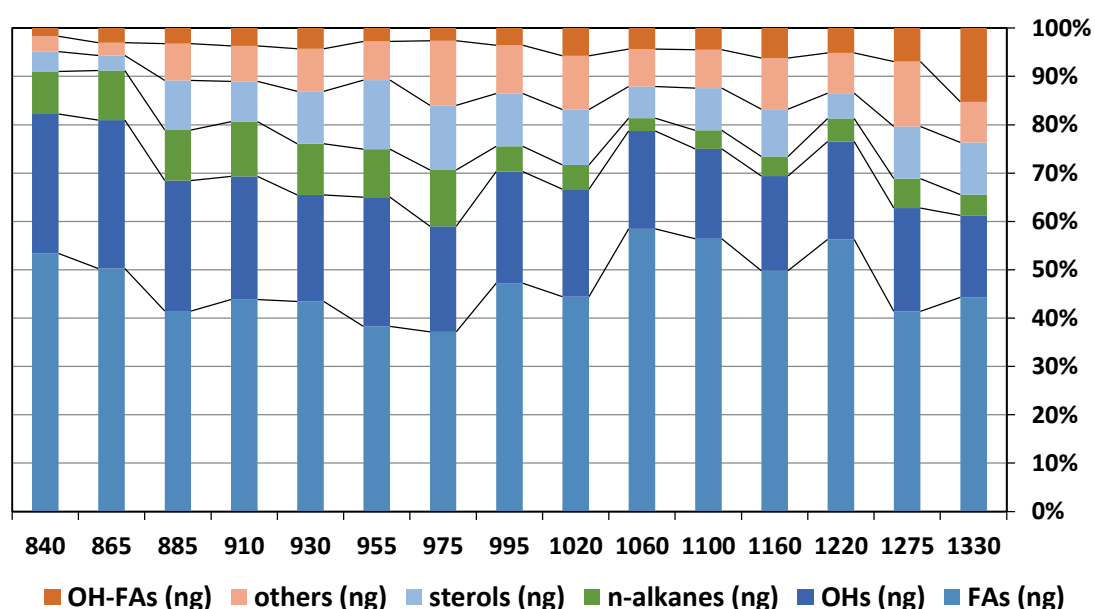


FIGURE III- 3. VARIATION ON THE COMPOSITION OF TOTAL LIPID OF SITE Lz1120 OVER TIME

TABLE III- 1. COMPOUNDS QUANTIFIED IN TOTAL LIPID EXTRACT FROM Lz1120 CORE IN  $\text{ng.g}^{-1}$  SEDIMENT AND CONCENTRATION OF TOTAL LIPID PER GRAM SEDIMENT AND PERCENTAGE OF THE TOC CONTENT

Age	840	865	885	910	930	950	975	995	1020	1060	1100	1160	1220	1275	1330
<b>Lipid <math>\mu\text{g. g sed}^{-1}</math></b>	2.5	5.8	1.4	1.8	1.3	1.1	1.5	5.1	3.7	9.0	8.4	8.1	8.9	6.1	14.5
<b>Lipid <math>\mu\text{g. \%TOC}^{-1}</math></b>	10.6	17.5	4.7	5.6	4.8	4.0	3.2	13.9	5.0	10.1	9.2	9.0	10.0	6.5	12.8
<b>FA (<math>\text{ng.g}^{-1}</math> sed)</b>															
$\Sigma$ C14-C19	887.2	1167.6	308.1	888.0	1051.1	695.4	602.2	2112.9	1948.0	4523.0	4968.2	3855.3	3696.6	3351.4	2917.1
$\Sigma$ C20-C23	1152.8	2056.4	474.7	490.0	587.2	614.3	622.3	2279.2	2048.6	6039.6	5325.7	3875.9	5242.2	2549.5	3761.5
$\Sigma$ C24-C33	5226.9	7404.9	1926.4	1607.8	1391.5	966.9	1093.2	4315.1	3916.3	9455.4	7160.0	4836.4	9800.0	3283.1	7479.0
<b>Hidroxy Acids</b>															
$\omega$ -C16 OH-FA	47.8	46.1	36.0	3.4	3.8	23.7	29.9	65.5	62.1	69.8	74.2	79.8	62.3	86.2	82.3
$\omega$ -C18 OH-FA	18.9	21.2	9.5	14.0	12.8	13.6	12.1	62.1	63.1	37.9	46.6	50.0	52.2	38.5	43.5
$\omega$ -C20 OH-FA	47.9	77.1	26.0	24.2	24.4	22.0	21.3	58.3	51.0	144.8	103.2	153.2	153.2	79.0	104.8
$\omega$ -C22 OH-FA	35.3	22.6	41.0	40.0	51.3	39.8	48.7	173.8	364.1	376.7	405.0	529.8	520.9	468.9	430.1
$\omega$ -C24 OH-FA	95.2	91.1	83.8	77.3	68.5	43.2	53.3	167.3	367.3	321.1	350.4	312.8	360.9	460.2	450.7
<b>Branched FA</b>															
$\Sigma$ C15 branched FA (iso/anteiso)	16.8	26.7	25.8	53.4	88.7	39.4	60.6	338.7	208.3	614.8	695.1	668.0	602.5	525.7	466.5
<b>Alcohols</b>															
$\Sigma$ C12-C19	407.6	323.8	396.4	426.9	281.0	415.2	367.1	777.7	760.5	913.4	1330.1	1032.7	793.9	690.3	626.8
$\Sigma$ C20-C23	387.8	779.7	245.2	276.0	284.7	256.8	254.5	1136.1	997.0	2426.8	1635.3	2903.6	1958.3	1329.4	1415.3
$\Sigma$ C24-C33	3290.0	5565.8	1165.9	1077.9	999.2	940.0	765.6	2343.3	2189.6	3611.2	2781.0	5378.3	4001.0	2735.9	3376.3
<b>Alkanes</b>															
$\Sigma$ C12-C19	80.3	101.2	25.8	59.4	78.4	62.8	63.2	131.5	127.8	133.9	153.0	172.5	139.2	132.4	97.4
$\Sigma$ C20-C25	150.5	125.7	112.5	105.3	107.4	71.2	92.9	161.1	197.9	258.6	316.8	299.0	289.3	216.6	239.2
$\Sigma$ C26-C33	225.8	240.5	202.8	217.7	199.6	198.6	221.9	440.4	417.0	467.8	512.1	505.0	762.9	658.6	778.9

TABLE III.1 Cont...

Age	840	865	885	910	930	950	975	995	1020	1060	1100	1160	1220	1275	1330
<b>Sterols</b>															
<b>Epicholesterol</b>	8.1	8.10	8.60	8.4	12.1	64.6	88.5	46.0	121.9	108.4	132.0	1223	117.6	132.7	138.4
<b>Cholesterol</b>	89.2	136.2	100.1	97.4	106.0	83.8	82.2	279.8	254.5	432.6	431.5	421.5	425.6	353.4	530.2
<b>Cholestanol</b>	43.7	45.9	33.7	47.4	49.6	64.0	60.7	248.8	225.1	404.2	413.4	386.8	356.0	439.2	386.9
<b>Sitosterol</b>	124.5	123.9	175.2	119.8	197.8	178.6	165.3	483.4	454.7	591.5	586.4	608.5	619.9	599.6	828.0
<b>Stigmastanol</b>	80.3	75.4	80.4	81.4	87.2	83.0	88.1	243.0	226.9	286.9	294.3	282.0	333.7	316.7	220.4
<b>Others</b>															
<b>β Amirin</b>	86.6	85.5	91.3	92.4	98.9	82.1	87.1	240.3	224.4	230.0	2550	291.6	344.0	327.4	405.3
<b>Tetrahymanol</b>	20.6	21.8	71.4	43.9	55.5	91.8	386.3	352.2	587.0	562.1	540.0	538.7	581.1	575.8	514.9
<b>BISMH</b>	109.5	119.3	111.8	84.6	928	71.7	667	195.3	167.7	21.4	200.2	331.0	364.2	395.4	348.1
<b>BISMH acid</b>	218.6	246.9	221.8	253.4	280.9	228.3	247.4	723.0	641.7	1300.0	1201.9	1206.8	1143.8	981.5	1020.0

Note FA: fatty acids, BISH: 17β,21β-bismohopanoi, BISH acid: 17β,21β-bismohopanoic acid., sed: sediment.

### III.2.2.1 Fatty Acids and Alcohols

According to TABLE III-1, most classes of compounds present similar variation over time, with highest values after 995. The fatty acids ranged from 308 to 9800 ng.g<sup>-1</sup>, with carbon chain lengths between 14 and 32, whereas the alcohols ranged from 245 to 5.348 ng.g<sup>-1</sup> with carbon chain lengths between 12 and 33. For fatty acids and alcohols, the long chain and even homologues were dominant. Either, fatty acids and alcohols are abundant in most living organisms, accounting for abundance in recent sediments (ANDERSSON AND MEYERS, 2012; MATSUDO AND KOYAMA, 1997). Sources of these compounds include bacteria and phytoplankton for shorter chain, and higher plants for long chains (VOLKMAN *et al.*, 1998; MEYERS AND ISHIVATARI, 1993). Mid-chain compounds have variable sources, which include soil, microalgae and epiphytes (JAFFE *et al.*, 2001; REZANKA *et al.*, 1991).

### III.2.2.2 Branched Fatty Acids

Concentrations of branched fatty acids showed distinct behaviour before and after 975. Small concentrations (16.8-88.7 ng.g<sup>-1</sup>) were detected before 975, whilst higher concentrations were found after 975 (166.5 - 695.1 ng.g<sup>-1</sup>, TABLE III-1). Only the *iso* and *anteiso* – C15 branched FAs were detected. According to van Bergen *et al.*, (1997), these compounds derive from bacteria living in sediments as well as in soils.

### III.2.2.3 Hydroxy Acids

Hydroxy acids were found in minor proportion in the sediments in comparison with fatty acids and alcohols (FIGURE III-3). There is a lack of  $\alpha$ -hydroxy acids, however abundance of  $\omega$ -hydroxy acids were observed, such as  $\omega$ -C16 OH-FA,  $\omega$ -C18 OH-FA,  $\omega$ -C20 OH-FA,  $\omega$ -C22 OH-FA and  $\omega$ -C24 OH-FA. The concentrations of  $\omega$  hydroxy acids ranged from 9.5 to 520.9 ng.g<sup>-1</sup> over time, whereas highest concentrations were observed as from 995 (TABLE III-1). The main source of  $\omega$ -hydroxy acids are the biopolyesters cutin and suberin (RIEDERER *et al.*, 1993). Cutin is the structural component (cuticular waxes) of the terrestrial plants and contains mainly C16 and C18  $\omega$  hydroxy acids (KOLATTUKUDY, 1980), whilst suberin is found in root material and contains large amounts of C22 and C24  $\omega$ -hydroxy acids, as well as C16  $\omega$ -hydroxy acids (BULL *et al.*, 2000). Cutin and suberin reach the sediment through catchment run off, either by litterfall or, in the case of roots, by below-ground

inputs or when the roots are exposed (e.g., by removal of vegetation) (RIEDERER *et al.*, 1993).

#### III.2.2.4 Alkanes

Like  $\omega$  hydroxy acids, alkanes represent a minor proportion in the sediment when compared to other compound classes, with their concentrations ranging from 25.8 to 778.9ng g<sup>-1</sup>. Although the absolute alkane concentrations increase from 995 (TABLE III-1), the proportion of alkanes relative to the total lipids decreases after 975 (FIGURE III-3). The long chain compounds are dominant. The distribution showed an odd over even predominance and carbon numbers ranged from C17 to C31, with maximum at C29. Generally, alkanes with odd carbon numbers are derived from different sources, long-chain alkanes (C27, C29 and C31) are usually assigned to leaf waxes of higher land plants (RIELEY *et al.*, 1991). C15, C17 and C19 alkanes are present in algae and photosynthetic bacteria (EGLINTON AND HAMILTON, 1963), whereas submerged and floating aquatic plants commonly maximize at C21, C23 and C25 (FICKEN *et al.*, 2000b). Additionally to the use of alkanes to define sources of OM composition in the sediment, these class of compounds have also potential to reflect climatic change, once the component and concentrations of alkanes in lake sediments can be used to infer the source status and further link to environmental conditions (LIN *et al.*, 2008).

#### III.2.2.5 Sterols

Sterols are a class of compounds with more specific sources, hence, they provide insights about the OM origin and preservation (VOLKMAN *et al.*, 1998). Sterols composition can be employed to infer paleoenvironmental changes that have affected the sources of these biomarker compounds in sediments deposited at different times (MEYER, 1997). In the present study, epicholestanol (5 $\alpha$ -cholestan-3 $\alpha$ -ol), cholesterol (cholest-5-en-3 $\beta$ -ol), cholestanol (5 $\alpha$ -cholestan-3 $\beta$ -ol), sitosterol (24-ethylcholest-5-en-3 $\beta$ -ol) and stigmastanol (24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol) were detected in concentrations from 8 to 828ng g<sup>-1</sup>, with highest concentrations in samples close to the 995 horizon (TABLE III-1). Sitosterol was the dominant sterol (TABLE III-1). It is the major sterol in vascular plants (MEYER, 1997), but is found also in emergent water plants, microalgae and phytoplankton (VOLKMAN 1986; MEYER, 1997). Cholesterol and cholestanol do not have specific sources; however, they are present in both vegetal and animal realm (BIACHE AND PHILP, 2013). Cholesterol can be derived from the faeces and

carcasses of fish and zooplankton (MUDGE *et al.*, 1999) and sometimes is the major sterol in phytoplankton (KILLOPS AND KILLOPS 2005; RIELEY *et al.*, 1991). Cholestanol can be found in dinoflagellates, diatoms (THIEL *et al.*, 1997) and in mammals' faeces (CHAN *et al.*, 1998). Stigmastanol is present in high plants (HUDSON *et al.*, 2001), but certain diatoms produce this sterol in appreciable quantities (VOLKMAN *et al.*, 1998). Among the sterols quantified in this study, epicholestanol is more likely an indicator of bacterial alteration in lakes (CORDEIRO *et al.*, 2008). High amounts of epicholestanol have been found in *Chlorobium* spp., reducing bacteria whose abundance at the water/sediment interface are at a maximum during the anoxic periods (ROBINSON *et al.*, 1984).

#### III.2.2.6 Other compounds

Besides the sterols, alkanes and fatty acids, it were also found compounds such as 17 $\beta$ ,21 $\beta$ -Bishomohopanoic acid and 17 $\beta$ ,21 $\beta$ -bishomohopanol, which are triterpenoids synthesized by a diverse range of bacteria and are present in its cell membrane constituents as from bacteriohopanepolyols (BHP's) (SAITO and SUZUKI, 2007). Diagenetic process occur after the death of bacteria modifying the side chain structure of biohopanoids, leading to the formation of hopanols, hopanoic acids and others derivatives (FARRIMOND *et al.*, 2002). In this study the 17 $\beta$ ,21 $\beta$ -bishomohopanol ranged from 21.4 to 395.4ng g<sup>-1</sup>, while the 17 $\beta$ ,21 $\beta$ -bishomohopanoic acid presented higher concentrations between 218.6ng g<sup>-1</sup> and 1300ng g<sup>-1</sup> (TABLE III-1). Biohopanoids and their derivatives have been reported to occur in several environments, such as marine and lacustrine sediments (VAN DONGEN *et al.*, 2006), soils and peats (TALBOT *et al.*, 2005). Recent studies have reported that biohopanoids may occur in obligatory anaerobic bacteria such as planctomycetes and sulphate reducing bacteria (FARRIMOND *et al.*, 2002).

Tetraymanol (gammaceran-3 $\beta$ -ol) is the only known biological precursor for gammacerane, the corresponding hydrocarbon, which is often found in the free and sulphur-bound fractions of fossil samples from marine and lacustrine settings (THIEL *et al.*, 1997). The concentrations of tetrahymanol in Lake Ohrid ranged from 20.6ng.g<sup>-1</sup> to 587ng.g<sup>-1</sup>, and are higher in sediments younger than 975 (TABLE III-1). Tetrahymanol is a mainly lipid in bacteriovores ciliates in marine and freshwater (HANISH *et al.*, 2003). Protozoa such as ciliate can be considered as the main source for tetrahymanol in aquatic sediments (THIEL *et al.*, 1997), although it is also present in the anaerobic phototrophic purple bacterium *Rhodospseudomonas palustris* and in very low amounts in a fern (HANISH *et al.*, 2003).

Finally,  $\beta$ -Amiryn was detected in concentrations between  $82.1\text{ng g}^{-1}$  and  $405.3\text{ng.g}^{-1}$  (TABLE III-1). The main source of  $\beta$ -Amiryn is deciduous trees in the watershed, it is a biomarker for angiosperms (MEYERS AND ISHIWATARI, 1993), and hence represents a marker for allochthonous inputs to the sediments (HANISH *et al.*, 2003).

### III.3 EVIDENCE OF ANTHROPOGENIC AND CLIMATE CHANGE IN LAKE OHRID

Based on the low amount of calcareous fossils, mineralogy and morphology lacustrine of area studied, and as well as in previous studies in Lake Ohrid, the sources of  $\text{CaCO}_3$  in sediment are considered to be autochthonous or, more likely, its formed authigenetically by precipitation (LEZINE *et al.*, 2010; WAGNER *et al.*, 2008). According Lezine *et al.* (2010), in the authigenetic process, calcium and carbonate ions from limestone outcrops in the drainage basin are dissolved by rain water and are transported to the lake by run-off and via the karstic aquifer. Within the lake, the carbonate/bicarbonate equilibrium and photosynthesis reduce the free hydrogen ions available and the pH rise; dissolved carbonate and calcium ions are used by aquatic biota (ostracods, molluscs) or precipitate directly in the epilimnion. Once the calcium and carbonate ions are transported by run-off, the content of  $\text{CaCO}_3$  in the sediment is directly related with the precipitation level in the watershed. Thereby, the increase in the  $\text{CaCO}_3$  values as from 995 reflects high precipitation period and/or elevated temperatures, which can increase the  $\text{CaCO}_3$  concentrations on the lake by evaporation, and/or reduce the saturation threshold for  $\text{CaCO}_3$ .

The TOC data also record a sudden increase in the amount of OM in the lake sediments above the 995 time horizon (TABLE III-1). This event is also evident in the biomarker data and inorganic analysis (TABLE III-1, FIGURE III-1). The interpretation is that this event is directly related to anthropogenic and climate change in the watershed, over the period studied.

Although most of samples in this study (840-1330) are dated from the Medieval Warm Period - MWP (950-1400), the main change observed in the biomarker proxies and inorganic data occurred over a period considered to be the transition between the Dark Age (270- 950), cool and dry, and the MWP (950-1400), warmer and wetter (GARCIA *et al.*, 2007). Large changes in precipitation patterns were reported over this transition (BRADLEY *et al.*, 2003; CROWLEY and LOWERY, 2000), and the average temperature became 1 -  $2^\circ\text{C}$  warmer (LAMB, 1964). The more intensive level of climate change (High Medieval Period) occurred between 1100 and

1200 (BRADLEY *et al.*, 2003), quite close to the time of the main environmental change observed in this study (>995).

Thereby, climates change including an increase in average temperatures and precipitation levels was probably one of the factors, which contributed to the increase on OM burial in Lake Ohrid sediments from 995, whether by entrainment of terrestrial compounds due the increase of run-off on the watershed, or increase in the autochthonous production, due to higher nutrient supply. Biomarkers strongly associated with terrestrial sources, such as long chain alkanes that are usually ascribed to leaf waxes of higher plants (HANISH *et al.*, 2003; RIELEY *et al.*, 1991) exhibit the same overall trends as the TOC and CaCO<sub>3</sub> (FIGURES III-1, III-4), reflecting the increasing terrestrial contribution on the OM in the lake climate changes. Considering that both temperature and precipitation are the main factors, which control terrestrial vegetation development, long chains alkanes could reflect temperature and precipitation changes (LIN *et al.*, 2008). Evidences of a raise on the precipitation level and temperature in MWP at southeastern of Europe are also reported by Telelis (2005) and Magri (1996).

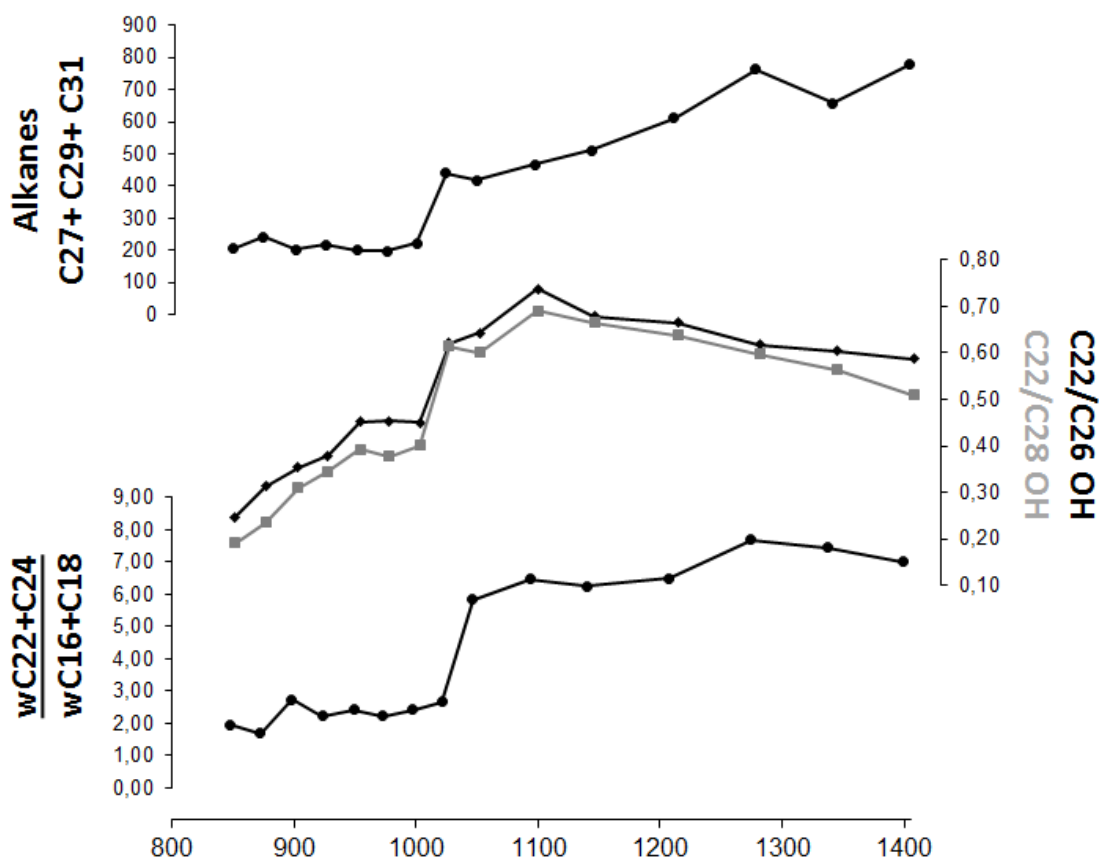


FIGURE III- 4. VARIATION OF TERRESTRIAL ALKANES RATIOS OF C22 PER C22 AND C28 ALCOHOL AND HYDROXY ACIDS, wC22+C24 PER wC16+C18 OVER TIME.

