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# **Investigating the use of coca and other psychoactive plants in Pre-Columbian mummies from Chile and Peru**

*An analytical investigation into the feasibility of testing  
ancient hair for drug compounds*

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# Abstract

## Investigating the use of coca and other psychoactive plants in Pre-Columbian mummies from Chile and Peru

Emma Louise Brown

Psychoactive plants have played a significant role in Andean cultures for millennia. Whilst there is evidence of the importance of psychoactive plants in the Andean archaeological record, none of these are direct proof that these culturally significant plants were used by ancient Andean populations.

This project utilised liquid chromatography tandem mass spectrometry (LC-MS/MS) to investigate the use of psychoactive plants in individuals from cemetery sites in Chile and Peru by analysing hair specimens for a variety of psychoactive compounds.

Hair specimens from 46 individuals buried at cemetery sites in the Azapa Valley (northern Chile) belonging to the Cabuza culture (c AD 300 – 1000) indicated around half of these people ingested coca, as evidenced by the detection of BZE in hair specimens. Two individuals from this population tested positive for bufotenine, the main alkaloid in *Anadenanthera* snuff. There is a specific material culture associated with snuffing. These findings confirm *Anadenanthera* was consumed in the Azapa Valley.

The 11 individuals from Peru came from the necropolis at Puruchuco-Huaquerones in the Rímac valley near Lima. These individuals belonged to the Ichma culture, but would have been under Inca imperial control during the Late Horizon. Although only a

small sample, two-thirds tested positive for BZE, suggestive that access to coca was widespread.

This project presents a synthesis of the archaeological evidence for the use of various psychoactive plants in Andes. Also presented is the first report of the detection of bufotenine in ancient hair samples and additional data contributing to the understanding of the use of coca in the Andes.

**Key words:** Inca, Cabuza, LC-MS/MS, human remains, coca, bufotenine, bioarchaeology

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# Chapter 1: Introduction

## Preamble

Human hair grows at a relatively constant rate, around 1 cm per month. It does not remodel once keratinisation has occurred, unlike other body tissues such as bone, dentine and muscle. Therefore, hair can provide a snapshot of information reflecting the last months or years of the life of an individual (Wilson 2005: 321). The robust chemical and morphological structure of hair is responsible for its preservation in archaeological contexts (Wilson 2005: 321).

Hair is not found in every archaeological context. However, when conditions are favourable, for example, where microbial activity is restricted, such as permafrost and arid environments (Wilson 2008: 137), hair survives and can provide a wealth of information on diet (Macko *et al.* 1999a; Macko *et al.* 1999b; Panarello *et al.* 2003; Schwarcz and White 2004; Wilson *et al.* 2007b), seasonality (White 1993; Williams and Katzenberg 2012), location, altitude and mobility (Fernández *et al.* 1999; Sharp *et al.* 2003; Ehleringer *et al.* 2008; White *et al.* 2009), stress levels (Webb *et al.* 2010), genetics (Gilbert *et al.* 2007a; Gilbert *et al.* 2007b; Gilbert *et al.* 2008; Rasmussen *et al.* 2010), disease (Horne 1979; Fletcher 1994; Araújo *et al.* 2000; Zink *et al.* 2008), radiocarbon dates (Taylor *et al.* 1995; Bonnichsen *et al.* 2001a), exposure to pollutants (Egeland *et al.* 2009) and poisons (Arriaza *et al.* 2010), use of cosmetics (McCreesh *et al.* 2011) and the ingestion of drugs (Cartmell *et al.* 1991b; Cartmell *et al.* 2001; Cartmell *et al.* 2005; Musshoff *et al.* 2009; Ogalde *et al.* 2009).

This thesis is concerned with the investigation of the use of psychoactive plants in ancient Andean populations by testing hair for drug compounds. Testing hair for drugs is common in forensic, clinical and sports medicine (Kintz 2007; Musshoff and Madea 2007; Pichini *et al.* 2007; Pragst and Yegles 2007; Kintz 2008). An early novel application of hair testing was the investigation of ancient coca chewing practices in the Andes (Cartmell *et al.* 1991a; Cartmell *et al.* 1991b). Coca (*Erythroxylum* sp.) is native to South America (Towle 1961: 58; Plowman 1986a; 1986b) and has an extremely long history of use, as confirmed by archaeological discoveries, such as coca leaves in secure early contexts on the north coast of Peru, and material culture associated with coca chewing (Wiedemann 1979; Belmonte *et al.* 2001; Dillehay *et al.* 2010) as well as the results of drug analyses of ancient hair from mummies from Peru and Chile (Cartmell *et al.* 1991a; Cartmell *et al.* 1991b; Balabanova *et al.* 1992b; Brown *et al.* 2008).

The subject of this thesis is the analysis of hair from mummified human remains from Chile and Peru for the active compounds in psychoactive plants such as *Echinopsis pachanoi*, *Anadenanthera* sp, *Erythroxylum* sp. thought to have been in use for ritual, social and medicinal use for thousands of years (Hastorf 1987; Torres 1995; Rivera *et al.* 2005; Dillehay *et al.* 2010). Despite the depiction of a number of psychoactive plants in Andean material culture, and some scant palaeobotanical evidence, relatively little is known about how psychoactive plants have been used by ancient Andean populations. Some preparations, particularly those that are imbibed as teas or decoctions do not survive in the archaeological record (Torres 1995). The analysis of hair using research grade instrumentation such as liquid chromatography tandem mass spectrometry can provide an insight to the history of

these practices that the analysis of material culture cannot (Cartmell *et al.* 1991b; Springfield *et al.* 1993; Brown 2007; Ogalde *et al.* 2009).

Hair samples were obtained from three Cabuza cemeteries in the Azapa Valley of northern Chile (c AD 300-1000), three individuals with little contextual information from sites around Iquique on the Chilean coast and “Inca” individuals from Puruchuco-Huaquerones (c AD 1400-1532) in the Rimac Valley from the central coast of Peru. These hair samples were tested for a number of naturally occurring active drug compounds using liquid chromatography tandem mass spectrometry (LC-MS/MS).

## **1.1. Statement of Project aims & thesis outline**

### **1.1.1. Project Aims**

The main aim of this project is to determine the feasibility of testing minimal ( $\approx 2$  mg) amounts of hair from Andean archaeological sites for drug compounds as a means of investigating the use of culturally significant psychoactive plants in archaeological populations. These data will be interpreted within the wider bio-cultural and archaeological context; taking into account biochemical and anthropological data, burial traditions, grave goods and other archaeological evidence to support or refute earlier findings.

### **1.1.2. Objectives**

- The development of an LC-MS/MS method suitable for the simultaneous detection of specific psychoactive compounds found in plants native to the Andes and western Amazon.
- The development of a suitable procedure for the extraction of psychoactive compounds from ancient hair.
- The application of the extraction and LC-MS/MS procedures to ancient hair samples to determine if any psychoactive compounds are present.
- Where sample size is sufficient, and there is an indication of psychoactive compounds in the hair, segmental hair analysis should provide a diachronic record of psychoactive use.
- A thorough review of the ethnobotany, chemistry and international legal status of key Andean/western Amazonian psychoactive plants, as many of the plants contain compounds that are controlled under both UK and International law. This has implications for this research project, as licenses are required from the Home Office for importing, purchasing and storing these compounds, Additionally, many traditional cultures still use these plants despite them being illegal under international law. This has obvious cultural implications for traditional societies today.
- A synthetic review of diverse literature for the material culture of psychoactive plant use in the Andes, placing this in the wider socio-cultural context of the period.

### **1.1.3. Thesis Outline**

Chapter 1 is an introduction to this thesis. It provides the research question, aims and objectives of the project, and describes the location, climate and environmental conditions for the area in which samples for this project originated. This chapter also discusses various timescales and chronologies used by Andean scholars. The main system, devised by John Howland Rowe in which periods are divided into Horizons and Periods is used, as this approach is understood by Andean scholars (although there are issues with this chronological system, see s1.3).

Chapter 2 discusses the botany and ethnography of the main psychoactive plants in the Andes and Amazon regions. General botanical information is provided, and the traditional preparation and medicinal and ritual uses of each plant as recorded in ethnographic literature is reviewed. The chapter ends with a brief discussion of psychoactive plants that were culturally significant in the past but are still lacking a definite botanical identification or phytochemical investigation.

Chapter 3 is a discussion of the biology and chemistry of the different alkaloid classes that are relevant to this research project. Their pharmacology is discussed, as are their legal statuses under UK and international law, and how these laws affect religious freedoms and traditional practices of South American peoples.

Chapter 4 is a discussion of the biology and chemistry of hair. The growth cycle is discussed, including factors that may affect growth rate. Drug incorporation and factors affecting the incorporation of drugs into hair and the survival of drug molecules in hair fibres within different depositional environments is reviewed.



Chapter 5 is a review of the literature regarding the testing of hair from South America for various psychoactive compounds. This chapter aims to review and summarise the most recent advances in drug testing ancient hair. The strengths and weaknesses of different analytical methods and the interpretation and integration of data into the wider biocultural context are discussed.

Chapter 6 deals with the depiction of psychoactive plants in the material culture of the Andes. The chapter is broken down into time periods established by John Howland Rowe (1962). Each section discusses significant events and cultural transitions, beginning from the Late Preceramic (c. 5000 BP). Relevant material culture is discussed, including ceramics, textiles, and architecture. In addition, palaeobotanical evidence of psychoactive plants is discussed, as are documents relating to psychoactive plants from the Colonial Period (15<sup>th</sup>-18<sup>th</sup> centuries).

Chapter 7 details the relevant cultural context of individuals from which hair samples were obtained. The location of sites, environmental conditions and what is known about each culture is discussed. Where possible, photographs, pathological information and radiocarbon dates for individuals in this study are presented.

Chapter 8 describes the methods and materials used to carry out the analyses that form the basis of this research project.

Chapter 9 reports the results from LC-MS/MS analyses that are the basis of this research project.

Chapter 10 discusses the data obtained from the LC-MS/MS analyses in terms of prior bioarchaeological research.

Chapter 11 offers conclusions from this research project and ends with suggestions for further work.