In terms of compounds classes, significant variations in the total lipid composition were not observed (FIGURE III-3). However, changes in composition of organic matter is evident on the isotopic signature of fatty acids and alcohols chains (TABLE III-2).  $\delta^{13}$  values of individual chains (OH and FA) are lighter in the sample 1160-1275 compared to the sample 910-955. Higher differences were observed for long chains fatty acids (C26: 1.55, C28:1.86) and middle chains alcohols (C22:3.72, C24: 2.14, TABLE III-2). It's suggest the input of new sources of organic matter or significant changes in the proportion of them, affecting mainly the sources of C22 alcohol, which presented the highest difference in samples prior and after 995.

In order to understand the changes occurred in organic matter composition, the distribution of alcohol and fatty acids were assessed. There was a strong linear correlation between the long-chain fatty acids and alcohols up until 995 ( $R=0.94$ ), after this linear correlation coefficient was lower ( $R=0.61$ ). Long-chain fatty acids and alcohols are biomarkers of terrestrial material (VOLKMAN *et al.*, 1998; MEYERS AND ISHIVATARI, 1993). The decrease in their linear correlation coefficient indicates that the OM delivery on the lake after 995 was less homogeneous, consistent with isotopic signature values. Fatty acid and alcohol distributions of representative samples show changes in distribution over time and particularly around 995 (FIGURE III-5). While long chain compounds decrease in relative abundance, the mid-chain homologues increase. The ratios of C22 to C26 compounds show the variation more clearly (FIGURE III-5). For the fatty acids the ratios are twice as high after 995, while for the alcohols after this time, the change is even more evident, the ratios being almost three times higher (FIGURE III-5). The relative depletion of the long chain alcohols (C26, C28) to their mid-chain counterparts (C22) is better illustrated by the ratios of C22/C26 and C22/C28 alcohols (FIGURE III-4). The pattern of these ratios shows the same trend as do TOC and  $\text{CaCO}_3$  contents (FIGURE III-1), indicating that the environmental change observed around 995 influenced not only the quantity, but also the composition of OM delivered to the lake. It is possible to infer that the proportion of terrestrial source materials also changed and favoured the sources of mid-chain alcohols and fatty acids relative to their longer chain counterparts.

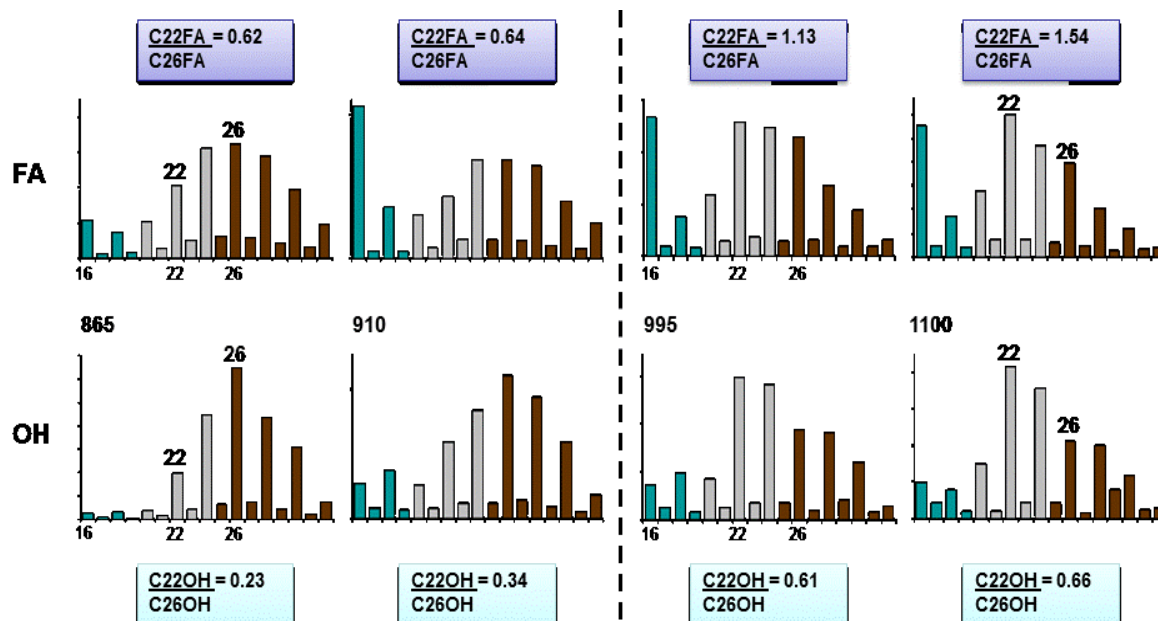


FIGURE III- 5. DISTRIBUTION OF FATTY ACIDS (FA) AND ALCOHOLS (OH) IN KEYS SAMPLES (865, 910, 995 and 1100) FROM Lz1120 CORE. DOTTED LINE ASSISTS TO DIFFER SAMPLES IN TWO GROUPS. C22/C26 OH AND FA RATIOS REFLECT THE CHANGE IN ORGANIC MATTER COMPOSITION. COLLUNMS IN GREEN, GREY AND BROWN COLOR REPRESENT: SHORT, MIDDLE AND LONG ALKANE CHAINS, RESP.

Mid-chain length fatty acids and alcohols do not have a unique source, having been identified in soil, microalgae and epiphytes (CHIKARAISHI and NARAOKA, 2006; JAFFE *et al.*, 2001). Rezanka *et al.*, (1991) found high amounts of C22 and C24 fatty acids and alcohols in bacteria from soil, whereas Jaffe *et al.*, (2001) attributed the occurrence of the C22 alcohol in sediments of Florida Bay to be from *Chara sp.*, a genera of aquatic macrophytes.

Although currently lake Ohrid is rich in *Chara sp.* and present a large amount of this epiphytes on the bottom and in the littoral zone (FIGURE III-6) (ALLEN and OCEVSKI, 2006), in this study, the origin of C22 alcohol, from *Chara sp.* is unlike. The simultaneous biomarkers analysis, compared of samples from potential sources of fatty acids and alcohols, point to soil as the predominant source of alcohols and fatty acids middle chains and consequently the main source of OM delivered on the lake as from 995. Total fatty acids and alcohols ratios (FA/OH) in samples from sediment, forest soil, leaf and reed lives of lake Ohrid watershed are showed on the TABLE III-2. Values of 9, 3 and 1 were found for forest soil, leaf and reed lives respectively. FA/OH ratios in sediment samples show a mixture of forest soil and reed lives sources between 840 and 975. After 995 the FA/OH ratios suffer an increase reflected by the raise on the forest soil sources contribution. This contribution is evident on the isotope composition of C22 alcohol chain (TABLE III-2). The depletion in  $\delta^{13}$  values suffered by C22 alcohol chain, after 995 is higher (3.72‰) than other chains (-0.64‰ to 2.14‰, TABLE III-2),

showing that this compound present high potential to reflect soil input and hence can be used as a biomarker for this source of OM in the lake Ohrid watershed.



FIGURE III- 6. CHARA BELT IN OHRID LAKE.

TABLE III- 2. FA/OH RATIOS IN ALL SEDIMENT SAMPLES, AND STABLE CARBON ISOTOPIC RESULT IN PER MIL (‰) OF SAMPLES AT 910-955 AND 1160-1275.

Age	FA/OH	$\delta^{13}$ Fatty Acids (‰)					$\delta^{13}$ Alcohols (‰)				
		C20	C22	C24	C26	C28	C20	C22	C24	C26	C28
1330	2.61										
1275	1.93										
1220	2.77	-34.86	-31.17	-31.40	-30.76	-30.74	-35.23	-35.28	-33.10	-33.40	-34.12
1160	1.35										
1100	3.04										
1060	2.88										
1020	2.00										
995	2.05										
975	1.67										
955	1.41										
930	1.94	-35.53	-31.99	-31.84	-32.31	-32.60	-35.82	-39.00	-35.24	-34.04	-34.33
910	1.68										
885	1.50										
865	1.59										
840	1.78										
$\delta^{13}$ Difference		0.67	0.82	0.44	1.55	1.86	0.59	3.72	2.14	0.64	0.21

The dominance of soil as a main source of C22 alcohols is also confirmed by others biomarkers. C16 and C18  $\omega$ -Hydroxy acids represent terrestrial plant sources (KOLATTUKUDY, 1980), while C22 and C24  $\omega$ -hydroxy acids derive from roots, hence suggest soil as dominant source (BULL *et al.*, 2000). The ratio of  $\omega$ C22+C24/ $\omega$ C16+C18 (FIGURE III-4), increases after 995 and reflects the increase of soil contributions relative to plants, following the same pattern as TOC for this period (FIGURE III-1).

Terrestrial sources of OM to lakes normally lead to high ratios of TOC/TN (>20) as higher plants contain cellulose which is lean in nitrogen (HAHN *et al.*, 2013). However, the results in this study suggest terrestrial OM input of soil as the main source, or the replacement of plants by soil input over time, lead to low values (5-10). Compared to vascular plants, soil microorganisms are nitrogen rich, with C/N ratios ranging from 5:1 to 15:1 for bacterial dominated population (HEDGES and OADES, 1997). Retention of nitrogen, during degradation of vascular plant tissues is thus a key strategy for terrestrial microorganisms, because nitrogen is limiting for vascular plant production in most soils occur predominantly in organic form (REZANKA *et al.*, 1991). According to Sollins *et al.*, (1984) a decrease in C/N values can occur due the immobilization of nitrogenous material accompanied by the remineralization of carbon by microbial growth in soil, which can contribute to explain the low values of TOC/TN ratios observed in this study. In general, changes in inorganic compounds are unusual (MEYERS AND ISHIVATARI, 1993), but when a higher amount of the soil is delivered on the lake, the effect in changes may be significant. Evidence of soil bacteria may be seen by the presence of the C15 branched fatty acids (*iso* and *anteiso*), which derive from bacteria living in sediment as well in soil (HOLTVOETH *et al.*, 2010) and have similar variation of the TOC and TOC/TN (TABLE III-1), suggesting the same influence.

#### III.4 EVIDENCES OF CHANGES IN PRIMARY PRODUCTIVITY

An increase in primary productivity is also reflected in biomarkers distributions. Short chain fatty acids and alcohols are strongly correlated ( $R=0.91$ ,  $p<0.001$ ), and showed the same sudden increase in concentration from 996 onwards (TABLE III-1). The main sources of these classes of compounds are bacteria and phytoplankton (VOLKMAN *et al.*, 1998; MEYERS AND ISHIVATARI, 1993).

Cholesterol, cholestanol, sitosterol and stigmastanol are also strongly correlated each other ( $R>0.90$ ,  $p<0.001$ ), indicating similar sources (BIACHE and PHILP, 2013; HUDSON *et al.*, 2001). Of these, sitosterol has been used as a

biomarker for terrestrial inputs, most likely higher plants (MEYER, 1997), however, this sterol can be found also in microalgae and phytoplankton (VOLKMAN 1986; MEYER, 1997), in oligotrophic environments green algae (mainly *Pramnesiophyceae*) and cyanobacteria usually dominate the phytoplankton, and contain moderate amount of sitosterol (VOLKMAN, 1986; HALLEGRAEFF, 1981).

In general, sources of Cholesterol, Cholestanol, Sitosterol and Stigmasterol can be allochthonous, from land plants mainly, or autochthonous, from phytoplankton/bacteria (BIACHE and PHILIP, 2013; HUDSON *et al.*, 2001). Nevertheless, other biomarkers such as  $\beta$ -amyrin for instance, have more specific sources. It is a "higher plant" triterpenoid, which is usually ascribed to terrestrial sources (MEYERS and ISHIWATARI, 1993). Hence, the ratio sterols (Cholesterol+Cholestanol+Sitosterol+Stigmasterol) to  $\beta$ -amyrin was used in order to assess the variation of autochthonous sources (phytoplankton/bacteria) relative to the terrestrial contribution by plants, which are being replaced by soil (FIGURE III-7). Again, the same behaviour for TOC was observed, indicating that although these sterols have terrestrial contribution, the autochthonous production (phytoplankton/bacteria) is predominant and reflects the amount of OM delivered on the lake. The absence of other "higher plant" sterols such as stigmasterol and campesterol reinforces this conclusion.

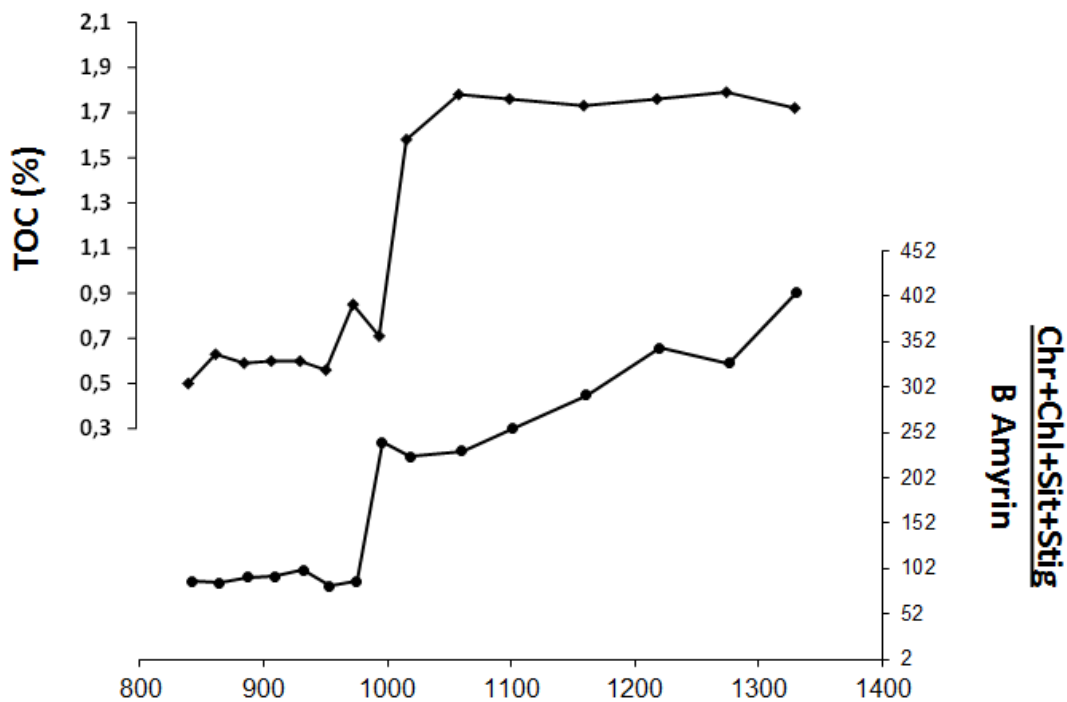


FIGURE III- 7. VARIATION OF STEROLS (CHOLESTEROL, CHOLESTANOL, SITOSTEROL, STIGMASTEROL) PER  $\beta$ -AMYRIN, OVER TIME.

Among the quantified sterols, epicholestanol showed lowest correlation with other sterols ( $R=0.80-0.86$ ,  $p<0.001$ ). One explanation for this could be the high amounts produced in anoxic environments by a specific reducing bacteria *Chlorobium* sp. The typical epicholestanol/cholestanol ratio *Chlorobium* bacteria is 0.37 (ROBINSON *et al.*, 1984). TABLE III-3 show the Epicholestanol/Cholestanol ratios found in this study. Values close to 0.37 as from 995, suggest significant production of epicholestanol by *Chlorobium* sp. Anoxic conditions at the water/sediment interface in Lake Ohrid apparently have occurred in the past since by sulphur concentrations, which are strongly correlated with epicholestanol values ( $R=0.90$ ,  $p<0.001$ ), and showed the same temporal trend of TOC (FIGURE III-1). The presence and variation on the concentration of others biomarkers also reflect the anoxic condition in Lake Ohrid, as well as bacteria grown. Tetracymanol is produced by anaerobic purple bacterium *Rhodospseudomonas palustris* and by protozoa such as ciliate, in aquatic sediments (HANISH *et al.*, 2003; THIEL *et al.*, 1997). It is strongly correlated with sulphur values ( $R=0.96$ ,  $p<0.001$ ) and epicholestanol ( $R=0.95$ ,  $p<0.001$ ), suggesting suffer the same environmental influences such as increase in amount of OM and changes in the redox conditions on the sediment.

TABLE III- 3. EPICHOLESTANOL/CHOLESTANOL RATIOS IN SEDIMENT SAMPLES, OVER TIME. IN BOLD VALUES NEXT TO 0.37 SUGGEST PRODUCTION OF EPICHOLESTANOL BY *Chlorobium* sp (ROBINSON *et al.*, 1984).

Time(Year)	Ratios
1330	<b>0.36</b>
1275	<b>0.30</b>
1220	<b>0.33</b>
1160	<b>0.32</b>
1100	<b>0.32</b>
1060	<b>0.27</b>
1020	<b>0.54</b>
995	<b>0.18</b>
975	1.46
955	1.01
930	0.24
910	0.18
885	0.26
865	0.18
840	0.18

Evidence the presence of bacteria as from 995 is observed also by  $17\beta,21\beta$ -bishomohopanoic acid and  $17\beta,21\beta$ -bishomohopanol. These triterpenoids are synthesized by a diverse range of bacteria (SAITO and SUZUKI, 2007), have been

reported in several environments (VAN DONGEN *et al.*, 2006), including soil and peat (TALBOT *et al.*, 2005) and may occur in obligatory anaerobic bacteria such as planctomycetes and sulphate reducing bacteria (FARRIMOND *et al.*, 2002). There are strong correlations between 17 $\beta$ ,21 $\beta$ -bishomohopanoic acid and 17 $\beta$ ,21 $\beta$ -bishomohopanol and sterols (Cholesterol, Cholestanol, Sitosterol and Stigmastanol), with R values ranging from 0.94 to 0.98 ( $p < 0.001$ ).

### III.5 BIOMARKERS AND HISTORIC EVENTS

Higher amounts of soil being delivery on the lake indicate a change on cover or land use in the watershed and removal of vegetation becoming the soil more exposed. Some historic events on Lake Ohrid watershed may be related to this anthropogenic changes. The Dark Age to Medieval Warm Period transition in Europe coincide with the establishment of the medieval monastery as an institution managing land and agricultural production on an unprecedented scale and initiating the deforestation of extensive areas. From 900 and 1010 years, the monastery of Saint. Naum was founded at the southern shores of Lake Ohrid (PEARSON, 2004). This event raised the social condition of the area contributing for the town of Ohrid became the capital of Bulgarian Empire in 996 (ZLATARSKI, 1918). These historic events could be resulted in the growing anthropogenic impact in the area as from 995, when people moved from a defensive hill-top settlement towards the lake to establish what is now the town of Pogradec (RUNCIMAN, 1930). The growth of anthropogenic impact in the region as from 995, resulted in deforestation on large areas in a short period led to enhanced lake catchment erosion and higher soil contribution in OM toward the lake. This change on the OM fluxes is reflected on the lipid biomarkers and isotopic composition. Considering the heterogeneous composition of soil, microorganisms and nutrients supply, could be affected the primary productivity and, consequently also the formation of authigenetic carbonates, resulting on the changes on the values of biomarkers observed in this study. Additionally, the proximity of the Saint Naum from the local of core collection (FIGURES II-1 and II-3) may also have affected hydrologic conditions, which control amounts of OM and carbonate precipitation, contributing for higher amounts after 995. Wagner *et al.*, (2008), observed the same influence.

Besides the change in land use, the type of vegetation on the lake Ohrid watershed also may have been changed. Fatty acids and alcohols long chains (C26, C28) presented  $\delta^{13}\text{C}$  values ranged from -34.33 to -30.74 (TABLE III-2), typically from C3 plants (COLLISTER *et al.*, 1994). However, C3 plants can has been partly replace by C4 plants after 995, once the  $\delta^{13}\text{C}$  values of C26, C28 fatty acids in sediment

sample 1160-1275 are 1.55‰ and 1.86‰ respectively lighter than sediment sample 910-955 (TABLE III-2). This difference is close to the difference observed by Kramer and Gleixner (2006) of 2.4‰, in soil, which suffered change of 15% of vegetation C3 to C4, in Halle (Germany). Deforestation (C3 plants) in order to agricultural use land (C4 plants) is consistent with the historic events mentioned (RUNCIMAN, 1930; ZLATARSKI, 1918). Moreover, higher temperatures favour plants depleted in  $\delta^{13}\text{C}$  values (C4 type), which exist universally and their distribution vary with altitude (SUKUMAR *et al*, 1993).

An increase on temperatures and precipitation levels on Dark Age to MWP transition has been reported (BRADLEY *et al*, 2003; CROWLEY and LOWERY, 2000). Rise on the rainfall level associated to the soil exposure lead to the higher amounts of soil delivery to the lake. Magri (1996) suggests that the reduction of woodland in Medieval Warm Period in southeastern of Europe show positive correlation with the climatic conditions, mainly the mean annual precipitation values, which were a major factor in determining the history of vegetation in that time. Thereby historic events related to the removal of vegetation in large areas associated with the climate changes such as precipitation level and temperature were the most provable contributors for the change in the amount, as well as the composition of OM delivered on the lake Ohrid during the transition between the Dark Age and the Medieval Warm Period.