## **1.2. Geography, Climate & Environmental Conditions**

### ***1.2.1. Ecological Zones in Peru and Chile***

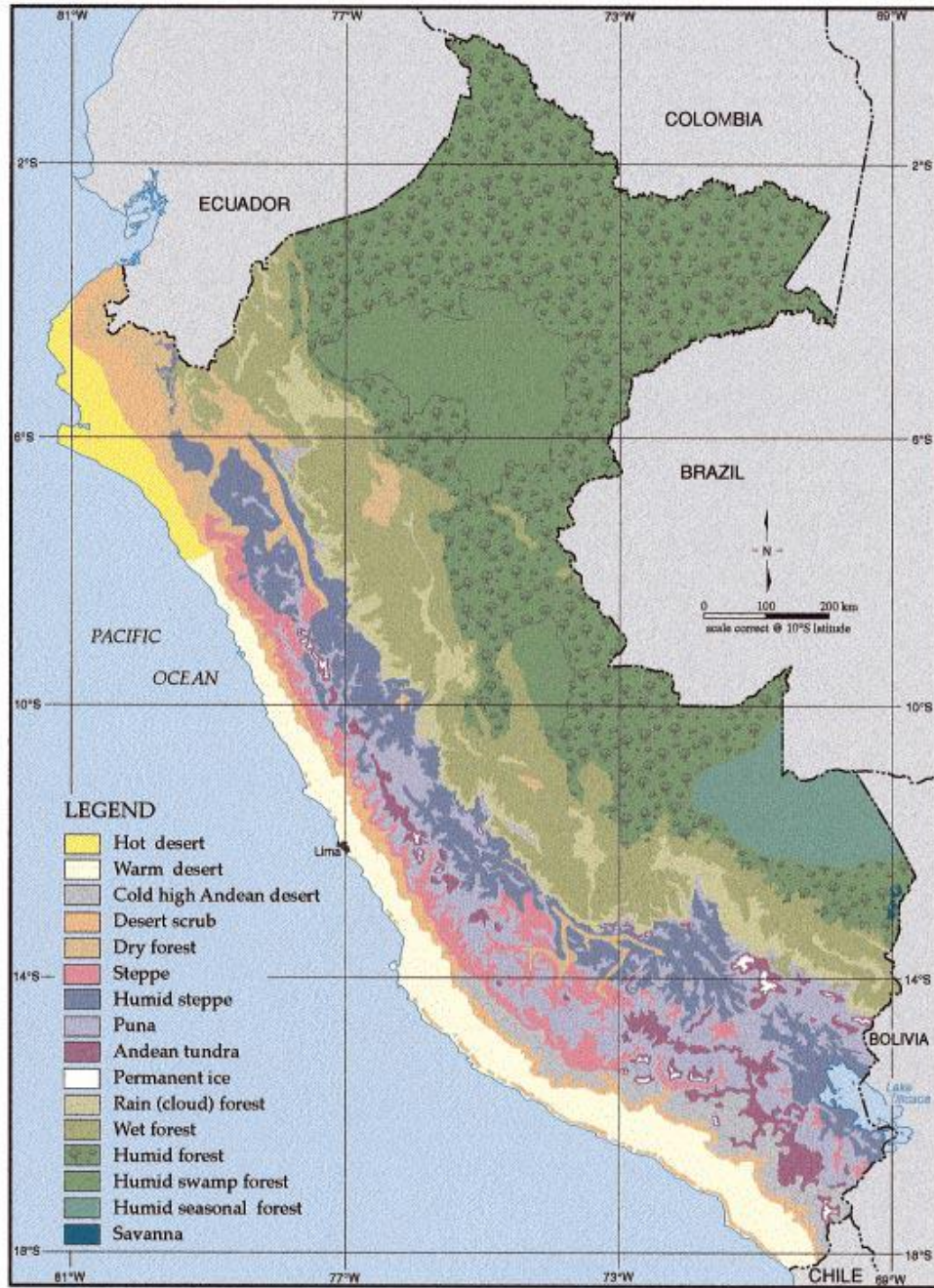
The tropical Andean region of South America is the most biologically diverse region on Earth, containing a sixth of the world's plant species in less than 1% of the total area of the planet (Conservation International 2007). The range of biodiversity is due in part to the wide range of ecological zones in South America. At a basic level there are three biomes: the coast, the Andes and Amazon. These lie within a relatively small area; in some parts of Peru it is possible to pass through all three biomes within 200 km (Williams 2005: 40). Using Quechua terminology that is widely understood throughout South America, Pulgar-Vidal (1987) identified eight ecological zones (see table 1.1 below). This system is widely used by various disciplines, including archaeology (e.g. Williams 2005).

An alternate system proposed by Rodríguez and Young (2000) uses 16 major zones in Peru (see Fig. 1.2). This system is more specific, in that it breaks down each ecozone into a percentage of the total area it represents in Peru. Pulgar Vidal's system is more general. When combined, both systems provide a detailed map of different ecozones in Peru. The largest ecological regions are humid tropical forests in the Amazon lowlands (32%); wet forests, including those in the eastern Andean foothills (13%); warm tropical desert (7%), swamp forest in the Amazon lowlands (6%); humid steppe (6%), and rain or cloud forest in the eastern montane and

premontane belts (5%). The remaining 10 ecological regions collectively cover about 20% of Peru and are distributed in the coastal plain, the Andes mountains, and in small pockets in the Amazon lowlands (Rodríguez and Young 2000).