### III.6 CHAPTER SYNTHESIS

This chapter clearly illustrates how organic matter fluxes in Ohrid lake watershed were affected by response of natural climate change as well as in response of human populations. The integration of organic geochemistry of the lake Ohrid sediments with isotopic and inorganic analysis leads to convincing evidence of a changing in the catchment area around last 1,000 years ago. Most of biomarker assessed show a similar pattern quantitative over time, which reflect a rise on the OM input. In response, an increase on the productivity of lake was observed, reflected by sterols, branched C15 fatty acids, tetraymanol and bacteriohopanepolyols (BHP's) derivatives values.

Although significant changes on the total lipids composition were not observed, the classes of major compounds such as alcohols and fatty acids present long chains being replaced by middle chains after 995. It implies in a change on the terrestrial sources of OM, where plants are partly replaced by soil input. Among the biomarkers assessed, the C22 alcohol best reflected this change and demonstrated high potential to be used as a specific proxy of soil input in lake Ohrid watershed.

Moreover, deforestation and farming resulted of Saint Naum monastery foundation and the Bulgariam Empire capital condition of the Ohrid city appear to be related to the increase on the erosion of catchment area. Despite human impact, appear to be the direct cause, climatic effects also have contribution. The period of change observed overlap with the transition of Dark Age, colder and drier and the Medieval Warm Period, warmer and wetter.

Overall, this chapter demonstrate that the amount and composition of OM toward the lake Ohrid are controlled by an interaction of multiple natural and anthropogenic factors and the biomarkers assessed have high potential to reflect changes on these factors.

## **CHAPTER IV**

# **CLIMATE AND VEGETATION CHANGES IN BARIGUI RIVER BASIN IN THE LITTLE ICE AGE AND MODERN TIME**

## IV.1 CHAPTER INTRODUCTION

Chapter 4 describes the beginning of the study in the upper part of the Barigui River watershed. First, an evaluation of the condition of the area is presented to provide support for the further approaches. Nutrient concentrations were used as evidence of the earliest meaningful human impact in the area. Following the sequence, the level of diagenetic process that occurred was assessed by sterols ratios reported in the literature.

However, the main approach of this chapter is the assessment of shifts in vegetation of the study area in response to climatic changes during the transition of the Little Ice Age (LIA) to Modern Time (1600-2012). LIA is a climatic event that occurred globally but with variable intensity in time and space. Therefore, this chapter provides new evidences of the real effects of the LIA in southern Brazil in terms of temporal variation of temperature and its impact on vegetation cover, since changes in vegetation are considered as a response to variations in climate in an environment without significant anthropogenic impact.

In this chapter, a past scenario was reconstructed in order to report the onset of the significant human impact in the area, level of diagenetic processes and natural environmental changes throughout the distribution of nutrients, sterols, alkanes and indexes of glycerol dialkyl glycerol tetraether compounds (GDGTs). Such biomarkers consist of compound classes from different sources and processes; however, they seem to reflect coherently the environmental changes that occurred in the study area.

## IV.2 ANTROPOGENIC IMPACT RECORD

The nutrient load in a river is closely linked with the land use and occupation in the catchment watershed. Thus, variations in amount and composition of nutrients toward the river may provide evidence of the human activities in the area. In this way, total organic carbon (TOC), total nitrogen (TN), and inorganic (IP), organic (OP) and total phosphorus (TP) contents were assessed in order to detect temporal variation on the nutrient load into the Barigui River. The results are presented in TABLE IV-1. Those parameters are impacted directly by human activities, such as deforestation and untreated sewage discharges. The contents of TOC ranged from 0.73% to 6.4%, those of TN from 0.03% to 0.33%, and those of TP from 0.25% to 1.9% (TABLE IV-1).

TABLE IV- 1. TEMPORAL VARIABILITY OF TOTAL, ORGANIC AND INORGANIC PHOSPHORUS, TOTAL CARBON AND TOTAL NITROGEN, IN PERCENTAGE, AND RATIOS TOC/TN AND IP/TP, DEMONSTRATING NUTRIENT INPUT IN PT CORE OVER TIME.

Age	IP (%)	OP (%)	TP (%)	TOC (%)	TN (%)	TOC/TN	IP/TP
2012	0,75	0,15	0,90	4,28	0,20	21,42	<b>83,27</b>
2000	0,72	0,31	1,03	5,05	0,21	23,73	<b>70,00</b>
1995	1,18	0,38	1,57	<b>6,38</b>	0,22	28,93	<b>75,44</b>
1985	0,94	0,59	1,53	5,19	0,22	23,06	<b>61,56</b>
1980	1,40	0,31	1,71	4,37	0,26	16,90	<b>81,85</b>
1970	0,83	0,20	1,03	3,54	0,17	21,41	<b>80,31</b>
1965	0,46	0,09	0,55	2,86	0,13	22,35	<b>83,73</b>
1955	0,61	0,38	0,99	3,61	0,15	24,79	<b>61,27</b>
1945	0,24	0,01	<b>0,25</b>	<b>0,73</b>	0,09	<b>7,94</b>	<b>94,50</b>
1940	0,30	0,04	0,34	1,05	0,05	20,66	<b>89,42</b>
1930	0,07	0,26	0,33	0,89	0,04	22,17	<b>21,70</b>
1920	0,56	0,05	0,60	0,88	0,04	24,19	<b>92,25</b>
1910	0,47	0,19	0,66	1,87	<b>0,03</b>	<b>55,53</b>	<b>71,58</b>
1900	0,43	0,20	0,64	1,41	0,05	27,69	<b>68,36</b>
1895	0,45	0,22	0,67	2,10	0,06	37,50	<b>66,67</b>
1890	0,46	0,25	0,71	1,99	0,06	32,85	<b>65,15</b>
1880	0,54	0,15	0,69	1,60	0,07	23,87	<b>78,73</b>
1875	0,70	0,22	0,92	4,21	0,09	47,20	<b>76,42</b>
1870	0,50	0,11	0,61	4,20	0,16	26,59	<b>81,56</b>
1855	1,07	0,19	1,26	5,85	0,15	39,06	<b>85,09</b>
1840	0,94	0,14	1,08	4,66	0,20	22,92	<b>87,01</b>
1820	0,12	0,65	0,77	2,23	0,15	14,88	15,05
1805	0,03	0,14	0,17	4,36	0,29	15,30	18,41
1790	0,12	0,44	0,56	5,22	0,28	18,86	20,93
1770	0,04	0,63	0,67	4,73	0,26	18,27	6,45
1730	0,05	0,86	0,91	5,32	0,29	18,52	5,23
1690	0,98	0,95	<b>1,93</b>	5,75	<b>0,33</b>	17,19	50,71
1645	0,13	1,28	1,41	2,20	0,13	17,29	9,29
1605	0,06	0,39	0,45	2,40	0,13	18,46	13,22

Note: In bold maximum and minimum limits and period of antropoc influence in IP/TP ratio.

Variability in productivity as well as sources of organic matter can be detangled by TOC/TN. These ratios give insight into the abundance of terrestrial relative to aquatic components of OM, and its variation may reflect changes on the land use and anthropogenic impacts (MEYERS and ISHIWATARI, 1993). TOC/TN ratios greater than 20 indicate terrestrial source predominance, including fecal contamination (HAHN *et al.*, 2013), whereas values between 5 and 10 are attributed to phytoplankton and primary productivity (MEYERS and ISHIWATARI, 1993). However, these values are only a guide since organic matter originates in a mixture of sources. In this study, the TOC/TN ratios ranged from 7.9 to 55.5 (FIGURE IV-1). In the period from 1605 to

1820, these ratios are consistently less than 20, while from the period 1840 to 1920 they suddenly increased, and a peak was observed in 1910 (FIGURE IV-1).

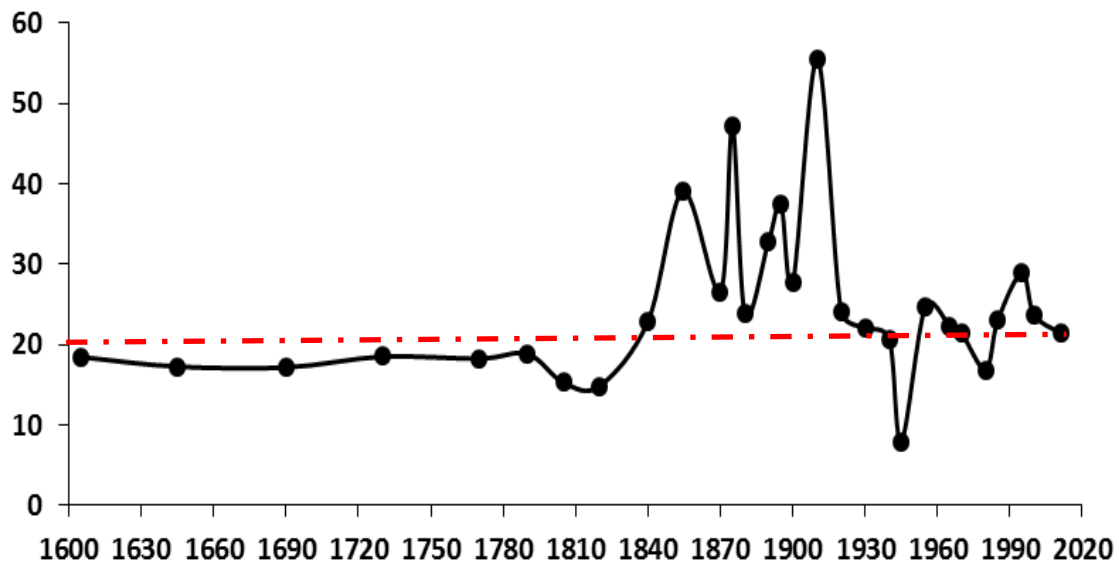


FIGURE IV- 1. TEMPORAL VARIABILITY OF TOC/TN IN PT CORE OVER TIME. THE DOTTED LINE ASSIST IN UNDERSTANDING OF LIKELY ORGANIC MATTER SOURCES. TOC/TN > 20 INDICATE TERRESTRIAL SOURCES.

Among the terrestrial sources of organic matter, detritus from deforestation is the main cause of nutrient input into lakes and rivers because plants are removed and entrained throughout the watershed, and soil is exposed, contributing to erosion in the catchment area. Records from the Institute of Land and Cartography Geosciences of Parana (ITCG, 2012), report an increase in deforestation in the studied area mainly after 1890. Therefore, the increase of the TOC/TN ratios in the period 1840 to 1920 observed seems to be related to the removal of vegetation whether by deforestation or bush fire also recorded in local history (FIGURE IV-2).

The anthropogenic influence observed in the TOC/TN ratios is also detected with the phosphorus content. Considering sediments without sewage contamination, the IP contents represent approximately 60% of the TP, while higher values suggest fecal contamination (MATER *et al.*, 2004). In PT sediment core, values greater than 60% are observed as from 1840 (TABLE IV-1), consistent with the changes that were observed in the TOC/TN ratios (FIGURE IV-1).

Thus, according to the phosphorus analysis and TOC/TN ratios, the evidence suggests that since 1840, anthropogenic interferences have changed the studied area. Deforestation and sewage contamination may be the result of urbanization during the expansion of the city, once population in Curitiba grew 40% in 1850, while population in Almirante Tamandaré grew 20% in 1900 and almost 80% in 1920 (IPPUC, 2009).

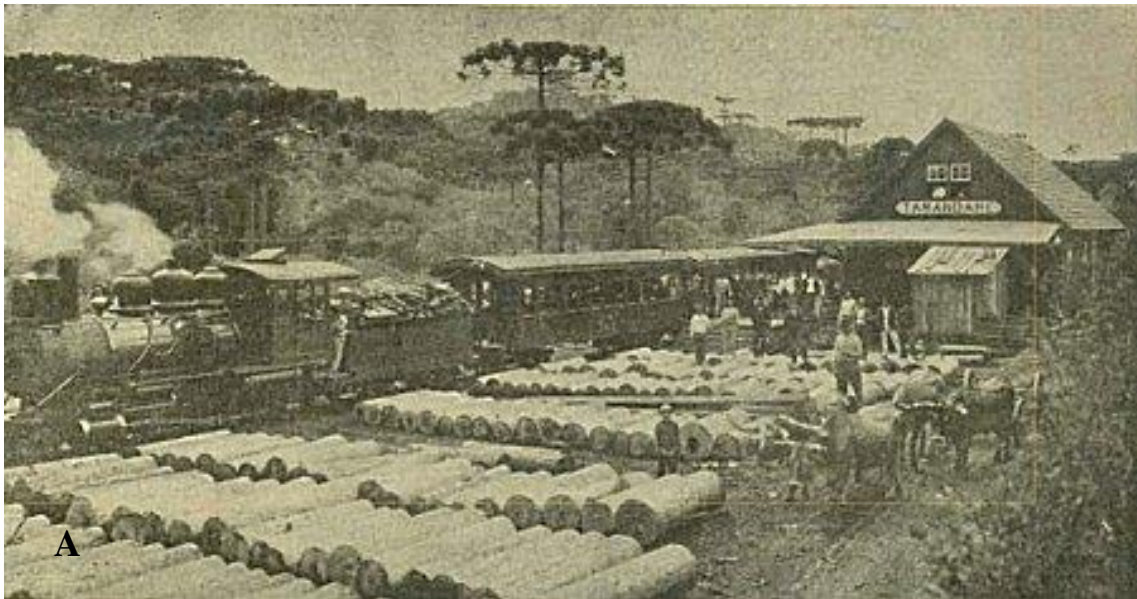


FIGURE IV- 2. EVIDENCES OF DEFORESTATION IN ALMIRANTE TAMANDARE (A) AND AROUND CURITIBA (B) IN 1910. SOURCES: KOTOVISKI, 2013; VALENTINI, 2009.

### IV.3 DIAGENETIC PROCESSES

Diagenesis is the process affecting the organic matter in sediment that takes place prior to deposition and during the early stages of burial under conditions of relatively low temperature and pressure, leading to biological, chemical and physical processes of transformation (KILOPS and KILLOPS, 1993). Microbial activity is one of the major agents altering sedimentary organic matter during the early stages of diagenesis near the sediment-water interface where more biochemically labile compounds are consumed, leaving behind the more biochemically stable materials and varieties of alteration products (ALI and MUDGE, 2005). Despite the wide use of sterols as biomarkers, this class of compounds presents higher propensity to suffer diagenetic processes compared to most conventional biomarkers. Some sterols are converted in other sterols under anoxic conditions. Hence, some ratios between sterols are able to represent the level of diagenetic transformation in the sedimentary environment (WAKEHAM and CANUEL, 2006). Among sterols, Cholestanol/Cholesterol (Chol/Chor) is commonly used for this goal, once it is in the “fresh” organic matter, which was recently produced by autotrophic process, the Chol/Chor ratio is between 0.1 and 0.5 (ARAUJO *et al.*, 2011).

In this study, the Cholestanol/Cholesterol ratio was calculated, and its temporal variability is shown in FIGURE IV-3. Values greater than 0.5 were observed between 1940 and 1980, indicating a significant influence of diagenetic processes during this period (CARREIRA *et al.*, 2001). This increase in the diagenetic process may be related to an early increase in the TOC/TN ratio between 1840 and 1920 (FIGURE IV-1), when terrestrial OM input, with microorganisms and nutrients in the soils, could have affected the primary productivity and redox conditions at the water/sediment interface (ALI and MUDGE, 2005). Soil presents a heterogeneous composition of microorganisms and nutrients supply and could affect the primary productivity and redox conditions in water/sediment interface. Considering that microbial activity is one of the major agents of diagenesis (ALI and MUDGE, 2005), the early increase on the TOC/TN values may have contributed to the rise on the diagenesis of sterols by soil microorganisms and nutrient supply. In most of the period studied, the Cholestanol/Cholesterol ratio was between 0.1 and 0.5, suggesting no significant diagenesis (ARAUJO *et al.*, 2011).

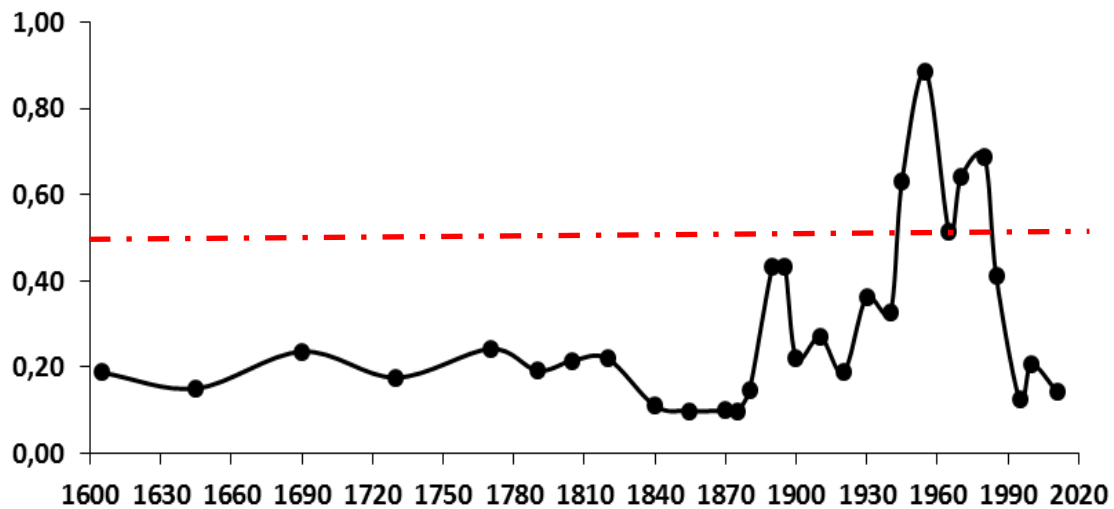


FIGURE IV- 3 TEMPORAL VARIABILITY OF CHOLESTANOL/CHOLESTEROL RATIO IN PT CORE, OVER TIME. THE DOTTED LINE ASSIST IN DEFINITION OF SIGNIFICANT DIAGENETIC PROCESSES (> 0.5).

### IV.3 MEAN AIR TEMPERATURE RECONSTRUCTION

MBT and CBT indexes were used to obtain mean annual air temperature (MAAT) in the studied area in the last 400 years. The temperature variation is shown in FIGURE IV-4 (b). Until 1730, MAAT remains around 14°C, then decreases. A minimum temperature is observed in 1800 (10°C), followed by a sharp rise to 13°C in 1850 and increase roughly at a maximum of 17°C in modern time. However, throughout this period, it presented two meaningful drops: in 1890 and 1970.

Peterse *et al.* (2009a) studied a series of soils and confirmed that MBT is controlled by soil temperature and pH, whereas CBT is related to pH. Moreover, in recent studies, Peterse *et al.*, (2009b) observed that the MBT/CBT ratio should only be used at sites with a catchment area where sufficient soil formation takes place and the soil thereby contains substantial amounts of branched GDGTs. In this way, to estimate the input of allochthonous terrestrial isoprenoid GDGTs, Weijers *et al.*, (2006) suggested the BIT index. This index is based on the relative abundance of crenarchaeol and the three main, branched GDGTs produced by anaerobic soil bacteria. Values of this index range from 0 to 1. The lowest value means no input of soil organic matter, while the highest value indicates a dominant soil organic-matter input. In this study, the BIT index was higher than 0.96, accounting for substantial soil input. Although the data are reliable to the reconstructed MAAT, the analytical error for both the CBT and MBT index must be also considered (approximately 0.01). These values can be translated into MAAT; hence, a local validation is recommended (WEIEJERS *et al.*, 2011). The recent MAAT values observed in this study were compared with in situ measurements at 2002 to

2011 (SIMEPAR, 2014), and the difference ranged between 1.5 and 1.8°C higher than the MAATs reconstructed in this study. Standard error of up  $\pm 5^\circ\text{C}$  is reported between reconstructed and measured MAAT (WEIEJERS *et al.*, 2011; TIERNEY *et al.*, 2010).

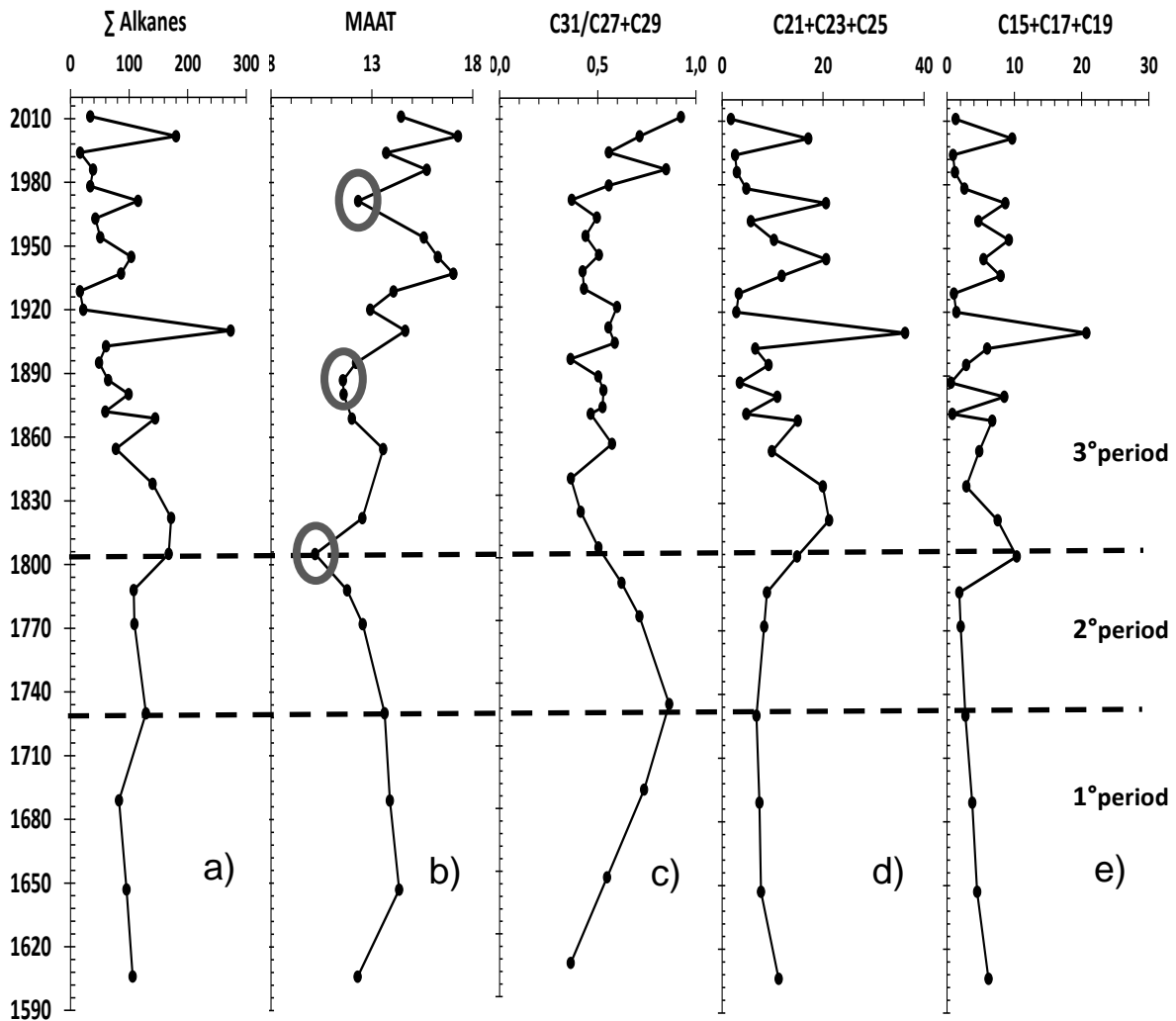


FIGURE IV- 4. TEMPORAL VARIABILITY OF  $\Sigma$  n-ALKANES ( $\mu\text{g.g}^{-1}$ ) (a), MAAT ( $^\circ\text{C}$ ) (b), n-ALKANE PROXIES FROM LONG CHAINS (c), MIDDLE CHAIN (d) AND SHORT CHAINS (e) IN PT CORE. CIRCLES INDICATE MINIMUM IN MAAT.

#### IV.4 SOURCES AND ENVIRONMENTAL SIGNIFICANCE OF ALKANES

The relative abundance of n-alkane homologues in some samples is shown in FIGURE IV-5. In all samples analyzed, n-alkanes ranged from C15 to C36 and the total concentration varied from 0.1 to 270  $\mu\text{g/g}$  (FIGURE IV-4 (a)). The most significant change in total concentration of n-alkanes was observed in the segment corresponding to years 1800 onward.

The n-alkanes distribution is dominated by middle-long carbon chains ( $>C20$ ), predominately C27 and C29 (FIGURE IV-5). It is known that n-alkanes with different

carbon chain length have different biotic sources. Generally, aquatic sources, such as algae and bacterias, are dominated by C15, C17 and C19 n-alkanes (EGLINTON and HAMILTON, 1963). Aquatic macrophytes commonly maximize at C21, C23, and C25 n-alkanes (FICKEN *et. al.*, 2000b) whereas vascular plants contain large proportions of C27, C29, and C31 n-alkanes in their epicuticular wax (RIELEY *et. al.*, 1991). The profile of n-alkane distribution (FIGURE IV-5) points to vascular plant and aquatic macrophytes as predominant sources of organic matter. Algae and photosynthesis bacteria have less contribution along the core.

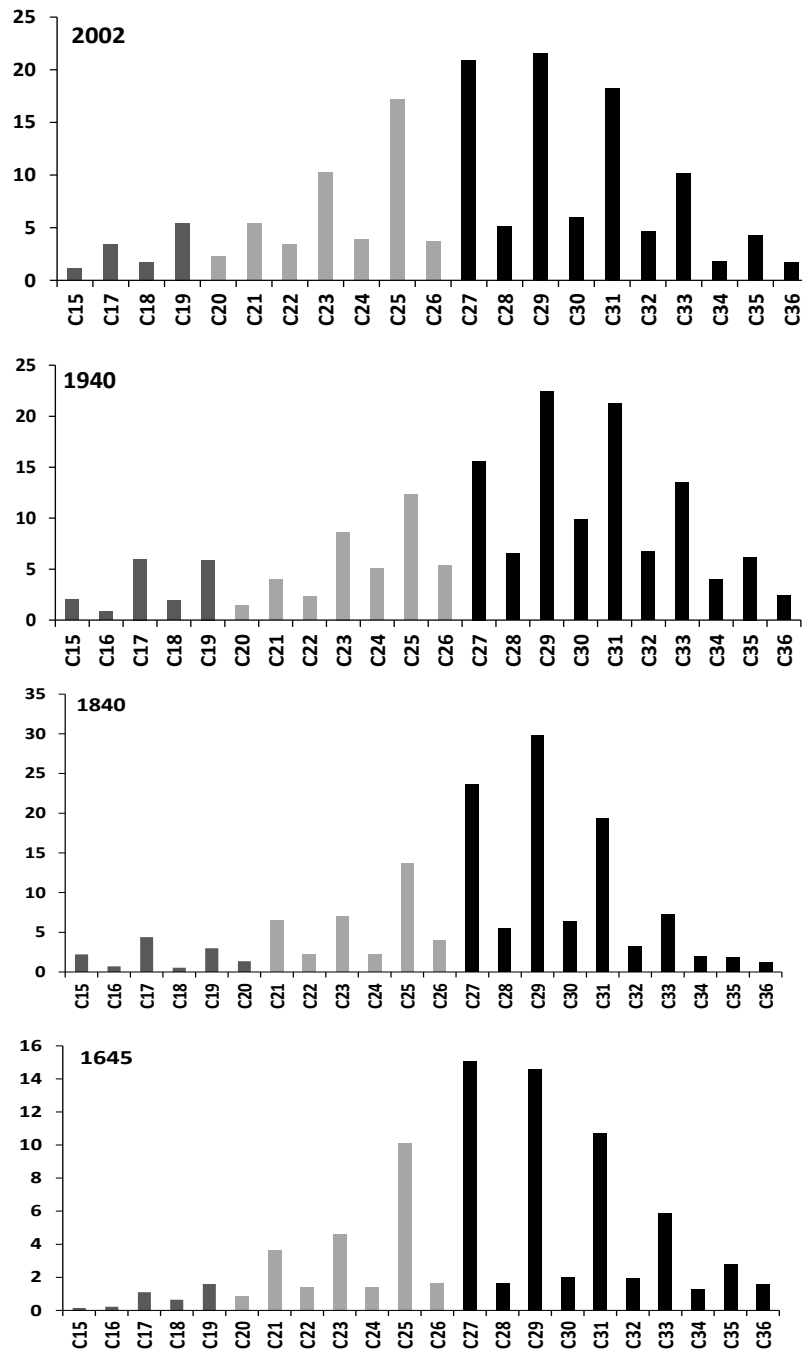


FIGURE IV- 5. n-ALKANE DISTRIBUTION OVER TIME OF SOME SELECTED SAMPLES FROM PT CORE.

Although non-emergent macrophytes (submerged and floating leaved) display enhanced abundances of middle chains (C21, C23 and C25), emergent macrophytes may have an n-alkane distribution similar to those of terrestrial plants, typically dominated by long chains (>C27) (SU *et al.*, 2005). Thus, Ficken *et al.* (2000b), formulated a proxy ratio ( $P_{aq}$ ) to discriminate the relative contribution of non-emergent/emergent macrophytes and terrestrial vegetation in middle chains distribution, using the relative proportion of mid-chain to long-chain homologues, as shown in Equation 7.

$$P_{aq} = \frac{C_{23}+C_{25}}{C_{23}+C_{25}+C_{29}+C_{31}} \quad (\text{Equation 7})$$

Where  $P_{aq} < 0.1$  corresponds to terrestrial plants, 0.1–0.4 to emergent macrophytes and 0.4–1 to submerged/floating macrophytes (FICKEN *et al.*, 2000b). In this study,  $P_{aq}$  values ranged from 0.15 to 0.43 (average 0.23), indicating a mixture of inputs, where emergent macrophytes are a predominant source of middle chains, with less contribution of submerged/floating macrophytes.

The carbon preference index (CPI), Equation 8, was used to better understand and reconstruct the sources of carbon content in core sediment. Terrestrial plants generally contains long alkane chains with a strong odd over even carbon number predominance, therefore CPI values are indicators of organic matter sources. CPI values higher than five can be attributed to terrestrial sources, while CPI close to unity can be associated with petroleum contamination (MOLDOWAN *et al.*, 1985).

$$CPI = \frac{2(C_{21}+C_{23}+C_{25})}{C_{20}+2(C_{22}+C_{24}+C_{26})+C_{28}} \quad (\text{Equation 8})$$

The CPI index temporal variability is present in FIGURE IV-6. Values varied from 4.0 to 9.0 indicating terrestrial sources predominance. Higher values are between 1840 and 1930, concomitant with a prominent shift in TOC/TN ratios (FIGURE IV-1) and supporting the evidence of terrestrial input in this period. Although a predominance of terrestrial plant source is evident (CPI>5), a slight decline in CPI values is observed mainly after 1930, which may be related to a contribution of petrogenic sources due to economic development of the region in this period.

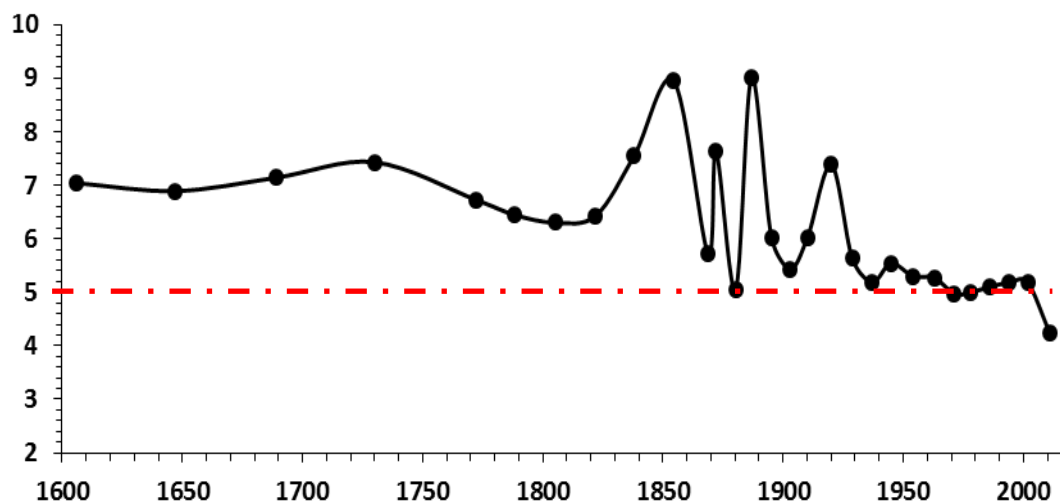


FIGURE IV- 6. TEMPORAL VARIABILITY OF CPI VALUES. THE DOTTED LINE ASSIST IN DEFINITION OF TERRESTRIAL (CPI>5) AND PETROGENIC (CPI  $\approx$ 1) ALKANES SOURCES

The Barigui River watershed is located in the first plateau of Parana state so that the declivity decreases from north to south. As mentioned earlier, significant anthropogenic influence in this area such as deforestation and fecal pollution started after 1840 with the rise in population. Thus, before 1840, temperature and precipitation were the main factors controlling terrestrial vegetation development (BERTOLO *et al.*, 2005).

The C27, C29 and C31 were the predominant n-alkanes. Paleoenvironmental studies have reported that long-chain n-alkane could reflect temperature and precipitation change once vegetation cover is sensitive to climatic changes and play an active role in the climate system via feedback mechanisms (SUN *et al.*, 2011; ZOU *et al.*, 2009; SCHWARK *et al.*, 2002).

Woody plants are much more resistant to low temperature and humid conditions and are the sources for C27 and C29 n-alkanes while herbaceous plants are the main source of C31 n-alkanes and are more sensible to cold and arid weather (ZHANG and GAO, 2005). Thus, changes in organic matter composition from terrestrial plants can be associated to changes in climate and hydrologic conditions. According to the literature (SUN *et al.*, 2011; LIN *et al.*, 2008; ZHANG *et al.*, 2005), the ratio of C31/(C27+C29) reveals the proportion of woody and herbaceous plants, reflecting changes in temperature and precipitation in a positive correlation. In this study, the trend of C31/C27+C29 ratio are showed in the FIGURE IV-4 (c), the values increase until 1730 and then gradually decrease. After 1840 oscillations in ratios values are observed probably due human intervention such as deforestation.

The biomass of aquatic plants (emergent and submerged/floating) is influenced by temperature, sunlight intensity, nutrients supply and dissolved inorganic carbon

(DIC) (COOPS *et al.*, 2007). The main source of nutrients for aquatic plants is the surface of sediment. In Barigui River due to the high biomass of aquatic macrophytes (MALTCHIK *et al.*, 2005 and FIGURE IV-7), the nutrients supply in surface sediments is a restriction, once significant amounts of nutrients are necessary to their growth. The main type of DIC used by macrophytes aquatic is  $\text{HCO}_3^-$  (LIN *et al.*, 2008). Due small amounts of DIC necessary for macrophytes photosynthesis (PIERINI and THOMAZ, 2004) DIC naturally dissolved in the water of Barigui River is enough to supply aquatic plants development (MULLER *et al.*, 2008).



FIGURE IV- 7. EMERGENT AND SUBMERGED MACROPHYTES FROM BARIGUI RIVER.

Although sunlight intensity under water is very important to aquatic plants, the Barigui River is shallow. Nevertheless, their germination, flowering and dormancy are restricted by temperature (COOPS *et al.*, 2007). Thus, nutrients supply and temperature are obvious limiting factors for macrophytes aquatic in Barigui River. Higher nutrients supply, on the other hand, is often associated with higher precipitation by run off into the river (LEROY *et al.*, 2011; LIN *et al.*, 2008; MALTCHICK *et al.*, 2005). Thereby, the sum of middle chains alkanes (C21+C23+C25) from aquatic plants could be associated with temperature and precipitation. This sum is presented in FIGURE IV-4 (d), the values are constant until early 1800, suggesting lesser temperature and precipitation. After that, a sharp rise to 1820 and oscillation until nowadays are observed, indicating an increase in temperature/precipitation. .

In the PT core, the sum of short chains alkanes (C15+C17+C19), which represent primary productivity, presented trend similar to the middle chains (FIGURE IV-4 (e)), since biomass of algae and photosynthesis of some bacteria are also influenced by nutrients and temperature (LIN *et al.*, 2008).

## IV.6 CLIMATIC AND VEGETATION COVER CHANGES

Three periods could be distinguished in FIGURE IV-4, showing environmental changes since 1600 by n-alkanes distributions and MAAT reconstructed. Those periods seem to be a consequence of the Little Ice Age event (LIA), in which the Earth became, on average, slightly colder (WHITE, 2014). It is generally agreed that there were three minima in LIA, beginning around 1600, 1770 and 1850, each of them separated by slight warming intervals (PEREIRA *et al.*, 2009), although the worldwide effects of climatic events might diversify in time and magnitude.

Thus, the first period, between 1600 and 1730 seems to be an improvement in climatic conditions after the first minimum of LIA occurred before 1600. The MAAT around 14°C indicate a slight warming, which is reflected in type of vegetation. Herbaceous plants steadily increased and predominate until 1730, overlapping wood plants, according the C31/C27+C29 ratio (FIGURE IV-4 (c)). Such behavior was also observed by Behling *et al.*, (2004) in an area with similar characteristics of the area of this study (south of Brazil). Between 1520 and 1770 in south of Brazil, *Weinmannia sp.*, an endemic grass plant became a common taxa in the Araucaria forest, suggesting a shift to warmer climate conditions, consistent with the change observed in this study. Despite the variation of long chains, middle and short chains of alkanes distribution (FIGURE IV-4 (d, e)) reveal that aquatic plants and phytoplankton development were

restricted by temperature and more liked by scarce supply of nutrients due to less humid climatic characteristics, consistent with LIA period.

The second period is between 1730 and 1800. This period encompasses a steady decline toward the second minimum in temperature of LIA ( $\approx 1770$ ) reported by Pereira *et al.*, (2009). In 150 years (1650 to 1800) the mean temperature dropped 4°C (FIGURE IV-4 (b)). This temperature define the time of minimum temperature of LIA in the studied area in 1800. According to Mann *et al.*, (2009), the minimum of LIA in Pacific and Atlantic Ocean occurred around 1800 coincident with this study. The pattern of decline in temperature is also reflected in the decline of C31/C27+C29 ratio (FIGURE IV-4 (c)), supporting the positive correlation between them and indicating low temperatures and less humid climatic, characteristics in this period, once in anomalous climatic conditions, such as cold and aridity, woods plants tend to be more resistant than herbaceous plants (SUN *et al.*, 2011; LIN *et al.*, 2008). Machado *et al.*, (2014), in the same area reported a slight increase in the concentration sitosterol, stigmasterol and campesterol typically for high plants from 1730, concomitant with the decline in the C31/C27+C29 ratio in this study. It is reasonable to suppose that the increase of high plants sterols in the sediment might be related to the death (e.g. deposition, biodegradation) of herbaceous plants, due decline in the temperature and less humid conditions observed in the second period. Pereira *et al.*, (2009), also observed similar shifts in the vegetation composition in southeast of Brazil in the same period of first and second period of this study. Initially, a reduction of the forests and an increase of the savannah/fields were noticed. After that, the forest started to increase and finally, there was a recovery of the dense forest and a reduction of the savannah/fields. It is mean that the effects of LIA were similar in several regions from Brazil, although, Brazil is a large country and present climatic differences in its extent. The absent of blooming in aquatic plants and phytoplankton, reflected by middle and short chains of alkanes respectively, in the second period (FIGURES IV-4 (d, e)) indicates that less humid conditions remains. Still with regard to the LIA in Brazil, studies performed in both northeastern and southeastern regions that the coast experienced a sea-level regression and/or drier conditions with less rainfall during the LIA (OLIVEIRA *et al.*, 2014; PEREIRA *et al.*, 2009; COHEN *et al.*, 2005), consistent with the findings of this study.

The third period is between 1800 and nowadays, it corresponds to the end of the LIA, reported in several studies between 1850 and 1890 marked by warming and wetter climate (WHITE, 2014; MANN *et al.*, 2009; BEHLING *et al.*, 2004). Both middle and short chains of alkanes presented an increase from 1800 to 1850 reflecting terrestrial and submerged aquatic plants blooming (FIGURE IV-4 (d, e)), due warming

and higher precipitations from 1800, also reported in Rio de Janeiro (PEREIRA *et al.*, 2009) and in Amazonia (COHEN *et al.*, 2005). Increase in precipitation affects the runoff and consequently leads to higher nutrients input in river, which associated to the warming results in a rise in primary productivity (phytoplankton and macrophytes aquatic). In this period the temperature increased roughly until the present moment (FIGURE IV-4 (b)), although meaningful drops are observed in two moments. First one in 1890, probably related to the third minimum of LIA (PEREIRA *et al.*, 2009), and observed by Mann *et al.*, (2009) in the Atlantic and the Pacific Ocean. Second moment around 1970, leading to one of the coldest winters in the history of the region, including the presence of snow (FIGURE IV-8) (SENKO, 2012; YANG, 2005), which is rare due to the warm-temperate regional climate (SILVA and GUETTER, 2003).



FIGURE IV- 8. SNOW IN CURITIBA IN 1975. SOURCE: WACHOWITCZ, 1995.

After 1850, it is not more possible to assess and confirm climate variations through the response of terrestrial and aquatic vegetation, due to anthropogenic influence in the studied area from 1840. However, the warming observed in reconstructed temperature data of this study is consistent with local measurements from 1970, confirming the results (IAPAR, 2014; SIMEPAR, 2014)

#### **IV.7 CHAPTER SYNTHESIS**

A scenario of vegetation evolution and paleoenvironmental changes in south Brazil, since 1600, was reconstructed by n-alkanes distribution and GDGT indexes. Correlated studies, historic records and measurements in situ support these changes. Three distinct periods were observed in terms of changes in climate and vegetation, which correspond to the LIA period. The first period (1600–1730) presents slight warming (around 14°C) and less humid conditions; it seems to be an improvement of the minimum temperatures previous to 1600. In this period herbaceous plants overlapped wood plants. After that, in the second period (1730–1800) the temperature decreased gradually toward a value of 10°C in 1800. Concomitantly, wood plants predominated over herbaceous plants due to the decline in temperature and drier conditions. In the third period, (1800–nowadays) the end of LIA is evident throughout warming and higher precipitation levels reflected in a blooming of phytoplankton and submerged aquatic plants.

Although some LIA paleoenvironmental information has already been reported in South Brazil, the studies are scarce and therefore this study provides new information about climate and vegetation evolution features during LIA and transition to the modern time.

## **CHAPTER V**

# **ATMOSPHERIC COMPOSITION CHANGE IN BARIGUI RIVER BASIN IN THE LITTLE ICE AGE AND MODERN TIME**

## V.1 CHAPTER INTRODUCTION

Chapter V is an extension of the previous chapter. Middle and long-chain alkanes from aquatic and terrestrial plants studied in Chapter IV are studied in this chapter from an isotopic point of view. The temporal variation of soil pH was also assessed in order to provide subsidiary information.

First, the results of soil pH and middle/long alkanes isotopic composition were explained; then their temporal variability to detect trends and meaningful changes was discussed. In sequence, some environmental factors, such as temperature, humidity and atmospheric CO<sub>2</sub> concentration, with a high potential to affect isotopic ratios of plants were evaluated to identify their influence in prominent shifts. Among them, changes in atmospheric CO<sub>2</sub> levels over time seem to have been accountable for temporal variability in isotopic ratios of plants in the watershed. Although such changes have affected all plants simultaneously, the magnitude of the response to this change in aquatic and terrestrial plants is quite different and some comments about this approach were made.