**Table 1.1.** – Major ecological zones in the Andes and their key features (Pulgar Vidal 1987).

<b>Zone</b>	<b>Altitude (meters above sea level)</b>	<b>Features</b>
Chala	0-500	Extremely arid, varies in width from north to south.  Agriculture is possible but only with irrigation.
Omagua	80-400	Lush tropical rainforest. Various crops cultivated, especially near rivers and floodplains.
Rupa-rupa	400-1000	Very steep slopes covered by low trees and ferns, little cultivation or habitation in this area.
Yunga	500-2300	Midvalley zone found on western slopes of Andes and intermontane valleys. Warm, sunny, sheltered. Highly valued crops such as coca are cultivated here.
Quechua	2300-5300	Lower slopes and valley floor. Frost-free environment, cultivation with irrigation.
Suni	3500-4000	Steep slopes and hollows devoted to agricultural production of tubers, quinoa and lupines.
Puna	4000-4800	Year-round grasslands that are the natural habitat of wild camelids. Frost occurs in this area, diurnal fluctuations in temperature, but snow is rare. Soil is poor for cultivation
Janca	>4800	Permanently snow covered. Uninhabitable.



**Fig. 1.1** – Simplified map of Peru showing different ecological zones (Rodríguez and Young 2000).

There is in the order of 20,000 plants species in Peru, including flowering plants and ferns (Rodríguez and Young 2000). South America is second only to Mexico in biodiversity of psychoactive plants (Schultes *et al.* 2001: 27). Richard Evans Schultes, the eminent Harvard ethnobotanist suggested that there were some 130

psychoactive plant species in the New World, as opposed to less than 50 in the Old World (Schultes *et al.* 2001: 30) .

Based on ethnohistorical accounts written after the arrival of the Spanish in the early 16<sup>th</sup> century, John Murra proposed a vertical economic model, in which he suggests that Andean communities established colonies in remote and non-contiguous ecological zones to gain access to goods produced in these areas (Murra 1956). This idea has been immensely influential in Andean archaeological research, but has been criticised, most notably by Mary Van Buren (1996), who doubted that vertical economies provisioned for whole communities. She has argued that they produced “goods that were critical to the maintenance of political power” – i.e. high status or specialist items rather than provisioning an entire community.

### **1.2.2. The Peru Current and El Niño**

The coastal region of Peru and Chile is extremely arid with low precipitation. The Atacama Desert makes up a section of the north Chilean coast. The verticality model as proposed by John Murra helps to explain how communities living in this extreme environment obtained food, building materials and prestige objects from other nearby ecozones.

The Atacama lies on the west slope of the central Andes between 15 and 30 °S at elevations between sea level and 3500 meters above sea level. On average the Atacama receives less than 200 mm rainfall every year (Houston and Hartley 2003; McKay *et al.* 2003). The area around the Loa River and the coastal town of Iquique

receives even less than other regions, in the region of 1mm per year (Cereceda *et al.* 2008).

The hyperaridity is caused by the Peru Current, (also known as the Humboldt Current), which is an upwelling of polar-fed bottom waters characterised by low temperatures, regardless of latitude or season. This nutrient-rich water creates one of the largest and most productive marine ecosystems, which in turn supports a wide variety of marine life, including plankton, marine mammals, fish (particularly anchovies, sardines and mackerel) and birds (WWF 2001; Spangler 2003: 436).

The Peru Current flows along the western South American coast northwards from Antarctica. The current diverges westward just south of the Galapagos Islands off the coast of Ecuador and has a significant cooling effect on the coasts of these countries (WWF 2001). The air cooled by the cold water carries less moisture, which is partly responsible for the extreme aridity of the western Pacific coast. As cooler clouds move inland, they warm slightly, increasing their ability to retain moisture. The result is very little precipitation at any time of the year. However, during the winter months (June to November), these clouds are trapped beneath warmer air, producing a thick blanket of fog, (*garua*) that inundates the central coast (generally late June to November). This *garua* produces a fine mist or drizzle that can increase the overall humidity of the central coast. During the summer months (December to May), these clouds are able to rise, cooling gradually, producing rain in the highlands and preventing rain along the coast (Williams 2005: 45).

The Peru Current is periodically disrupted by major climatic events called El Niño/El Niño southern oscillation (ENSO) events, which occur approximately every five

years. El Niño is characterised by the warming of sea temperatures, resulting in substantial climatic change. Flooding, mudslides, agricultural damage, increased sea levels, growth of toxic plankton and the death of marine life and seabirds are associated with ENSO events (Rohli and Vega 2012: 64).

La Niña events are the opposite of El Niño events, in that sea temperatures are cooler. During La Niña events there is a large scale strengthening of trade winds, an upwelling of cold water and high pressure systems, causing suppression of rainfall in the eastern Pacific, resulting in drought along the coastal regions of Ecuador, Peru and Chile (D'Aleo and Grube 2002: 3).

### ***1.2.3. Location of sites and research samples***

The target region for this research project was the hyperarid coastal desert of northern Chile and southern Peru, and the valleys that bisect them. Specifically: the Azapa Valley of northern Chile (49 specimens from three cemetery sites), ending at the port city of Arica, and the Rímac Valley (13 specimens from one site) on the central Peruvian coast near the capital city of Lima (see fig. 1.1). Three additional samples believed to be contemporaneous with those from the Azapa Valley from sites around Iquique, Chile were also included. These samples had already undergone a number of analyses, including a radioimmunoassay test for a coca marker (benzoylecgonine, BZE), one of which one tested positive. This sample acted as a positive control for BZE.