Overall, an environmental change during the transition of LIA to Modern Time in atmosphere is the main finding of this chapter. Changes in atmospheric composition in the region are also discussed as possible sources of enhanced CO<sub>2</sub>. Such sources were identified as globally and locally influenced, according to their scope and comparison with detailed studies of climate changes in Antarctic. Among the global sources, natural events such as volcanism seem to be related to the increase of atmospheric CO<sub>2</sub> in the earliest time once volcanism is reported in literature as a possible contributor for an LIA event.

Considering the spatial extent of the atmosphere, changes in CO<sub>2</sub> concentration observed in this chapter have regional scope, which can be extended for South Brazil where, as mentioned in the previous chapter, there is little information about environmental changes in the period studied and in terms of atmospheric composition.

## V.2 SOIL PH RECONSTRUCTION

The temporal variability of reconstructed pH, based on CBT index, is showed in FIGURE V-1. In the studied period (1605-2012), pH values ranged between 6.20 and 7.00. Around 1650, reconstructed pH presented a smaller value (pH ≈ 6.2), then steadily increased until 1730, where the maximum is observed (pH≈6.8). After that, reconstructed pH decreased to a minimum value of 6.2 in 1855 and increased again roughly with drops in 1890 and 1955, reaching values around 7.0 in the present day (FIGURE V-1). Although CBT index is widely used for pH reconstruction (NAEHER *et al.*, 2014; SHOON *et al.*, 2013; ZHU *et al.*, 2011), it

is recommended to get a local validation of pH in order to assess potential errors (WEIJERS *et al.*, 2011). Such validation was made in five surface soil samples collected from different places within the studied area. Three samples were collected in an open field and presented pHs of 6.86 ( $\pm 0.1$ ), 6.76 ( $\pm 0.1$ ) and 6.68 ( $\pm 0.1$ ), while two samples were collected from forest areas, resulting in pHs of 6.06 ( $\pm 0.1$ ) and 5.98 ( $\pm 0.1$ ). The lower pH values in areas covered with forest are probably related to acidification of soil by trees (OLIVEIRA and CARVALHO, 2011; FARIA *et al.*, 2009; SOUZA *et al.*, 2006), even considering their high capacity to recycle nutrients and alkalis (SOUZA *et al.*, 2006) and the potential to release organic acids by roots (OLIVEIRA and CARVALHO, 2011). Reconstructed pH by CBT index for 2012 is approximately 0.3 higher than pH measured in soil surface. The literature estimates that uncertainties could be up 0.8 pH units for reconstructed CBT pH (WIEJERS *et al.*, 2011; TIERNEY *et al.*, 2010; WEIEJERS *et al.*, 2006).

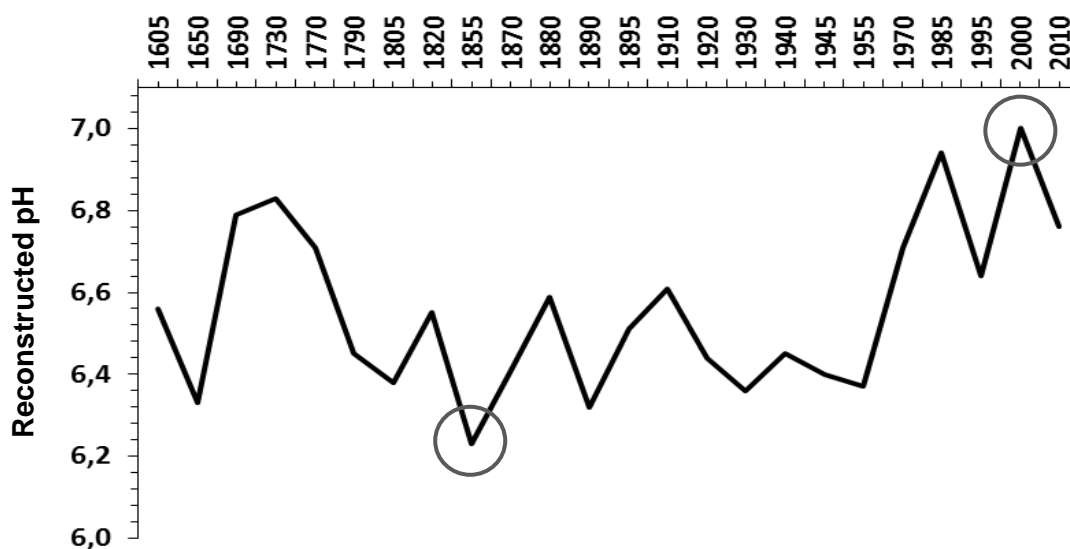


FIGURE V- 1. TEMPORAL VARIABILITY OF RECONSTRUCTED pH FROM GDGTs INDEXES. CIRCLES DISPLAY MINIMUM AND MAXIMUM VALUES.

### V-3 ISOTOPIC SIGNATURE OF ALKANES - BULK AND TREND

Odd n-alkane chains C15 to C31 length were quantified in the core sample; however, only C21 to C31 n-alkanes presented reliable isotopic ratio data (CV<3%).  $\delta^{13}\text{C}$  temporal variability of those alkanes is presented in FIGURE V-2. Middle alkanes chains (C20-C25) derive mainly from macrophytes (AICHNER *et al.*, 2010), whereas long alkane chains (C27-C31) derive from leaf waxes of terrestrial plants (SUN *et al.*, 2011; MEAD *et al.*, 2005). Thus, temporal variability of C27, C29 and C31 chains are similar. These terrestrial alkanes are  $^{13}\text{C}$ -depleted as from 1600 and trend toward minimum negative values around 1880. After that, C27, C29 and C31 chains are sharply  $^{13}\text{C}$ -enriched, achieving a maximum  $\delta^{13}\text{C}$  value in

1930 and then decreasing roughly again to values that are more negative until the present day. This  $\delta^{13}\text{C}$  temporal variability might be summarized with a difference of  $\approx 4\text{‰}$ , in three periods, an earlier drop period (1600 to 1880), followed by a rise period (1880-1930) and finally, another drop period (1930 to the present day). In terms of  $\delta^{13}\text{C}$  values, terrestrial alkanes present values consistent with terrestrial C3 plants (typically  $-33 \pm 2.3\text{‰}$ ,  $n = 320$ ) (TIPPLE and PAGANI, 2010) although  $\delta^{13}\text{C}$  values vary among the chains. C27 and C29 ranged from  $-36\text{‰}$  to  $-30\text{‰}$ , whereas C31 presented higher  $\delta^{13}\text{C}$  values of  $-33.5\text{‰}$  to  $-29\text{‰}$  (FIGURE V-2). According to Zhang and Gao (2005), woody plants are the main source of C27 and C29 alkanes while herbaceous plants are the main source of the C31 alkane. Although C3 plant groups encompass 85% of all plants species in most ecosystems (AICHNER *et al.*, 2010) and tend to be more resistant to colder and drier climate conditions, characteristic of the LIA period (CERLING *et al.*, 1997), both wood and herbaceous plant groups present C3 and C4 plants species, which differ widely in their isotopic signature. C3 plants are relatively  $^{13}\text{C}$ -depleted ( $-33 \pm 2.3\text{‰}$ ,  $n=320$ ) while C4 plants are  $^{13}\text{C}$ -enriched ( $-21.7 \pm 2.4\text{‰}$ ,  $n= 58$ ) (TIPPLE and PAGANI, 2010). Therefore, it is reasonable to suppose that the contribution of C4 plants species is more important as a source of C31 than C27/C29. It is reflected in the slight  $^{13}\text{C}$ -enrichment of C31 alkanes of  $\approx 2\text{‰}$ . Kramer and Gleixner (2006) observed a  $2.4\text{‰}$  difference in long chains alkanes from soil after 15% replacement of vegetation C3 by C4 plants in Halle (Germany).

The prominent shift in  $\delta^{13}\text{C}$  values observed in long chains alkanes is also apparent in middle chains, however, with some peculiarities.  $\delta^{13}\text{C}$  temporal variability of C21 and C23 alkanes is quite similar between them and vary roughly in the period 1600-1890. In both cases, after that period,  $\delta^{13}\text{C}$  values sharply increased reaching higher values around 1930, followed by a sudden drop toward lowest values in 1960 and a new sharp rise until the present day (FIGURE V-2). Conversely, C25 alkane showed a similar temporal behavior of long chains, except by  $^{13}\text{C}$ -enrichment after 1960, in contrast with long chains alkanes, which present  $^{13}\text{C}$ -depletion (FIGURE V-2).

In Chapter IV (Section IV.4), the proportion of emergent and submerged/floating macrophytes as a source of middle chains alkanes in PT core was assessed, and it was observed that emergent macrophytes are the major source with less contribution of submerged/floating macrophytes. Emergent macrophytes present similarities with terrestrial plants; therefore, C25 chains seem to have a higher contribution of emergent macrophytes compared to C21 and C23 chains. In terms of  $\delta^{13}\text{C}$  values, middle chains ranged from  $-33\text{‰}$  to  $-27.5\text{‰}$ , consistent with defined values for macrophytes (AICHNER *et al.*, 2010) and are usually within the range for C3 plants although they are slightly heavier than long chains (FIGURE V-2).

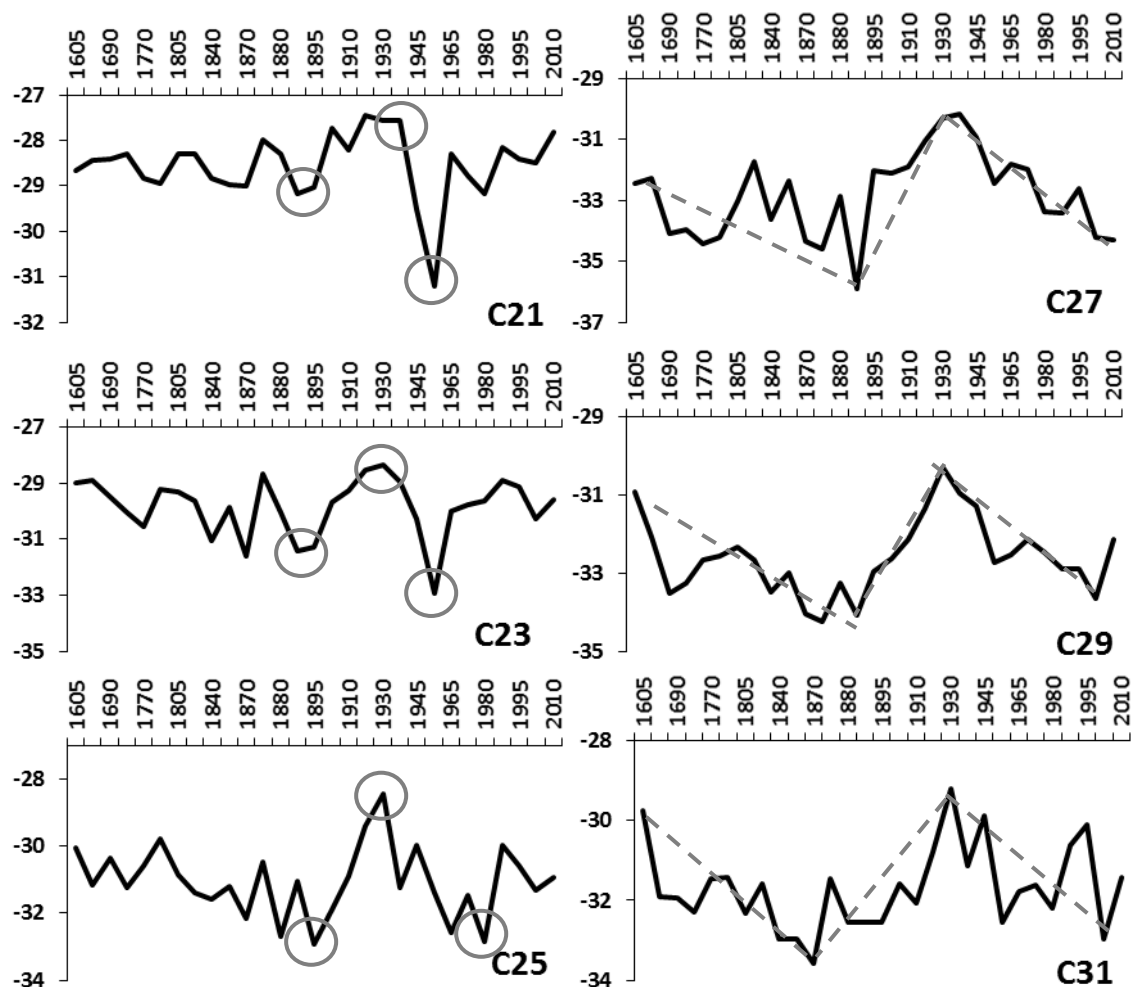


FIGURE V- 2.  $\delta^{13}\text{C}$  TEMPORAL VARIABILITY OF MIDDLE CHAINS (LEFT) AND LONG CHAINS (RIGHT) ALKANES. CIRCLES DISPLAY SIGNIFICANT PEAKS AND DASHED LINE THE TEMPORAL TREND.

#### V-4 TEMPERATURE AND HUMIDITY INFLUENCE IN ISOTOPIC COMPOSITION OF PLANTS

Two significant drops of  $\approx 4\text{‰}$  in  $\delta^{13}\text{C}$  values were observed in middle and long alkane chains between 1660 and 1880 and between 1930 and the present day (FIGURE V-2). In general, several factors contribute to changes in the  $\delta^{13}\text{C}$  of those chains from aquatic and terrestrial plants. The main environmental factors are vegetation type, concentration of  $\text{CO}_2$  in the atmosphere, temperature, humidity, water salinity and pH, soil fertility and air pollution (STREET-PERROT *et al.*, 2004; LOPEZ and CECILIO, 2002). These factors affect important biological processes of aquatic and terrestrial plants (e.g., photosynthesis, growth, development and nutrients absorption), leading to shifts in the isotopic discrimination and in different values of  $\delta^{13}\text{C}$  for middle and long alkane chains (CERNUZAK *et al.*, 2013). The magnitude of isotopic discrimination by C3 plants varies significantly in response to

environmental factors, especially moisture stress and temperature (STREET-PERROT *et al.*, 2004).

Chapter IV describes climatic changes in transition of LIA to Modern Time. Briefly, three periods were observed: i) warmer/drier period between 1600 and 1730; ii) colder/drier between 1730 and 1800; and iii) warmer/wetter after 1800 until the present day. In terrestrial plants, drier conditions leading to  $^{13}\text{C}$ -enrichment and consequently higher  $\delta^{13}\text{C}$  values, due the stomatal conductance reduction as a mechanism to avoid water loss by transpiration, however reducing also the  $\text{CO}_2$  diffusion (CERLING *et al.*, 1997). It similarly occurs with the temperature. Decrease in temperature causes increase in the water viscosity, slowing the transport of water from soil to the evaporative sites in leaves (RODERICK and BERRY, 2001); consequently, stomatal conductance and diffusion of  $\text{CO}_2$  in to leaves are reduced, resulting also in higher  $\delta^{13}\text{C}$  values (CERNUZAK *et al.*, 2013). Decreasing temperature and drier conditions were observed between 1600 and 1800. In this period,  $\delta^{13}\text{C}$  values of terrestrial chains steadily decreased instead to increase (FIGURE V-2). After 1800, temperature and humid conditions increased constantly until the present day, while  $\delta^{13}\text{C}$  values decreased only after 1930 (FIGURE V-2). The lack of a consistent correlation indicates that temperature and humid conditions are not the factors responsible for drops in  $\delta^{13}\text{C}$  values of terrestrial alkane chains.

In aquatic ecosystems, moisture stresses reduce the level of the water, while low temperatures lead to a higher amount of dissolved  $\text{CO}_2$ . Aquatic macrophytes can utilize both atmospheric carbon and the carbon pool in the water (AICHNER *et al.*, 2010). In the Barigui River, potential carbon sources for macrophytes are ambient  $\text{CO}_2$ , methanogenic sources and bicarbonate from rock and soil weathering (SUN *et al.*, 2013). In reduced water levels, consequently enriched in dissolved  $\text{CO}_2$ , concentration of bicarbonate and methanogenic carbon also tends to increase (STREET-PERROT *et al.*, 2004). The use of these carbon sources by aquatic macrophytes enhances isotopic discrimination and results in higher  $\delta^{13}\text{C}$  values (CERNUSAK *et al.*, 2013; AICHNER *et al.*, 2010). However, the decrease in temperature toward minimum values in 1800 and drier conditions is parallel to a rough variation in  $\delta^{13}\text{C}$  values of middle chains that rise instead, indicating no correlation. Moreover, temperature and precipitation rise progressively after 1800, while  $\delta^{13}\text{C}$  values of middle chains vary widely until the present day (FIGURES V-2 and V-3).

Thus, it seems that temperature and humidity do not account for drops in  $\delta^{13}\text{C}$  values of middle and long alkane chains although influences in  $\text{CO}_2$  diffusion by aquatic and terrestrial plants shift their  $\delta^{13}\text{C}$  values in a significant way.

## V-5 CO<sub>2</sub> ATMOSPHERIC INFLUENCE IN ISOTOPIC COMPOSITION OF PLANTS

It is known that atmospheric CO<sub>2</sub> plays an important and complex role in photosynthesis. Several steps are involved in incorporating it into plant material (TIPPLE *et al.*, 2010). Discrimination against <sup>13</sup>CO<sub>2</sub> occurs in all steps that transfer CO<sub>2</sub> from the atmosphere to the chloroplast and in the carboxylation reactions (FUNG *et al.*, 1997). It implies that shifts in isotopic composition of atmospheric CO<sub>2</sub> strongly influence the isotopic discrimination by plants and are reflected in their δ<sup>13</sup>C values. Several studies report the influence of atmospheric CO<sub>2</sub> in isotopic composition of plants (TIPPLE and PAGANI, 2010; ZHAO *et al.*, 2001; BERLING and WOODWARD, 1995). In fact, increases in atmospheric CO<sub>2</sub> cause decreases in δ<sup>13</sup>C values in alkanes from terrestrial and aquatic plants (WEISENBERG *et al.*, 2008a). Zhao *et al.*, (2001) assigned the depletion of 2.5-2.8‰ in δ<sup>13</sup>C values in wheat, after 153 years, to an increase of CO<sub>2</sub> atmospheric in Rothamsted (England). Berling and Woodward (1995) reported a small decrease in δ<sup>13</sup>C values of 17 C<sub>3</sub> plants (grass and herb species) due to an increase of atmospheric CO<sub>2</sub> in a growth chamber experiment, while Weisenberg *et al.*, (2008b) observed depletion of 2-6‰ in δ<sup>13</sup>C of n-alkanes from plant waxes under ambient with elevated CO<sub>2</sub> conditions.

Thus, among the factors that affect isotopic composition of plants, earlier mentioned, atmospheric CO<sub>2</sub> seems to be the most likely accountable for drops in δ<sup>13</sup>C values of middle and long chains observed in FIGURE V-2. Therefore, it is supposed that increases in atmospheric CO<sub>2</sub> occurred around the periods of 1600-1880 and 1930-present day, intercalated for an interval with a decrease in atmospheric CO<sub>2</sub> levels between 1880 to 1930, tightly coupled with drops and rise of δ<sup>13</sup>C values from alkane chains shown in FIGURE V-2.

Several studies report an increase of atmospheric CO<sub>2</sub> concentration over time (TIPPLE and PAGANI, 2010; WEISENBERG *et al.*, 2008b; ZHAO *et al.*, 2001). The Intergovernmental Panel on Climate Change (IPCC) presented in 2007 a detailed assessment of past climate change and its causes, in order to be followed as a reference for all concerned with climate changes in the world. The temporal variability of atmospheric CO<sub>2</sub> in the last 600 years by IPCC (2007) on the Antarctic Continent is presented in FIGURE V-3, overlapped by temporal variability of δ<sup>13</sup>C values from C<sub>27</sub> alkane in order to assess the influence of global levels in the CO<sub>2</sub>. The C<sub>27</sub> chain was chosen among the alkanes chains because terrestrial plants are more dependent of atmospheric CO<sub>2</sub> than aquatic plants (AICHNER *et al.*, 2010; STREET-PERROT *et al.*, 2004). Moreover, although C<sub>27</sub> varies in concert with remaining terrestrial chains, among them, C<sub>27</sub> supposedly presented less contribution of C<sub>4</sub> plants source, which are more resistant to CO<sub>2</sub> variation than C<sub>3</sub> plants (TIPPLE and PAGANI, 2010).

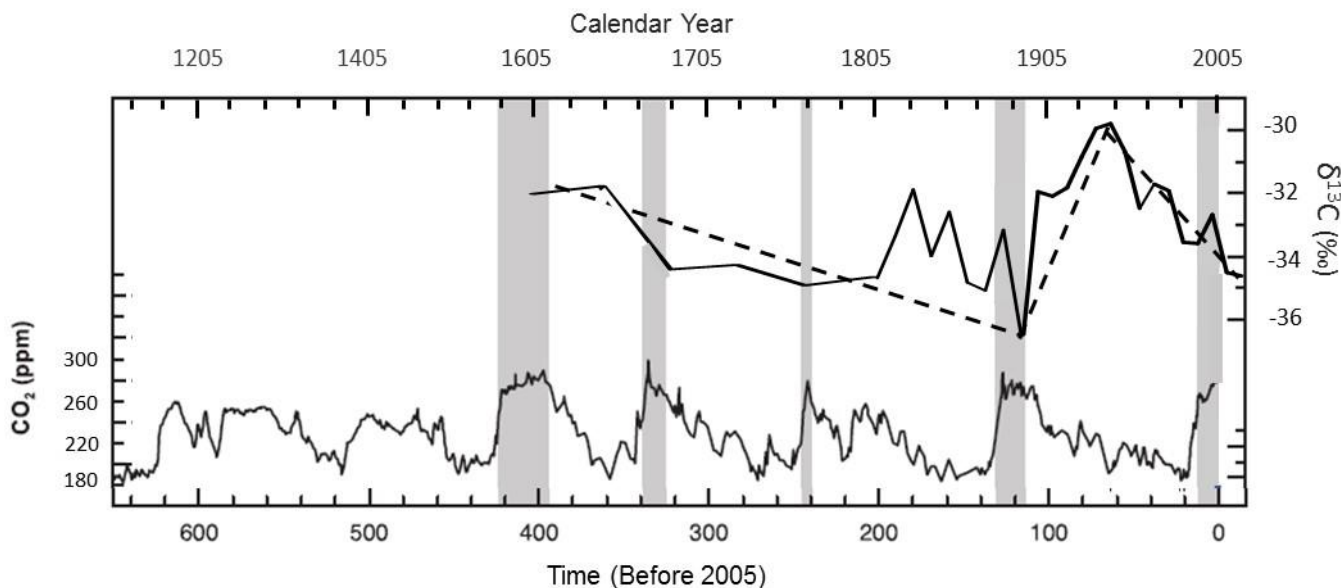


FIGURE V- 3. TEMPORAL VARIABILITY OF ATMOSPHERIC CO<sub>2</sub> IN ANTARCTIC CONTINENT IN LAST 600 YEARS BY IPCC (2007), OVERLAPPED BY  $\delta^{13}\text{C}$  TEMPORAL VARIABILITY OF C27 ALKANE OF THIS STUDY.