The samples from these sites are not representative of entire populations living in either the Azapa or Rímac Valleys, as is the case with most cemetery sites. A

comprehensive biocultural interpretation comparing use within and between populations is not possible. This project is intended as a preliminary study into the use of coca and other psychoactive plants in ancient individuals using LC-MS/MS. This study is the first time that this method, normally used in forensic and clinical research, has been used on groups of ancient peoples.

### **1.3. Andean Chronologies**

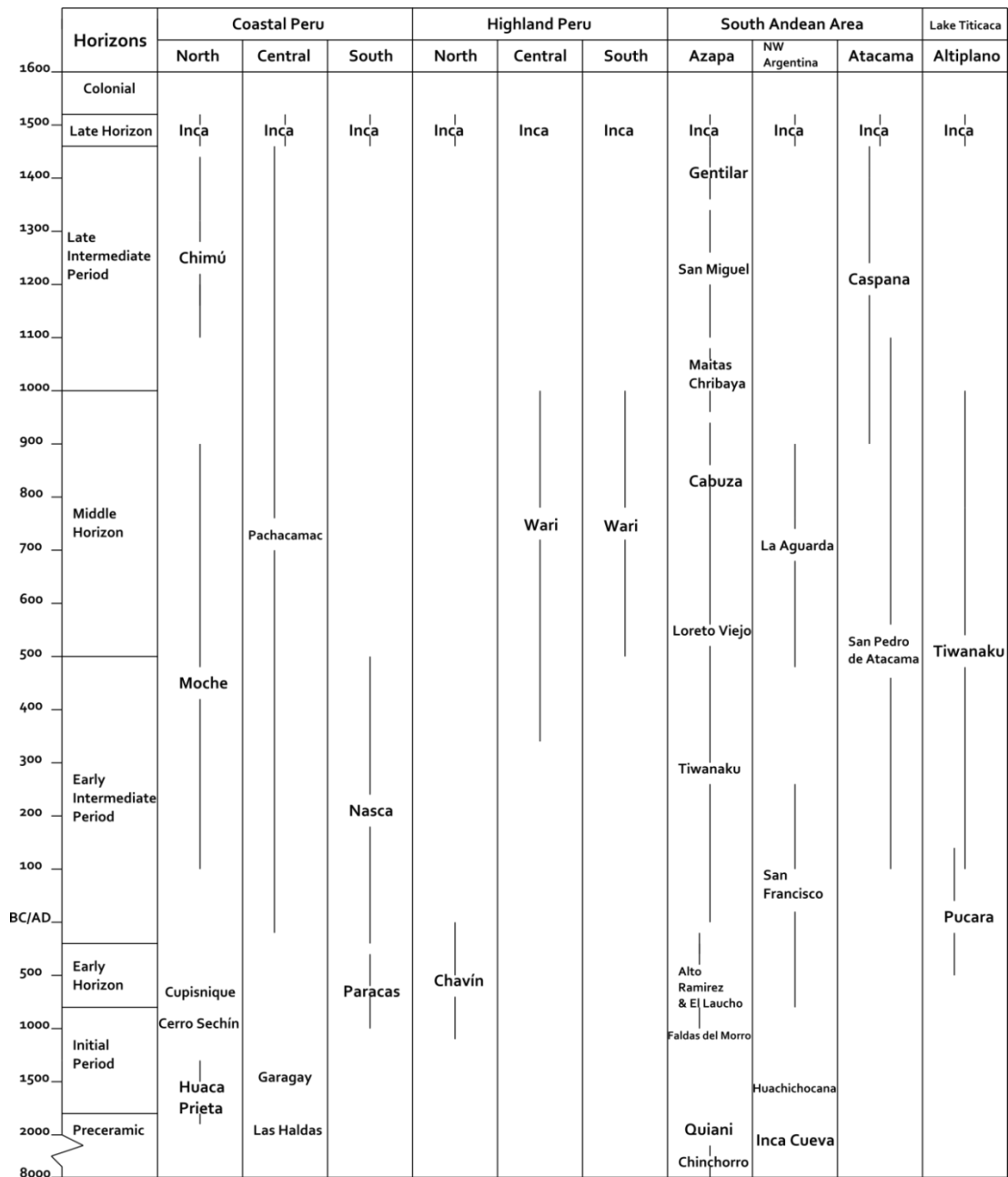
A number of different chronologies have been developed to describe the cultural developments in the Andes, as significant events such as the introduction of pottery and the cultivation of certain crops happened at different times (Lumbreras 1989: 12). As a result, many of the coastal valleys along the Peruvian and Chilean coast have their own chronologies. However, the most widely understood system is that devised by John Howland Rowe (1962). Rowe's system divides time into periods and horizons (Initial Period, Early Horizon, Early Intermediate Period, Middle Horizon, Late Intermediate Period, and Late Horizon). This system was based on a ceramic master sequence from the Ica Valley, on the central coast of Peru, as it was the best defined at the time (Pozorski and Pozorski 1987: 6). Edward Lanning (1967) refined the absolute dates associated with each period using available radiocarbon dates. This refined system is widely accepted and used. However there have been criticisms of this system (Pozorski and Pozorski 1987: 6)

Gordon Willey (1948: 8) describes a horizon as "abstraction based upon the recurrence of specific features of style or manufacture in prehistoric artefacts, mainly pottery, from one region to another so that the phenomena become pan-

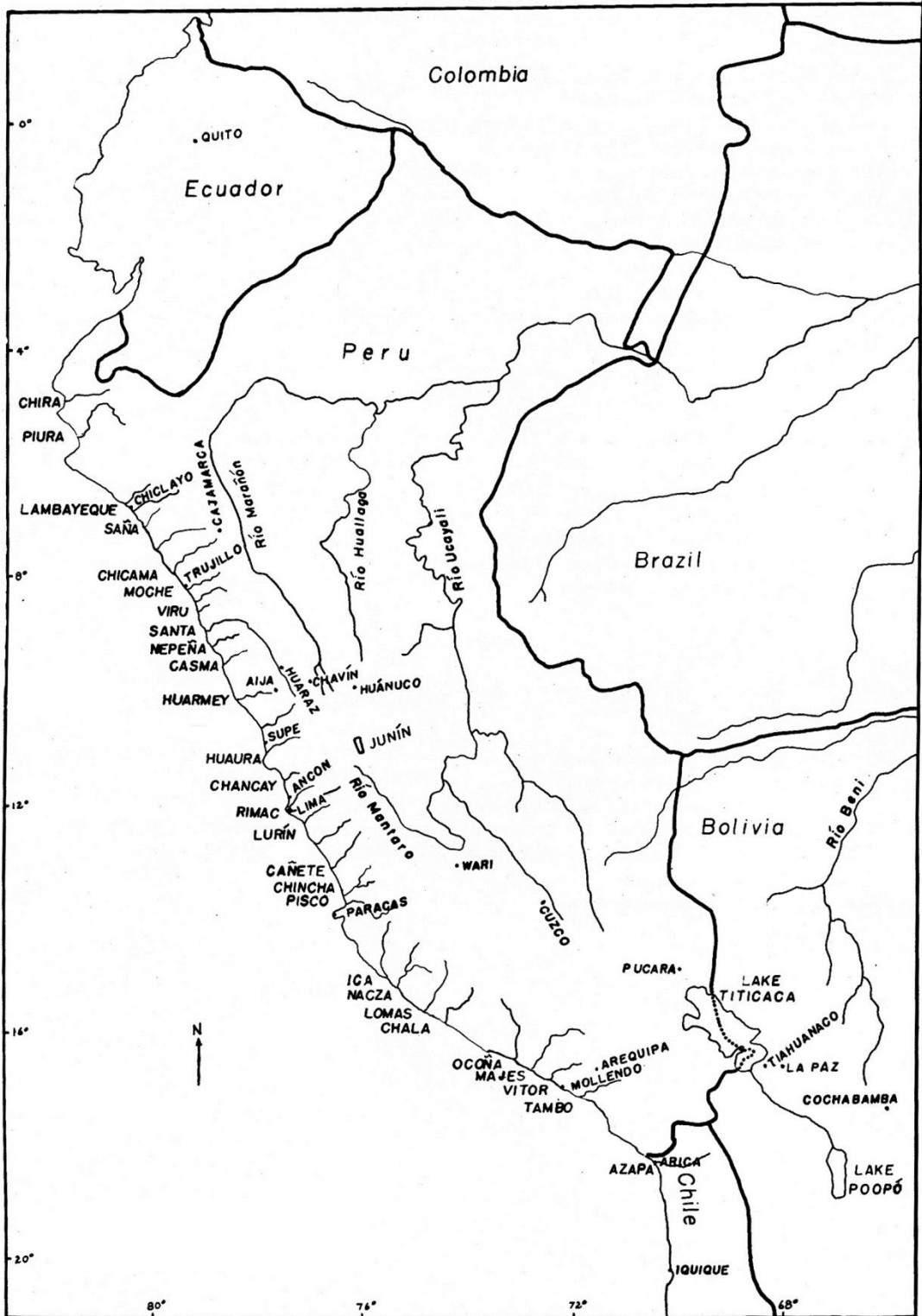


Peruvian in scope". The system works well for the central coast as it was developed using materials from this area. However, as Lanning (1967: 25) noted that as one gets farther from the central coast, finding stylistic influences becomes ever more tenuous. He also noted that radiocarbon dates may be lacking or inaccurate, as no dating may have been undertaken as part of the excavation or samples submitted for dating that were not necessarily from secure contexts. However, technological advances in scientific dating techniques and the use of well-defined sampling strategies informed by site stratigraphy and an understanding of some of the pitfalls of different dating techniques and samples (i.e. reservoir effects) have improved the dating evidence available from archaeological excavations in South America (Molto *et al.* 1997). However, there is still a large amount of "rescue archaeology" undertaken in this region. The Cabuza specimens donated by Arthur Aufderheide and Ichma/Inca specimens from Puruchuco-Huaquerones were obtained from rescue excavations (Focacci 1990; Cock 2002).

In recent years some Andean scholars have abandoned Rowe's chronology and developed alternative regional chronologies, for example, Stanish's chronology for the Upper Moquegua Valley in northern Chile (Stanish 1991) and Bauer's chronology for the Cuzco region in the Peruvian highlands (Bauer 1999). For this thesis, Rowe's chronology will be employed as it is still widely used. The chronology for the northern Chilean coast is slightly different. The Chilean chronology replaces the Initial Period, Early Horizon and Early Intermediate Period with one broad period, termed the Formative Period (Sutter 2005a).