According to FIGURE V-3, global levels of atmospheric CO<sub>2</sub> measured in Antarctic continent seem to have a strong influence on the levels of CO<sub>2</sub> in the study area. Five peaks of 280 ppm of CO<sub>2</sub> around 1600, 1670, 1760, 1890 and 2000 are likely responsible for the decrease in  $\delta^{13}\text{C}$  values of aquatic and terrestrial chains. Emphasize for the peak in 1890, which caused a sharp drop in  $\delta^{13}\text{C}$ . After 1890, the reduction of global CO<sub>2</sub> allowed an increase in  $\delta^{13}\text{C}$  values of C27 until 1930, followed by a new decrease probably due to air pollution from local sources of CO<sub>2</sub>, better explained later in this chapter. Additionally, there was a peak in global levels of CO<sub>2</sub> around 2005. Etheridge *et al.*, (1996), observed similar variation in global atmospheric CO<sub>2</sub> in the last millennium, also from air in Antarctic ice measurements.

Several studies present an investigation of gross <sup>12</sup>C and <sup>13</sup>C exchange between the atmosphere and biosphere and their influence on the  $\delta^{13}\text{C}$  variations in the atmosphere (DRIGO *et al.*, 2010; FREEMAN and HAYES, 1992; KEELING, 1961). Fung *et al.*, (1997) calculated that depletion of 3% in  $\delta^{13}\text{C}$  values of terrestrial biosphere would imply in an increase of 20% of atmospheric CO<sub>2</sub> levels. Applying this model in the results of this study, variations around 2% in atmospheric CO<sub>2</sub> in theory, occurred in South Brazil in the last 400 years, obtained from an average of  $\approx 13\%$  in variation of  $\delta^{13}\text{C}$  terrestrial chains (4‰). However, this is just a rough estimate. This approach depends upon accurate estimates of the regional and temporal pattern of isotopic discrimination by terrestrial photosynthesis.

## V-6 DIFFERENCES IN CO<sub>2</sub> DISCRIMINATION BY AQUATIC AND TERRESTRIAL PLANTS IN RESPONSE TO ATMOSPHERIC CHANGES

Taking into account the concentration of atmospheric CO<sub>2</sub> as the main factor responsible for variation in  $\delta^{13}\text{C}$  of middle and long alkane chains, it is possible to get a better understanding of the differences in their temporal variability. Middle alkane chains (C21-C25) from aquatic plants presented smaller temporal variation of  $\delta^{13}\text{C}$  values compared to the long chains (C27-C31) from terrestrial plants and an opposite trend after 1960 (FIGURE V-2). Terrestrial plants use only atmospheric CO<sub>2</sub> as carbon substrate and are, hence, extremely sensitive to changes in atmospheric composition (STREET-PERROT *et al.*, 2004). Similarly, aquatic plants use atmospheric CO<sub>2</sub> (emergent macrophytes), but also may use other carbon sources, such as dissolved CO<sub>2</sub>, bicarbonate, biogenic CO<sub>2</sub> emitted by plant and bacterial respiration and methanogenic carbon (submerged/floating macrophytes) (AICHNER *et al.*, 2010).

Higher amounts of CO<sub>2</sub> in the atmosphere lead to perturbation in the carbonate system in the aquatic environment (CO<sub>2</sub>(aq), HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>-2</sup>). Dissolved carbonate species are isotopically lighter than atmospheric CO<sub>2</sub> and increase  $\delta^{13}\text{C}$  values of aquatic plants (STREET-PERROT *et al.*, 2004). Thus, while the increase in atmospheric CO<sub>2</sub> implies a direct decrease of  $\delta^{13}\text{C}$  values in terrestrial plants, aquatic plants had this effect mitigated by assimilation of bicarbonate and dissolved CO<sub>2</sub>, resulting in a  $\delta^{13}\text{C}$  temporal variability with lesser magnitude, between 1600 and 1930.

Immediately after 1930, a sharp drop in  $\delta^{13}\text{C}$  values was observed in middle chains, while in long chains this drop was smoother and extended until the present day (FIGURE V-2). It could be assigned to the use of methanogenic carbon by aquatic plants in this period. In anoxic conditions, anaerobic respiration produces methane that is <sup>13</sup>C-depleted, which can be incorporated into surface waters and cause a decrease in  $\delta^{13}\text{C}$  of algae and aquatic plants (SUN *et al.*, 2013). Anoxic conditions in sediment surface may be related to a meaningful rise in sewage pollution in the area, reported by Machado *et al.*, (2014). After 1930, fecal sterols rise significantly due to fast demographic growth and poor sanitation assigned to regional economic development. As mentioned in Chapter IV (Sections IV.2 and IV.3), sterols and carbon/nitrogen ratios also indicated an increase in primary productivity and diagenetic process in response to a higher amount of organic matter delivered in the river. Thus, depletion in  $\delta^{13}\text{C}$  values of aquatic plants due to a rise in primary productivity could be related to those facts. A similar case was also reported by Sun *et al.*, (2013).

Since anthropogenic pollution has contributed to sharp drop of  $\delta^{13}\text{C}$  values in middle chains after 1930, it is reasonable to suppose that the reduction in fecal pollution, observed

by fecal sterol data (MACHADO *et al.*, 2014), is one of the factors that increased  $\delta^{13}\text{C}$  values of aquatic plants after 1960. Higher assimilation of lighter carbon sources such as bicarbonate and mainly biogenic  $\text{CO}_2$  may have contributed. Although anoxic conditions produce methane that is isotopically heavier, significant amounts of  $\text{CO}_2$  isotopically lighter are also produced (AICHNER *et al.*, 2010). In this approach, temperature may play an important role, controlling the solubility of these gases in the water. After 1940, temperature in the study area started to decrease and dropped almost  $4^\circ\text{C}$  between 1955 and 1970. This cooling may have resulted in an increase in  $\text{CO}_2$  solubility and consequently in the reduction in methanogenic carbon assimilation by aquatic plants. Therefore, effects of water pollution and temperature seem to be responsible for isotopic differences in the magnitude of response to atmospheric changes, between middle and long alkane chains.

## V-7 EMISSION AND UPTAKE SOURCES OF ATMOSPHERIC $\text{CO}_2$

Atmospheric  $\text{CO}_2$  can be from natural or anthropogenic sources. In periods with less anthropogenic impacts (before 1750), natural sources associated with climatic events have been reported as responsible for an increase of atmospheric  $\text{CO}_2$  (LOPES and CECILIO, 2002). Geological processes such as hydrothermal outgassing, weathering and volcanism are some of the main natural inputs of  $\text{CO}_2$  in long timescales (PEARSON and PALMER, 2000). Conversely, in modern times, especially after the industrial revolution, anthropogenic sources of  $\text{CO}_2$  are more significant (WEISENBERG *et al.*, 2008b; ZHAO *et al.*, 2001). Emissions of  $\text{CO}_2$  from burning of fossil fuel and unplanned land use are the primary anthropogenic sources (LOPES and CECILIO, 2002). Peaks in global atmospheric  $\text{CO}_2$  around 280ppm from the pre-industrial period to 2005 are observed in FIGURE V-3 and have continued to increase (ETHERIDGE *et al.*, 1996). It was estimated that about 2/3 of total anthropogenic  $\text{CO}_2$  emissions have come from fossil fuel burning and the rest from land use change (IPCC, 2007).

In the study area, significant anthropogenic impact, including possible bushfire, occurred from 1840, as discussed in Chapter IV (section IV.2). However, although biomass combustion is a source of  $\text{CO}_2$ , its magnitude in the region was not enough to change significantly the local atmospheric composition. Moreover, the period of biomass burning does not explain the increase in  $\text{CO}_2$  level before 1840. Therefore, the increase in atmospheric  $\text{CO}_2$  observed between 1600 and 1890 is mainly assigned to natural and anthropogenic sources in global level, with small local contribution.

Among natural sources of  $\text{CO}_2$  with global influence, volcanism may have been an important contributor, mainly because the period studied encompasses the LIA period, in

which, volcanism is reported as one responsible factors for cooling and reduction in precipitation land in global levels (MANN, 2002). Powerful volcanic eruptions typically have global effect once huge quantities of gases and ash are released into the stratosphere, where they produce optically significant aerosols, altering Earth's radiation balance and resulting in a cooling of the atmosphere and reduction in land precipitation (MANN, 2002; MINNIS *et al.*, 1993).

Regarding the high potential of volcanism to global climate changes, IPCC (2007) described the evolution of volcanism in the last millennium based on available proxies record (e.g., sulfate load), termed volcanic forcing (AMMANN *et al.*, 2003). Besides CO<sub>2</sub>, volcanic eruptions also emit huge amounts of sulphur dioxide (SO<sub>2</sub>), which is oxidized to sulfuric acid in the atmosphere, washout in precipitation and may acidify surface waters and soil (BOULON *et al.*, 2011). Thus, in order to assess the volcanism influence on the soil pH, a reconstructed pH curve of this study was overlapped by temporal variability of volcanic forcing reported by IPCC (2007) and is shown in FIGURE IV-4.

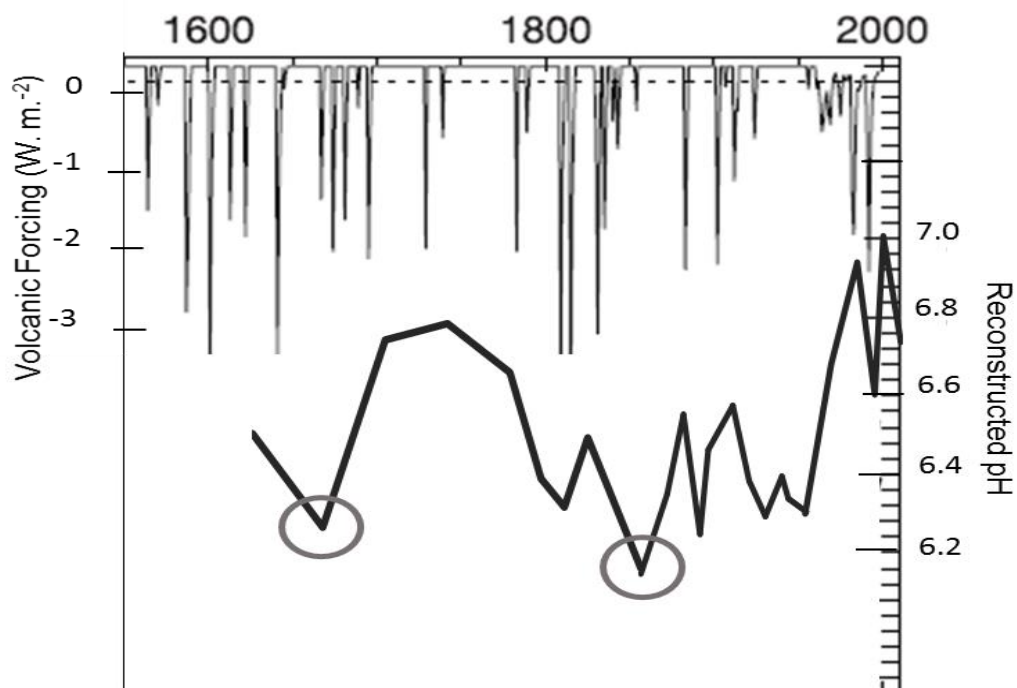


FIGURE V- 4. VOLCANIC FORCING IN LAST 400 YEARS BY IPCC (2007), OVERLAPPED BY RECONSTRUCTED pH OF THIS STUDY. CIRCLES DISPLAY MINIMUM pH VALUES

According to FIGURE V-4, temporal variability of volcanic forcing and reconstructed pH seems to be tightly coupled. Periods with intense volcanic forcing match with the lower values of pH. Two periods of higher volcanic forcing are observed in the 1600-1700 and 1800-1900 periods. The last one is probably related to Tambora (Indonesia, 1815) and Krakatoa (Indonesia, 1880) eruptions, two of the ten greatest eruptions in history

(CROWLEY *et al.*, 2000). Such intense volcanic activity is reflected in lower pH values in some moments (1650 and 1850). Maximum volcanic forcing around 2000, clearly reflected in pH-reconstructed, is probably related to the most violent eruption in the history of the planet in Mt. Pinatubo (Philippines) in 1991 (IPCC, 2007).

In contrast, higher values of pH are seen in those periods of volcanic quiescent, around 1750 and 1950 (FIGURE V-4). Additionally to the volcanic quiescent, deforestation in the area could be attributed for the rise in pH values around 1950, once trees reduce soil pH. Besides the literature (OLIVEIRA and CARVALHO, 2011; FARIA *et al.*, 2009), this effect was also observed in samples collected from surface soil for pH measures. Samples from forest areas presented lesser pH than samples from open field (section V.2). Therefore, the reduction of trees results in a rise in soil pH.

In the context above mentioned, volcanism seems to be an important contributor for the first CO<sub>2</sub> increase in the 1600-1890 period. Therefore, it is reasonable to suppose that reduction in volcanic forcing was one of the factors that may have caused depletion in atmospheric CO<sub>2</sub> in 1890-1930, probably associated with other environmental factors such as uptake processes by terrestrial biosphere and ocean. Ocean can remove up to 50% of CO<sub>2</sub> emissions. These removal processes are influenced by atmospheric CO<sub>2</sub> concentration and by changes in climate (IPCC, 2007).

The second increase in atmospheric CO<sub>2</sub> (decrease of  $\delta^{13}\text{C}$  values) occurred immediately after 1930 and is extended until the present day (FIGURES V-2 and V-3). Although lower volcanic forcing (FIGURE V-4) and global CO<sub>2</sub> (FIGURE V-3) are observed until almost 2000, in this period (1930-present day) the more likely sources of atmospheric CO<sub>2</sub> are local sources, mainly burning of fossil fuels, due to demographic growth in the studied area and a sudden economic development in Brazil. The evolution of anthropogenic pollution in the last 150 years in the study area will be described in more detail, in the following chapter, using polyaromatic hydrocarbons (PAHs) distributions as biomarkers. Anthropogenic CO<sub>2</sub> is <sup>13</sup>C-depleted and cause depletion in atmospheric CO<sub>2</sub>  $\delta^{13}\text{C}$  values (LOPES and CECILIO, 2002; ZHAO *et al.*, 2001)

Overall, two increases in atmospheric CO<sub>2</sub> were observed. The first increase (1600-1890) has more influence by global than local sources, mainly natural events (volcanism). In contrast, the second increase in atmospheric CO<sub>2</sub> (1930-nowadays) presents more influence by local than global sources.

## V-8 CHAPTER SYNTESIS

A temporal record of isotopic signature in terrestrial and aquatic alkanes was made together with soil pH-reconstruction from 1600 to the present day.  $\delta^{13}\text{C}$  values for terrestrial alkanes (C27-C31) presented drops (1600-1880 and 1930-nowadays), intercalated with increasing period (1880 to 1930). The decrease in  $\delta^{13}\text{C}$  values corresponds to an increase in atmospheric  $\text{CO}_2$  by global and local sources. Aquatic alkanes chains (C21-C25), conversely, presented lower isotopic variation, probably due to the use of lighter carbon sources by aquatic plants, such as bicarbonate and biogenic  $\text{CO}_2$ . Moreover, water pollution and temperature changes over time also contributed to differences in discrimination of  $\text{CO}_2$  by terrestrial and aquatic alkanes in response to atmospheric change.

Global  $\text{CO}_2$  sources seem to be predominantly responsible for decreasing  $\delta^{13}\text{C}$  values between 1600 and 1880. However, after 1930, although  $\text{CO}_2$  global sources contribute, local sources are predominant. Among global  $\text{CO}_2$  sources, volcanism may have a significant influence, once the temporal variation of both reconstructed soil pH and global volcanic forcing seem to be coupled. Characteristics of the LIA period corroborate with this. Local  $\text{CO}_2$  sources were assigned to anthropogenic action, mainly the burning of fossil fuels related to fast regional economic development, discussed in the next chapter.

Increase in atmospheric  $\text{CO}_2$  concentration has led to an intensive debate about climate change. However, studies that bring evidences of atmospheric  $\text{CO}_2$  variation in south Brazil from geochemical markers are scarce. Therefore, the findings of this chapter improve the knowledge about the influence of natural and anthropogenic  $\text{CO}_2$  sources, in global and local levels, on the atmospheric composition of south Brazil.

## **CHAPTER VI**

# **ANTHROPOGENIC IMPACT IN BARIGUI RIVER BASIN IN THE LAST 150 YEARS THROUGH PAHs RECORDS**

## VI.1 CHAPTER INTRODUCTION

In the last chapter, an increase in atmospheric CO<sub>2</sub> in modern times appears to be associated with regional sources and anthropogenic influence. Thus, the main objective of this chapter is to assess the evolution of anthropogenic impact in the studied area over the last 150 years, when most of the environmental changes due to demographic growth and economic development occurred.

Currently, concomitant to the cities' progress, the demand for fossil fuels and the settlement of land has led to an increase in PAH concentrations in soils and sediments. Such compounds are often used as chemical markers to identify potential sources of environmental pollution. In this chapter, the distribution and ratios among PAHs conventionally reported in literature were used to understand the evolution of those environmental pollution sources. First, an analysis of the pollution level was made in the area through PAH distribution and comparison with local values of surface sediment. Then the PAH temporal variability was discussed and the prominent peaks of concentration were associated with some regional historic events. The influence of grain size in PAH distribution was also noted.

Since PAH sources are tightly coupled to human processes, changes in anthropogenic activities may directly affect the proportion among individual PAHs, mainly compounds thermodynamically different. Thus, some ratios of PAHs were selected from literature to reconstruct a scenario of PAH source evolution over time, and throughout their environmental significance understand the relationship with regional development. Considering demographic growth as a factor ascribed to economic development and consequently anthropogenic impact, population data from Curitiba and Almirante Tamandare were compared with PAH total concentration over time.

## VI.2 PAHs DISTRIBUTION

The individual concentrations of PAHs ranged from 40 to 2,400ng.g<sup>-1</sup>, while the Σ16 PAHs varied from 1,800ng.g<sup>-1</sup> to 15,000ng.g<sup>-1</sup> from 1855 to 2012 (FIGURE VI-1). In the PAH distribution pyrene and phenanthrene were predominant compounds. Indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene and benzo[ghi]perylene were found only in two time intervals, 1910-1920 and the 1985 sediment horizon, ranging in concentration from 100 to 780ng.g<sup>-1</sup>.

The distribution of PAHs in the surficial sediment sample (0 to 2cm) was similar to those reported in other studies conducted in Brazil (SANTANNA *et al.*, 2010; NISHIGIMA *et*

*al.*, 2001; ZANARDI *et al.*, 1999). This study seems to be the first report of the temporal distribution of the PAHs in studied area (Curitiba and metropolitan region), although their distribution in surface sediments of the Barigui River has been described (LEITE *et al.*, 2009).

The main concern about the presence of PAHs in sediments is their toxicological impact on the ecosystem, which is dependent on their concentration. Hence, sediments can be classified as moderately contaminated when the PAH concentration is between 200 – 3,000ng.g<sup>-1</sup>, whereas a concentration higher than 3,000ng.g<sup>-1</sup> in sediments as highly contaminated (BENLAHCEN *et al.*, 1997).

In this study, most of the core samples can be classified as moderately contaminated; however, the samples from 0 to 10cm have higher concentrations of PAHs than those from the deepest section, suggesting higher pollution only in recent time.

Levels of PAHs depend on the potential sources of emission. Particular sources may be responsible for the simultaneous emission of several PAHs. According the matrix correlation presented in TABLE VI-1, the sedimentary concentrations of all PAHs, apart from fluoranthene, are significantly correlated ( $R>0.6$ ,  $p<0.01$ ), suggesting that the PAH contamination in the Barigui River originated from the similar sources during the period studied or were influenced by a similar deposition process.

### VI.3 TEMPORAL VARIABILITY OF PAHs

Based on the sediment chronology, changes in  $\Sigma 16$  PAH concentrations and those of the individual PAHs were determined (FIGURE VI-1).  $\Sigma 16$  PAH concentrations increased between approximately 1855 and 1910 (2,000ng.g<sup>-1</sup> to 7,200ng.g<sup>-1</sup>), decreased and then increased dramatically to 1,800ng.g<sup>-1</sup> in 1940. During this period, high molecular weight PAHs concentrations (>202) were dominant, mainly pyrene and fluoranthene. The  $\Sigma 16$  PAHs increased in concentration again after 1940, and the maximum was observed in 1971 (14,900ng.g<sup>-1</sup>); the concentration then gradually decreased to 5,100ng.g<sup>-1</sup> in 2012. After 1940, low molecular weight PAHs (<202) dominate, mainly naphthalene and phenanthrene (FIGURE VI-1).