**Fig. 1.2** – Timeline based on Rowe’s chronological system indicating key cultural groups in Andean areas.



**Fig. 1.3** – Map of Peru and northern Chile, with sites from which samples for this project were obtained: Puruchuco-Huaquerones (Lima, Rímac Valley, Peru), Azapa 6, Azapa 71 and Azapa 141, Azapa Valley, n. Chile) and Iquique (n. Chile) (after Bennett, 1948: x).

#### **1.4. The Colonial Period & the ‘Spanish Chronicles’**

The demise of the Inca Empire came in 1532-33 with the arrival of Francisco Pizarro and a small band of mercenaries, who captured and executed the *Sapa Inca* Atahualpa in July 1533 (D'Altroy 2002: 1). With his death came the end of Andean states and the beginning of the Colonial Period. As the native peoples of South America did not have a writing system that has been recognised (although *kipus*, knotted strings, were used to keep records), the accounts of various Spanish chroniclers have long been used as a source of information on the Inca Empire at the time of the conquest. The earliest eyewitness accounts were written by soldiers and scribes during the invasion of a land and culture far removed from their own “without time for reflection or understanding of the civilisation they were observing” (D'Altroy 2002: 10). The later accounts dating to the 16<sup>th</sup> and 17<sup>th</sup> centuries have been widely used to inform on life during Inca rule. In some cases these sources were written many years after the fall of the Inca and based on interviews with indigenous people rather than those who remembered life before the arrival of the Spanish. Terence D'Altroy (2002: 10) noted that by the time the Spanish took an interest in the Inca, those who were interviewed provided accounts coloured by time, political and economic objectives and wariness of Spanish oppression of their way of life and beliefs. However, with knowledge of how these accounts were gathered and an awareness of some of the inherent biases, the accounts can still provide a valuable insight into Andean beliefs and traditions.

### 1.5. Summary

The Andes and Amazon regions of South America are extremely biodiverse, with tens of thousands of plant species native to the region, including numerous species of psychoactive plants that remain culturally important to Andean and Amazonian communities even today. The people of these regions have employed many of these psychoactive plants for ritual, medicinal and social purposes since prehistory. There is evidence in the archaeological record of some of these plants, for example, the leaves of various species of *Erythroxylum*, as well as a specific artefacts associated with the use of other psychoactive plants (e.g. snuff trays for inhaling *Anadenanthera* or *Virola* snuff). However teas and decoctions made from psychoactive plants do not survive in the archaeological record and identifying their use through iconography is tenuous (Torres 1995).

The climatic conditions along the Peruvian and Chilean coast are very favourable for the preservation of human remains and their associated artefacts. It is possible to investigate the use of psychoactive plants in ancient populations by testing scalp hair for psychoactive compounds utilising analytical techniques normally used in forensic and clinical research.

## Chapter 2: Botany and Ethnography: South American psychoactive plants

### 2.1. Introduction

The Andes and Amazon regions of South America are some of the most biodiverse regions on the planet. Owing to this diversity, there are hundreds of plant species that contain psychoactive compounds. Some of these species are culturally significant in that they have played a role in the lives of native peoples from prehistory to the present day.

The methods by which psychoactive plants are prepared for ingestion vary greatly throughout South America. Many communities have their own methods of preparing and ingesting various snuffs, decoctions and enemas for both ritual and medicinal purposes. Some preparations, for example, *cimora* and *ayahuasca* are pharmacologically complex, owing to the use of admixture plants that have additional pharmacological effects (Schultes 1972; McKenna *et al.* 2008).

This chapter aims to describe the major psychoactive species native to the Andes and Amazon regions that are culturally significant. Trade routes between the coast, highlands and Amazon regions have existed for thousands of years, and many authors suggest that psychoactive plants have been traded via these routes (Lathrap 1973; Pillsbury 1996; Goldstein 2000). The distribution and major varieties of each species are described, as well as geographical and cultural variations in plant preparations. For some psychoactive plant species, such as *Erythroxylum*,

there is a lot of information available, as this species contains cocaine, which has been widely used and abused by Western societies for decades. There is also a large body of literature regarding *ayahuasca*, a combination of *Banisteriopsis caapi* and *Psychotria viridis*, which produces vivid hallucinations when consumed. The traditional uses of both coca and *ayahuasca* are emotive subjects, with proponents and detractors in equal measure. Some of the problems and benefits of these two plant preparations are discussed in some detail, as they are major issues in anthropology, ethnography and world drug policy. There is less information for drugs that are not as well recognised by western scientists as they either have no recognised medicinal use, or are not as attractive to people seeking to experience psychoactive plants.

This chapter focusses on plants that either alone or in combination produce hallucinations. Coca, which has been culturally significant in the Andes for at least 3,000 years, is also discussed.

Tobacco (*Nicotiana* sp.) has been suggested to be hallucinogenic (Janiger and Dobkin de Rios 1973; Elferink 1983; Wilbert 1990). However this has never been conclusively proved. The use of *Nicotiana* species in the Americas is very well documented; much is known about how tobacco is grown, prepared and used, as well as its pharmacology and effects on health (Wilbert 1990; 1993; Burns 2007; Benowitz 2008; Billings [1875] 2008). Caffeine containing plants such as *Ilex guayusa* have a very long history of use in the Andes (Wassén 1972), but is not considered here as it does not produce hallucinations. Similarly, xanthine

compounds, found in plants such as *Theobroma cacao*, have been detected in mummy hair (see s5.2), but again these are not discussed further here.