The peaks observed in  $\Sigma 16$  PAH concentrations coincide with particular historical events during the development and settlement of the region. For example, the first peak, which occurred around 1910, may reflect population growth due to immigration programs encouraged after 1890. Germans, Polish and Italian farmer immigrants occupied the northern region of Curitiba from 1870, giving origin to Pilarzinho and Abranches neighborhoods (IPPUC, 2009). In Almirante Tamandare, these immigrants settled from 1886, and besides

immigration, demographic growth was also a reflection of population expansion in Curitiba mainly from 1900 to 1960 (KOTOVISKI, 2013).

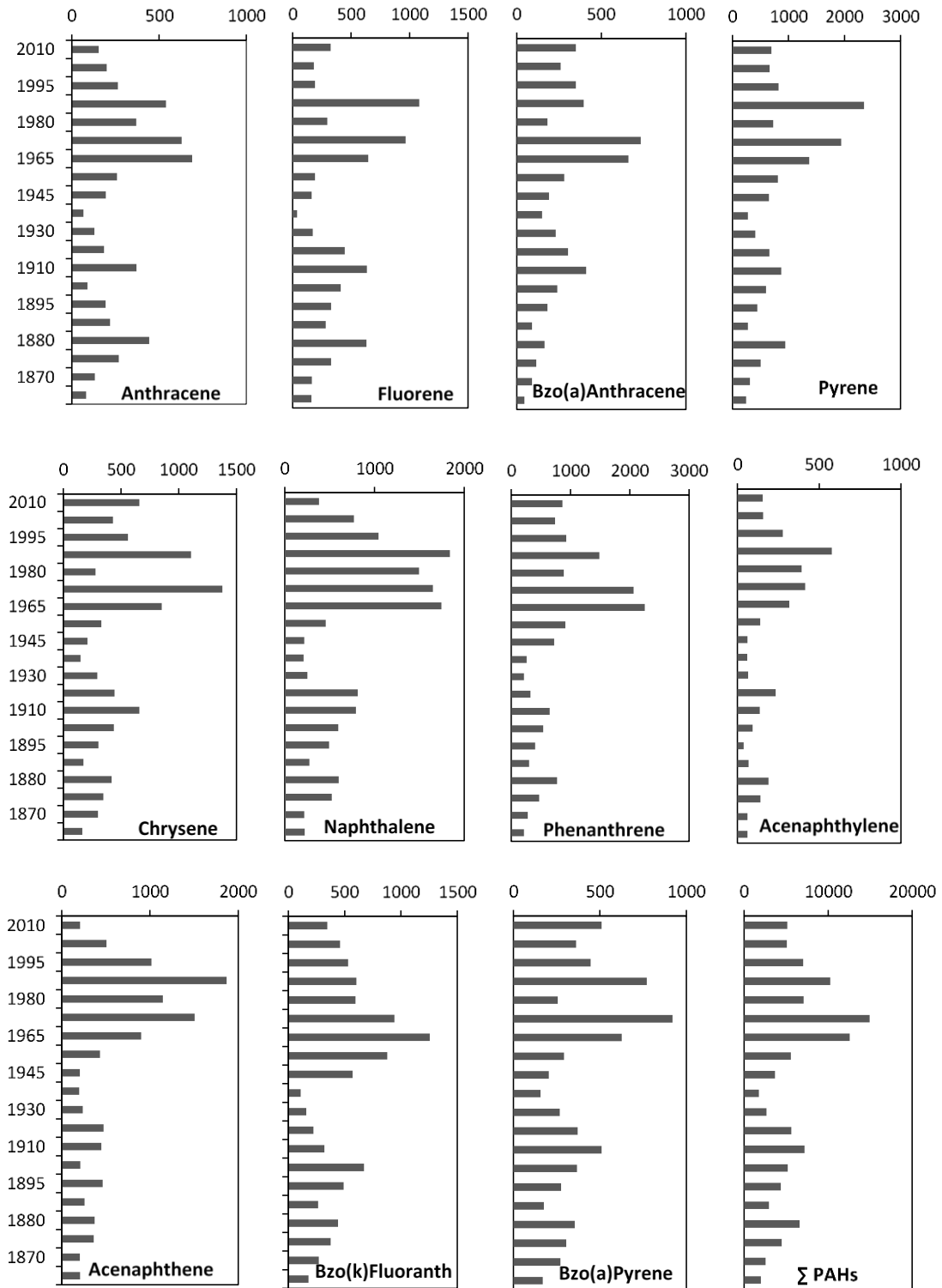


FIGURE VI- 1. TEMPORAL VARIABILITY OF PAHs IN PT CORE IN  $\text{ng.g}^{-1}$ .

From 1960 to 1980, Brazil experienced a sudden economic development, termed “Economic Miracle,” reflected in higher consumption of natural resources, industry development, urbanization and better economic power of population, which means larger fleets of vehicles (AZEVEDO and BARCELLOS, 2011). This could account for the highest PAH concentration, which apparently occurred in the 1970s.

The concentration of sedimentary PAHs is dependent on the sedimentation rates and grain size, physico-chemical properties, and emission factors (YUNKER *et al.*, 2002). The sedimentation rate in sampling station has been relatively constant over time ( $0.44\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$ ,  $\pm 0.09$ ), suggesting no influence on the PAH concentrations (e.g., by sediment dilution).

The core has significant variation in its granulometric composition according to FIGURE VI-2. In general, sand (63-2000  $\mu\text{m}$ ) and silt (4-63  $\mu\text{m}$ ) size fractions dominated the granulometric composition, while the clay content (<4  $\mu\text{m}$ ) was minor. Usually, hydrophobic compounds such as PAHs are sorbed by fine particles (silt and clay), and modern sediments with high silt and clay contents have high PAHs concentrations. However, there is no significant correlation between the PAHs and fine sediment particles in the PT core (TABLE IV-1), indicating that, although there is an influence, PAH concentration does not depend exclusively on sediment grain size. Such behavior was already observed by Santana *et al.* (2010). Hence, the controlling factors of the PAH concentrations might be outside the sedimentary environment, such as the pathway between the source and the sediment. (GELINAS *et al.*, 2001).

Variations in particle size distribution may be related to changes in land use and vegetation cover in the catchment area. In general, reduction in both herbaceous and woody vegetation increases local erosion and favors the input of large particles; on the other hand, soil compaction produces the opposite effect (BRITO *et al.*, 2009; FROEHNER *et al.*, 2008). Changes in hydrological conditions in the watershed might affect the deposition processes of sediment and cause variations in its grain size composition (SILVA *et al.*, 2005). Thus, the replacing of sand by silt in the PT core after 1930 (FIGURE VI-2) may be associated with changes in vegetation cover due to shifts in land use. The increased load of fine/small particles may also reflect transport over long distances and even changes in hydrologic or flux conditions.

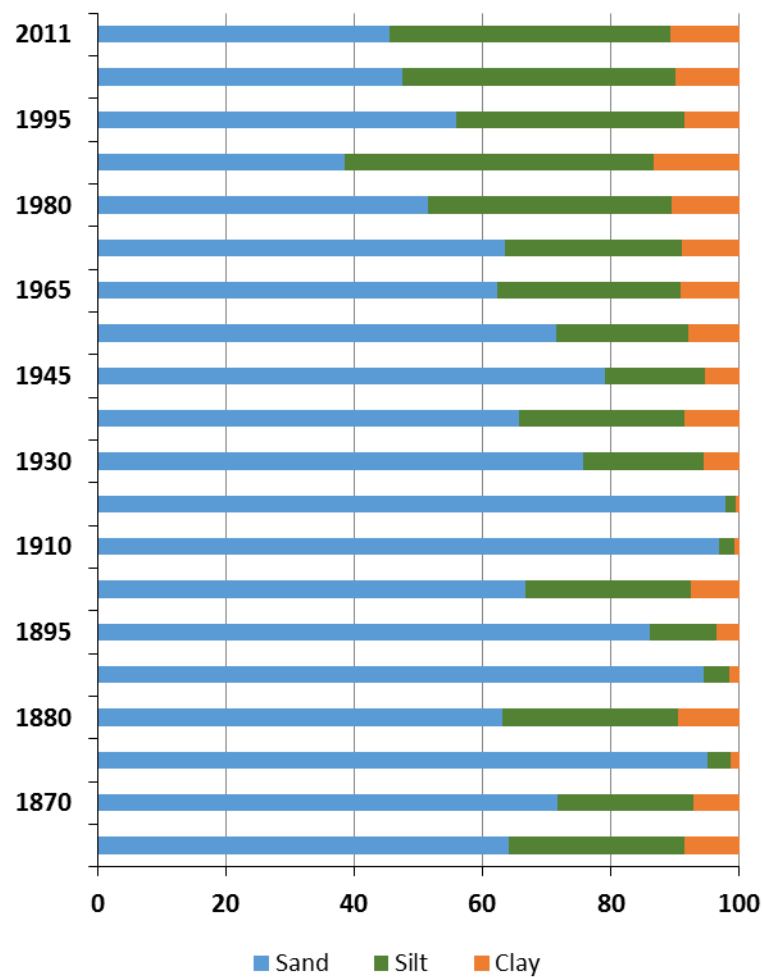


FIGURE VI- 2. TEMPORAL VARIABILITY OF GRAIN SIZE COMPOSITION IN PT CORE IN %

The chemical stability of PAHs is another factor that plays an important role in their preservation and therefore, sedimentary concentrations. Biomass and fossil fuel combustion produce aerosols with very different particle sizes and physical properties (SCHMIDT AND NOACK, 2000). These differences may affect both the dispersion pathways in the environment and the physical protection afforded to the PAHs (GELINAS *et al.*, 2001). The black carbon of wood soot consists of large, dark and refractory particles that provide a solid matrix that traps and stabilizes PAHs (SCHMIDT and NOACK, 2000). This protection is greater for high molecular weight PAHs than for those with low molecular weight (YUNKER *et al.*, 2002). Thus, the predominance of high molecular weight PAHs (HMW) concentrations (>202) until 1940 and low molecular weight (<202) PAHs after 1940 (FIGURE VI-1) may reveal influences of the source; alternatively, it may be related to the physical protection level of PAHs produced by grass and wood combustion. In addition, low molecular weight PAHs are more susceptible to degradation in the atmosphere and water column, as they are more volatile and soluble.

TABLE VI- 1. MATRIX CORRELATION OF PEARSON: PAHs, SAND, SILT AND CLAY

	$\Sigma$ PAHs	Sand	Silt	Clay	NAP	ANP	AEN	FLU	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF
Sand	-0.38															
Silt	0.37	<b>-1.00</b>														
Clay	0.40	<b>-0.98</b>	<b>0.96</b>													
NAP	<b>0.93</b>	-0.43	0.43	0.41												
ANP	<b>0.89</b>	-0.52	0.52*	0.51*	<b>0.93</b>											
AEN	<b>0.88</b>	-0.48	0.47*	0.47*	<b>0.92</b>	<b>0.95</b>										
FLU	<b>0.93</b>	-0.20	0.20	0.23	<b>0.83</b>	<b>0.76</b>	<b>0.78</b>									
PHE	<b>0.92</b>	-0.45	0.44	0.48*	<b>0.84</b>	<b>0.75</b>	<b>0.74</b>	<b>0.87</b>								
ANT	<b>0.91</b>	-0.23	0.21	0.28	<b>0.85</b>	<b>0.77</b>	<b>0.76</b>	<b>0.82</b>	<b>0.89</b>							
FLA	<b>0.67</b>	0.12	-0.13	-0.10	0.54*	0.51*	0.46*	<b>0.60</b>	0.43	<b>0.65</b>						
PYR	<b>0.96</b>	-0.44	0.43	0.47*	<b>0.85</b>	<b>0.88</b>	<b>0.88</b>	<b>0.86</b>	<b>0.85</b>	<b>0.84</b>	<b>0.66</b>					
BaA	<b>0.86</b>	-0.25	0.24	0.25	<b>0.75</b>	<b>0.63</b>	<b>0.63</b>	<b>0.90</b>	<b>0.88</b>	<b>0.76</b>	0.48*	<b>0.77</b>				
CHR	<b>0.93</b>	-0.36	0.36	0.35	<b>0.79</b>	<b>0.77</b>	<b>0.78</b>	<b>0.90</b>	<b>0.84</b>	<b>0.78</b>	<b>0.66</b>	<b>0.91</b>	<b>0.89</b>			
BbF	<b>0.89</b>	-0.18	0.17	0.20	<b>0.76</b>	<b>0.76</b>	<b>0.73</b>	<b>0.79</b>	<b>0.70</b>	<b>0.82</b>	<b>0.90</b>	<b>0.90</b>	<b>0.68</b>	<b>0.87</b>		
BkF	<b>0.73</b>	-0.30	0.28	0.36	<b>0.67</b>	0.52*	0.53*	<b>0.74</b>	<b>0.87</b>	<b>0.73</b>	0.28	<b>0.63</b>	<b>0.71</b>	<b>0.58</b>	0.47*	
BaP	<b>0.93</b>	-0.36	0.36	0.35	<b>0.79</b>	<b>0.77</b>	<b>0.77</b>	<b>0.89</b>	<b>0.83</b>	<b>0.78</b>	<b>0.66</b>	<b>0.91</b>	<b>0.89</b>	<b>0.95</b>	<b>0.87</b>	<b>0.58</b>

NAP: Naphtalene, ANP: Acenaphtylene, AEN: Acenaphtene, FLU: Fluorene, PHE: Phenanthrene, ANT: Anthracene, FLA: Fluoranthene, PYR: Pyrene, BaA: Benzo[a]Anthracene, CHR: Chrysene, BbF: Benzo[b]Fluoranthene, BkF: Benzo[k]Fluoranthene, BaP: Benzo[a]Pyrene.  
 Note: In bold: significant correlation  $p < 0.001$ ; \* significant correlation  $p < 0.05$

## VI.4 EVOLUTION OF PAHs SOURCES

Assessments of PAHs distributions and diagnostic ratios have been widely used for apportioning their sources in environmental samples. Different PAH sources may provide specific patterns or signatures, which can be used to identify the sources (LIU *et al.*, 2012; LIU *et al.*, 2005). According to Yunker *et al.* (1999), PAHs can be grouped in two subsets on the basis of their stability. The first is composed of less stable PAHs formed during combustion processes; the second includes more thermodynamically stable compounds. Considering the isomers formed by different process, the relative ratio of a given molecular mass can be used to identify the possible source and contribution of PAHs in the environment. Yunker *et al.*, (2002) measured some PAHs in conventional sources and environmental samples and reported ratios, which can be used as reference values in the identification of PAHs sources. These values are presented in TABLE VI-2.

TABLE VI- 2. PAHs RATIOS FOR PETROLEUM, SINGLE-SOURCE COMBUSTION AND ENVIRONMENTAL SAMPLES. SOURCE: YUNKER *et al.*, 2002.

Source	FLA/(FLA+PYR)	ANT/(ANT+PHE)	BaA/CHR
<b>Petroleum</b>			
Kerosene	0.46	0.04	0.35
Diesel oil	0.26 ( $\pm 0.16$ )	0.09 ( $\pm 0.05$ )	0.35 ( $\pm 0.04$ )
Crude oil	0.22 ( $\pm 0.07$ )	0.07	0.12 ( $\pm 0.06$ )
Lubricating oil	0.29 (0.17-0.30)	-	0.11-0.12
Asphalt	-	-	0.50
<b>Combustion (Pyrogenic)</b>			
Wood soot	0.50	0.26	0.43-0.49
Wood	0.51 ( $\pm 0.06$ )	0.14-0.29	0.40-0.52
Grasses	0.58 ( $\pm 0.04$ )	0.13-0.26	0.46 ( $\pm 0.02$ )
Gasoline	0.44	0.15	0.44-0.49
Kerosene	0.50	0.12-0.16	0.30-0.44
Diesel	0.39 ( $\pm 0.11$ )	0.01-0.27	0.38 ( $\pm 0.01$ )
Fuel oil	0.51	0.06	0.17 ( $\pm 0.05$ )
Crude oil	0.44 ( $\pm 0.02$ )	0.22 – 0.26	0.49 ( $\pm 0.01$ )
<b>Environmental samples</b>			
Bush fire	0.61	-	0.23
Road dust	0.42	0.18	0.13
Used engine oil, gasoline passenger car	0.30	0.22	0.50
Used engine oil, diesel car, truck and bus	0.37	-	-
Creosote	0.62 ( $\pm 0.01$ )	0.20 ( $\pm 0.05$ )	0.50 ( $\pm 0.03$ )

Following the relations proposed by Yunker *et al.* (2002; 1999), the fluoranthene/pyrene (FLA/PY), anthracene/anthracene + phenanthrene (ANT/ANT+PHE), benzo(a)anthracene/crysene BaA/CHR and low/high molecular weight (LMW/HMW) ratios were determined and presented in FIGURE IV-3.

The presence of PAHs in sediments with a ratio of FLA/(FLA+PYR) lower than 0.4 suggests a source typically from petroleum contamination Yunker *et al.* (2002; 1999); a FLA/(FLA+PYR) ratio higher than 0.5 indicates that the PAHs are mainly from the combustion of grass, wood and coal;  $0.4 < \text{FLA}/(\text{FLA}+\text{PYR}) < 0.5$  indicates the combustion of petroleum (YUNKER *et al.*, 2002). In PT core, the ratio of FLA/(FLA+PYR) was 0.53 to 0.68 ( $>0.50$  FIGURE VI-3) between 1855 and 1920, indicating a predominance of pyrogenic sources (biomass combustion), mostly wood combustion ( $0.51 \pm 0.06$ ), grass combustion ( $0.58 \pm 0.04$ ) and bush fires (0.61; TABLE V-2). The maximum observed concentration of  $\Sigma$ PAHs in approximately 1910 (FIGURE VI-1) could be associated with the sudden increase of the population of Curitiba and Almirante Tamandare by 102% and 40% (1900s) respectively (KOTOVISKI, 2013; IPPUC, 2009), leading to the removal of the vegetation in large areas (bush fire, wood and grass combustion). A FLA/(FLA+PYR) ratio of 0.62 is consistent with the fluorene and pyrene content in creosote used as wood preservative; such a signal post-1885 could reflect the period when the railway line was installed in Curitiba and metropolitan region (FIGURE IV-2, Chapter IV, section IV.2).

Over the period from 1920 to 2012, the ratio of FLA/(FLA+PYR) was 0.14-0.49 ( $<0.5$  FIGURE VI-3), consistent with a mix of combustion and contaminant petroleum sources. The period between 1965 and 1985, when the ratio of FLA/(FLA+PYR) was slightly lower than 0.5 (0.4-0.5, FIGURE VI-3), most likely reflects the increase in automobile traffic attributed to the rapid economic development of Brazil, the "Economic Miracle," in 1960-1980 (AZEVEDO AND BARCELLOS, 2011). Engine combustion and vehicle emissions are typical pyrogenic sources.

Anthracene and phenanthrene are low molecular weight isomers (178) (YUNKER *et al.*, 2002). While the ratio ANT/(ANT+PHE) can be used to identify sources of PAHs, the small difference in energy between pyrolysis and petrogenic process means that this ratio is less sensitive (YUNKER *et al.*, 2002). ANT/(ANT+PHE) values  $> 0.10$  indicate pyrogenic sources, while values  $< 0.10$  can be interpreted as typical of pyrolytic and petroleum sources, but care should be taken due to the insensitivity of this ratio (BUDZINSKI *et al.*, 1997). On the other hand in samples representative of the period between 1855 and 1930, ANT/(ANT+PHE) ratios were significantly higher than 0.1 (0.26-0.42, FIGURE VI-3), suggesting pyrogenic sources ( $>0.10$ ) and supporting the interpretation of the FLA/(FLA+PYR) ratios. After the 1930,

the ratios decreased (0.15-0.29), suggesting a mixture of combustion and contamination by petroleum sources such as used engine oil and gasoline vehicles (0.22), road dust (0.18), gasoline, diesel and crude oil combustion (0.11-0.27) and creosote (0.20) (TABLE VI-2).

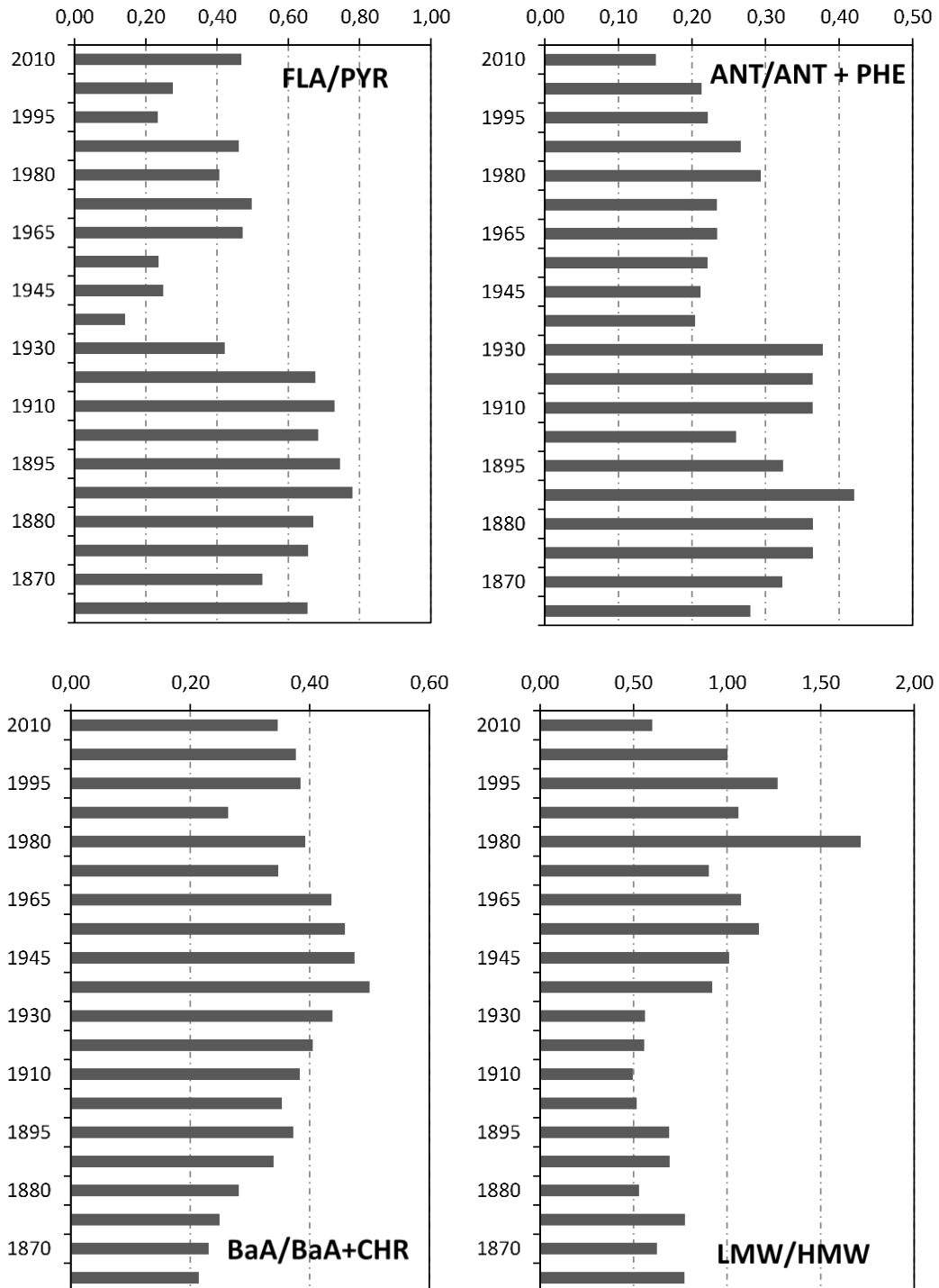


FIGURE VI- 3. TEMPORAL VARIABILITY OF PAHs RATIOS USED TO IDENTIFY POTENTIAL SOURCES OF CONTAMINATION

The ANT/(ANT+PHE) ratio can also be influenced by the preferential degradation/removal of phenanthrene due its higher solubility ( $1,1\text{mg}\cdot\text{L}^{-1}$ ) when compared to anthracene ( $0.045\text{ mg}\cdot\text{L}^{-1}$ ) (NETO *et al.*, 2000), particularly post the 1930, when the soot of wood and grass combustion contributions apparently decreased, also decreasing the physical protection of these PAHs. In consequence of decrease in physical protection, the PHAs became more exposed and those more soluble such as phenanthrene degrade easier.

PAHs of molecular weight 228 (chrysene and benzo[a]anthracene) are used less frequently as indicators of contaminant sources. In addition, they are usually minor components in refined petroleum products (GOGOU *et al.*, 2000), although they are present in significant amounts in higher boiling point fractions including asphalt and, possibly bitumen or coal (READMAN *et al.*, 1987). A BaA/CHR ratio higher than 0.35 suggests typical combustion sources, whereas a BaA/CHR ratio between 0.20 and 0.35 indicates a mixture of combustion and petroleum sources; values lower than 0.20 indicate petroleum sources (YUNKER *et al.*, 2002). In the PT core samples, the BaA/CHR ratios show a Gaussian distribution over time that is most likely associated to heterogeneous sources (FIGURE VI-3). According to Yunker *et al.*, (2002) such behavior shows the low value of such ratios. In 1855, the BaA/CHR ratio of 0.23 is typical of bush fire (TABLE VI-2); this ratio increased progressively to 0.5 in 1940, which likely can be attributed to wood combustion (0.40-0.52) and creosote ( $0.50\pm 0.03$ ). The peak (in approximately 1940) indicates the additional influence of gasoline and crude oil combustion (0.44-0.50) and asphalt ( $0.50\pm 0.03$ ) (TABLE VI-2). From 1940 to the present, a slight decrease in the BaA/CHR ratios (FIGURE VI-3), can most likely be attributed to the same sources as the Gaussian peak added to diesel combustion ( $0.38\pm 0.01$ ), fuel oil combustion ( $0.17\pm 0.05$ ) and contamination by petroleum sources such as kerosene (0.35), diesel oil ( $0.35\pm 0.04$ ) and lubricating oil (0.11-0.12) (TABLE VI-2), which have likely reduced the BaA/CHR ratios (FIGURE VI-3) through to present day.

The data presented are consistent with the ratio between low molecular weight PAHs (<202, LMW) and high molecular weight PAHs (>202, HMW). LMWs are the major constituents of petroleum, while HMWs are associated with pyrogenic sources. Ratios of LMW/HMW higher than 1 suggest petroleum sources, whereas ratios of LMW/HMW lower than 1 indicate pyrogenic sources (SILVA *et al.*, 2007). Like the other ratios (FLA/PYR, ANT/(PHE+ANT), BaA/CHR), the LMW/HMW ratio of this study showed an increase since 1940 (FIGURE VI-3), suggesting petroleum sources. According to the history of the region, the petroleum sources are likely asphalt and engine oil associated with the increase in paved streets and cars (FIGURE VI-4), in

addition to the petroleum contamination sources mentioned earlier. However, despite increased petroleum-based contributions after 1940, during all the periods studied, there is a predominance of pyrogenic sources (biomass and petroleum derivatives combustion), which is confirmed by the strong correlation between the total PAHs and benzo[a]pyrene ( $R=0.93$   $p<0.01$ , TABLE VI-1). Lesser concentrations of benzo[a]pyrene are found in petroleum sources, and thus, a significant amount in the environment indicates pyrogenic sources, since this PAH is preferably produced at lower temperatures (NETTO *et al.*, 2000).

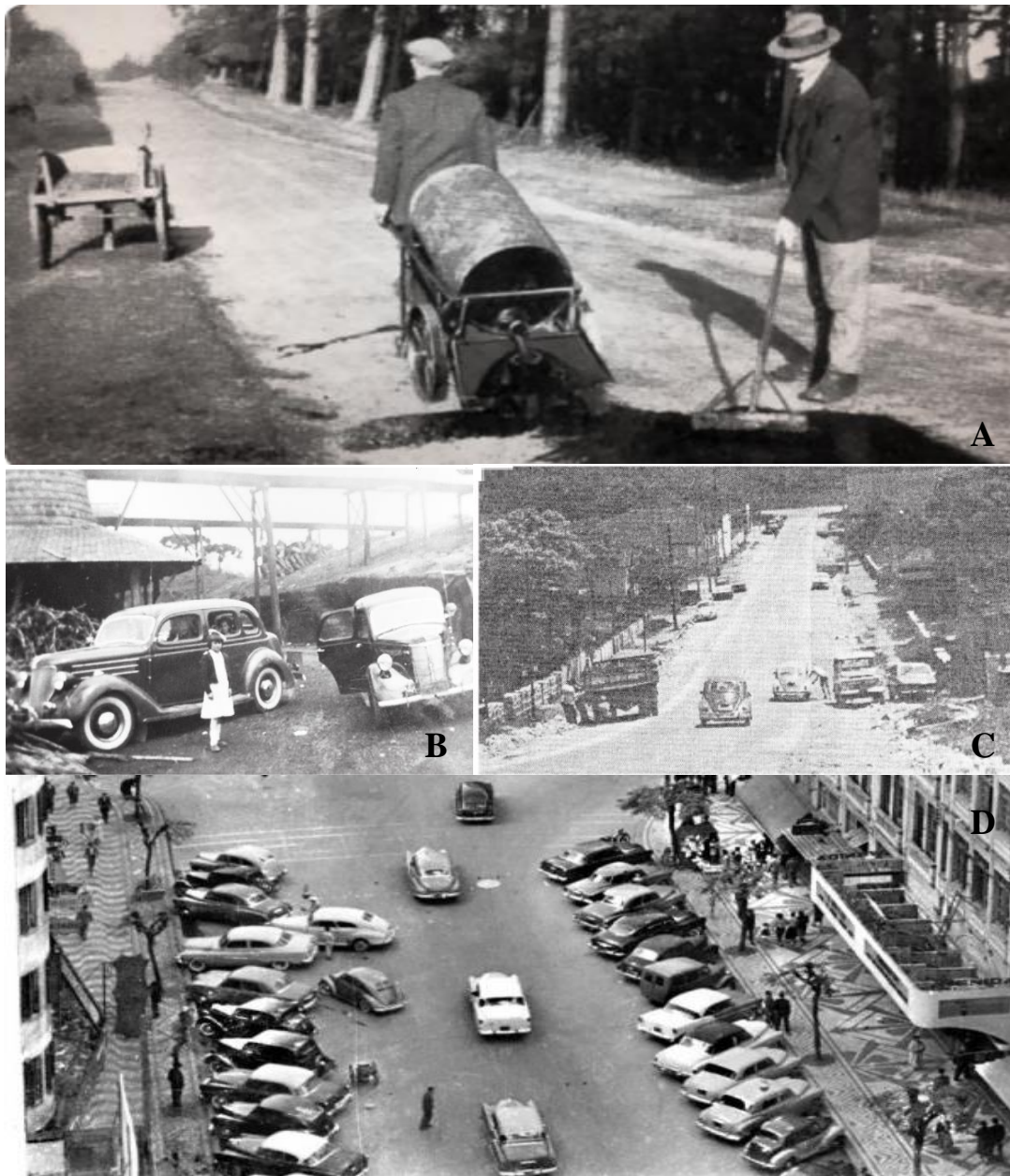


FIGURE VI- 4. ASPHALT IN THE REGION IN 1920 (A), ALMIRANTE TAMANDARE IN 1920 (B) AND IN 1970 (C), CURITIBA IN 1950 (D). SOURCES: KOTOVINCI, 2013; IPPUC, 2009; HLADCZUK, 2000.

Overall, all ratios studied showed the predominance of wood and grass combustion sources between 1855 and 1920 (FIGURE VI-3, TABLE VI-2). The period between 1920 and 1940 might be considered a transition period, with a mixture of biomass and petroleum combustion sources. Finally, after 1940 until present, petroleum derivative combustion and petroleum derivative contamination sources predominate.

## VI.5 RELATIONSHIP BETWEEN PAHs AND REGIONAL DEVELOPMENT

PAHs are produced mainly from the incomplete combustion of coal, petroleum and biomass materials and are used as a proxy to trace the impact of anthropogenic activities (LIU *et al.*, 2012; LIU *et al.*, 2005). The area studied has undergone significant social and economic development since its settlement. Previous studies have reported that the concentrations of PAHs in the environment are strongly influenced by the local human population (ZHANG *et al.*, 2013; LI *et al.*, 2008), industrialization activities (KUMAN *et al.*, 2008), the number of vehicles (LIU *et al.*, 2005), the length of highways, and energy consumption (LIU *et al.*, 2012).

Among the several indices used to represent human impact, demographic growth is closely linked to increased consumption of natural resources, deforestation, and waste production, resulting in greater pollution of water, air and soil in the region (ZHANG *et al.*, 2013). Population growth also can be considered a reflection of the region's economic development once it attracts people from other places and leads to urbanization (LI *et al.*, 2008). Thus, to assess the influence of regional development in the PAHs variability, population data from Curitiba and Almirante Tamandare in the last 150 years were compared with the total concentration of PAHs and showed in FIGURE VI-5. Pearson's correlation coefficient (R) was used to assess their relationship. A significant correlation between the population data and the total PAHs concentrations in the sediment core during all the periods studied (1850-2012) was not found. However, a strongly significant positive correlation was observed among the data between 1855 and 1970 (*Pop. R* >0.80, *p* = 0.002). After 1970, there is an opposite trend among the PAHs concentrations and population data. While the PAH concentrations decreased, the population data continued to increase from 1970 until the present (FIGURE VI-5).

Curitiba's population rose sharply from 1945, while Almirante Tamandare's population rose only after 1960, probably because in this period, the growth in this city is a reflection of the growth in Curitiba, mainly by attractive real estate. In contrast to the population data, the total concentration of PAHs declined after 1970 (FIGURE VI-

5). Considering vehicular emission as important PAHs sources, this decrease may be related to implementation of the vehicular emission control program (CONAMA, 1986), which imposed  $2.1 \text{ g.km}^{-1}$  as the limit for hydrocarbons, leading to several mechanical adjustments to reduce pollutants emissions. In addition, after 1975, the use of ethanol “in theory” as a cleaner vehicle fuel was encouraged in Brazil (IPPEA, 2011).

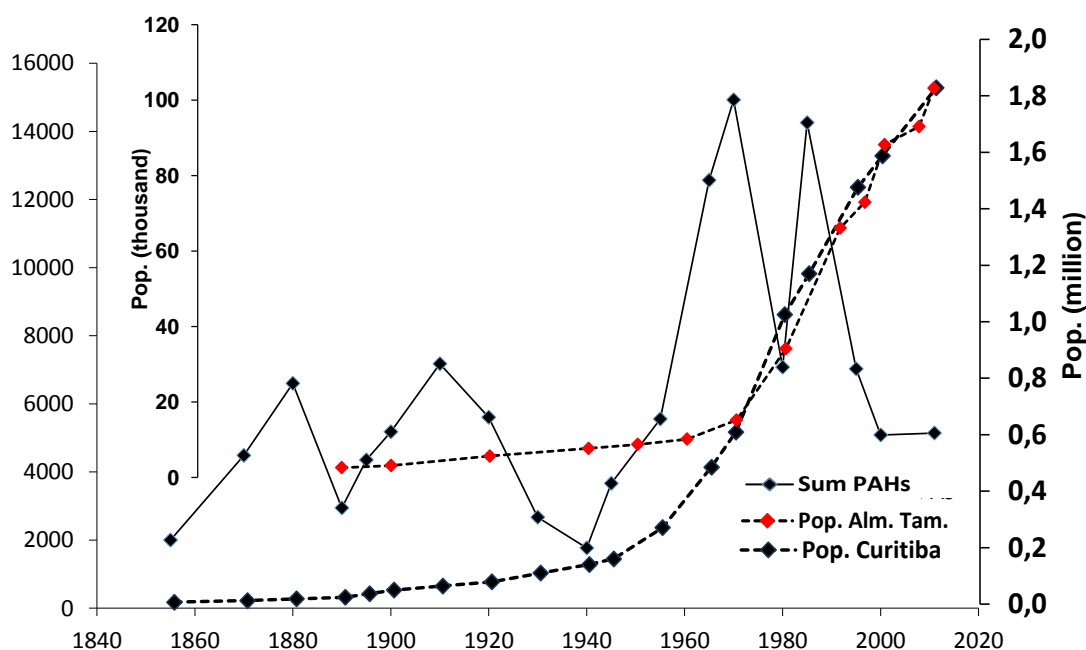


FIGURE VI- 5. RELATIONSHIP BETWEEN  $\Sigma$  PAHs CONCENTRATIONS ( $\text{ng.g}^{-1}$ ) IN PT CORE WITH POPULATION DATA FROM CURITIBA AND ALMIRANTE TAMANDARE.

Concomitant with the rise in population, economic growth is evident. The consumption of electricity in Curitiba increased by 163% between 1960 and 1970 (COPEL, 2010; IPPUC, 2009; PARIGOT DE SOUZA, 1996). In the same period, the number of connections to electricity supply increased 7.4 times for the population and 19 times for the industry in Paraná state (PARIGOT DE SOUZA, 1996). In industry, Curitiba and the metropolitan area grew almost 2% per year in the 1970s, resulting in  $\approx 47\%$  in 22 years (MEINERS, 1998). Highlight for chemical, mechanics, minerals and timber industries. This growth began in 1950 with the implementation of automakers in Sao Paulo, promoting an incentive for a network of suppliers in several areas of the country (BITTENCOURT, 2003). The increase in purchasing power of the population matched with vehicles' availability and resulted in an increase in the vehicle fleet in Paraná state, at a growth rate of 240% per decade (average), between 1950 and 1980. Thirty-one% of the total fleet of Parana state is from Curitiba and the metropolitan

region (ALFAVEA, 2009). Such a rise in fleet was reflected in region infrastructure with an increase of 150% in the number of streets in Curitiba and the metropolitan area between 1960 and 1970 (IPPUC, 2013; IPPUC, 2009).

Thus, considering the predominance of pyrogenic sources as mentioned in the previous section, it is observed that such sources from biomass burning predominated in the 1850-1920 period, while burning fossil fuels for mobile sources (e.g., vehicle) and stationary (e.g., industry) appears to be responsible for the largest generation of PAHs in more recent times.

Although the most significant economic growth in the region has occurred since 1960, increased concentrations of PAHs are observed from 1940 (FIGURE VI-1). According to the changes in the isotopic composition of plants, discussed in the previous chapter, local anthropogenic emissions since 1930 have contributed to the alteration of regional atmospheric CO<sub>2</sub> levels. Thus, the evolution of the anthropogenic impact revealed by PAH distribution is consistent with the results of isotopic analysis and complements the understanding of changes in regional atmospheric composition.

## VI.6 CHAPTER SYNTESIS

The record of environmental pollution in the north region of Curitiba and Almirante Tamandare during the last 150 years was assessed using PAH distribution as well as isomer ratios. The highest concentration levels of PAHs occurred in approximately 1970, corresponding to the sudden economic development of Brazil between 1960-1980, which resulted in more fossil fuel consumption and increased combustion of petroleum derivatives. Another peak with lower concentration (compared to the peak in 1970) was detected in approximately 1910, which may reflect the increase in population due to immigration programs in the 1890s.

The strong and positive correlations indicate that most PAHs derive from the same sources or were controlled by similar deposition process. The PAH isomer ratios showed a variation of PAH sources, suggesting three historical periods of contamination: (i) the predominance of wood and grass combustion sources (1855-1920), (ii) a mixture of biomass and petroleum combustions sources, considered a transition period (1920-1940) and (iii) the predominance of petroleum derivative combustion and contamination sources (1940-2012). The influence of the history of the region on the PAH sources was also apparent: increases in the population, the installation of railways, increases in the car fleet and paved streets. Although contamination from petroleum derivatives was observed, there was a predominance of

pyrogenic sources, confirmed by the strong correlation between the  $\Sigma$ PAHs and benzo(a)pyrene.

Finally, the results provide insight into the historical input of PAHs from local and long-range sources and indicate an anthropogenic environmental influence, which elucidates the understanding about changes in regional atmospheric composition after 1930, discussed in previous chapter.

## GENERAL CONCLUSION

Combined analysis of total lipids, isotopic ratios, and GDGT compound groups and inorganic analysis appear to be suitable to reconstruct past environmental changes in the two study selected areas. Changes in climate, land use, vegetation and atmospheric composition were revealed by biomarkers and supported by historic records and data from literature.

In Lake Ohrid watershed, the results showed how organic matter fluxes were affected by natural climate changes as well as the response of human population toward it. Around 1,000 years ago, organic carbon records showed distinct shifts that appear related to well-known Holocene climate fluctuations: the transition from the Dark Age (DA) to the Medieval Warm Period (MWP). The patterns observed in the lipids and inorganic analysis records may thus appear controlled by an entirely natural mechanism of ecosystem change associated with human interference such as deforestation and farming, which has the potential to tip the ecosystem out of its natural balance and, therefore, should be recognizable in biomarker profiles. Saint Naum Monastery foundation on the shores of Lake Ohrid and the shift in the social condition of Ohrid Town to the capital of the Bulgarian Empire appear to be responsible for the farming and deforestation in the area. Biomarkers in the Lz1120 core from Lake Ohrid allowed identification of changing contribution from the principal organic matter pools, i.e., aquatic and terrestrial biomass as well as soils, and suggested an increase in soil organic matter supply that reflects the substantial changes in land use, triggered by a shift toward more favorable climate.

In the Barigui River watershed, temporal variability of rates among alkanes and their distribution revealed changes in vegetation cover during the transition from LIA to Modern Time. The oscillations in alkane ratios suggested a substantial increase of wood-related herbaceous plants around 1800 in response to the minimum temperature (10°C) and dry conditions, characteristics of LIA. Real effects of this climatic event in south Brazil are scarce, and variations of past temperatures are unknown. Therefore, this study brought new evidence about the evolution of climate during LIA and Modern Time in the studied area, as well as showed the potential to GDGT indexes to reconstruct past temperatures. Since vegetation is directly affected by climate conditions, the similar pattern observed among alkanes and GDGT indexes used to assess vegetation and temperature changes respectively, demonstrates that these biomarkers are suitable to reconstruct past scenarios and offers a comprehensive multiproxy approach.

Despite vegetation and climate changes during the LIA period, changes in atmospheric composition were also detected in the Barigui River watershed, from shifts in isotopic ratios of alkanes. Long chains of alkanes from terrestrial plants reflected isotopically the changes in atmospheric CO<sub>2</sub> levels. Decreasing in the δ<sup>13</sup>C values indicated two rises in CO<sub>2</sub> levels. The variation of soil pH in the region revealed by the GDGT index presented a similar pattern to global volcanic activity assigning to volcanism the first increase of atmospheric CO<sub>2</sub> (1600-1880) as an important source, while the second increase (1930-2012) was assigned to dominance of local anthropogenic activities.

PAHs combined with organic carbon, nitrogen, and phosphorus ratios were used to assess the anthropogenic impact in the Barigui River watershed. Although the region was already populated previously, increases in TOC/TN and IP/TP ratios indicated that the human impact (main deforestation and fecal pollution) became significant in the region only after 1840 and PAHs ratios showed that this impact was more intense from the 1930s due to biomass/fossil fuel burning, petroleum and derivatives contamination. Significant correlation between total PAHs and demographic data from Curitiba and Almirante Tamandare indicated that effects by population growth and regional economic development were the main ones responsible for evolution of anthropogenic environmental changes.

Despite the representativeness and approach of the findings, this study presents a considerable contribution to demonstrate the potential use of biomarkers in dated sediments to construct past scenarios. Such information obtained can be useful to understand current climatic conditions and understand the evolution of human impact. Several reflections on the findings and evidence presented in this study can be made and linked to current environmental issues or provide support for deeper studies of this nature. Although the biomarkers used are from different classes of compounds and completely different sources (e.g., alkanes are from plants, while GDGT are from soil bacteria), the information obtained point the same way and are complementary, demonstrating consistency and reliability in the findings. Such findings can be used to reduce the uncertainties of forecasting models and allow a more accurate assessment for future implications and supporting decisions.

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