## 2.2. *Anadenanthera* and *Virola*

### 2.2.1. *Habitat and distribution*

The genus *Anadenanthera* is a type of tree native to South America. Two species are currently recognised: *Anadenanthera colubrina* (Vellozo) Brennan and *Anadenanthera peregrina* (Linnaeus) Spegazzini. Both of these species in turn has two geographically isolated subspecies, summarised in table 2.1. Both *Anadenanthera* species can grow up to 30m in height and bear large seeds pods which contain large red-brown to black seeds (see fig. 2.1), which are ground into powder and inhaled as a snuff.



**Fig. 2.1** – *Anadenanthera* seed pods, seeds and leaves (Safford 1916).



**Fig. 2.2** – *Virola surinamensis* leaves and fruit (Ayahuasca Info 2012).

*Virola* species (see fig. 2.2) belong to the Myristicaceae (nutmeg) family. Like *Anadenanthera* they are large trees (up to 30m in height) and all are native to tropical South America, particularly the western Amazon and Orinoco basin (Rätsch 2005: 529) There are at least 40 recognised species. The psychoactive



parts are the resin, inner bark and seeds which can be used to make snuffs and decoctions (Rätsch 2005: 530, see table 2.2).

**Table 2.1** - *Anadenanthera* species native to South America (Rätsch 2005: 50-56)

Species	Variety	Vernacular name	Distribution
<i>A. colubrina</i>	var. <i>colubrina</i> (Altschul)	Cebíl, villca, vilca, huilca, wilka	Eastern Brazil
	var. <i>cebil</i> (Grisebach)		Southern Andes & neighbouring areas (Argentina, Bolivia, Paraguay, Peru, SE Bolivia)
<i>A. peregrina</i>	var. <i>peregrina</i> (Altschul)	Cohoba, epená, ebena	Northern Brazil to the Antilles
	var. <i>falcata</i> (Bentham)		South America

### 2.2.2. Snuff preparation

*Anadenanthera colubrina* seeds contain the psychoactive tryptamine 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine) whilst *A. peregrina* contains *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), bufotenine and their *N*-oxides (Torres and Repke 2006: 108). *Anadenanthera* seeds are ground into snuff, which is then inhaled via the nose using single or Y shaped tubes. There is some geographical variation in the preparation and inhalation method. Baron von Humboldt witnessed the preparation of *yopo* snuff by the Maypure Indians of the Orinoco basin in 1801. Here, *Anadenanthera peregrina* pods were broken and left to ferment. The softened beans were then ground with lime and cassava flour and made into small cakes (Schultes *et al.* 2001: 119). In northern

Argentina, the Wichi shamans use ripe *A. colubrina* seeds to make snuff. The seeds are lightly roasted then ground into a fine powder. The snuff is then inhaled using short, hollow tubes. Occasionally the seeds are smoked. This requires less grinding and the coarse powder is mixed with tobacco and smoked as a cigarette (Schultes *et al.* 2001: 120; Räscht 2005: 50).

**Table 2.2** – Traditional use of *Virola* (Schultes 1969; Schultes *et al.* 1977; Räscht 2005: 530).

Species	Vernacular name	Use	Indigenous group
<i>V. calophylla</i> (Spruce) Warb.	epená	Snuff	Bora, Huitoto (Peruvian Amazon)
<i>V. divergens</i> Ducke <i>V. elongate</i> (Benth.) Warb.	Anya huapa, ko-de-ko	Treatment for arthritis; snuff; oral hallucinogen	Barasana, Huitoto (Peruvian Amazon)
<i>V. lorentensis</i> AC Smith		Hallucinogen	Huitoto (Peruvian Amazon)
<i>V. sebifera</i> (Aubl.) SW	Wircawei-yek; erika-bai-yek	Medicine	Venezuelan shamans
<i>V. duckei</i> AC Smith		Hallucinogen	Quechua peoples (Ecuador)
<i>V. theiodora</i> (Warb.)		Hallucinogen; arrow poison	Yanomamö (Orinoco basin, Brazil/Venezuela)
<i>V. surinamensis</i> (Rol. ex Rottb.) Warb.		Snuff	Bora, Huitoto (Peruvian Amazon); Warao (Orinoco basin)  Tradition in Iquitos, Peru

*Anadenanthera* powder has also been added to fermented beverages and administered as enemas (De Smet 1981; 1985; Torres and Repke 2006: ix).

*Virola* species have been found to contain 5-methoxy-*N,N*-dimethyltryptamine and *N,N*-dimethyltryptamine as the most abundant alkaloids. *Virola* also has trace amounts of other structurally related tryptamines (Aguirell *et al.* 1969; Kawanishi *et al.* 1985).

### **2.2.3. Cultural significance**

Both *Virola* and *Anadenanthera* are used to make hallucinogenic snuffs, both of which are used throughout the Andes and Amazon regions of South America. Many botanical descriptions and phytochemical investigations of *Virola* and *Anadenanthera* have been published over the years (e.g. Schultes 1969; Lai *et al.* 1973; Torres and Repke 2006), yet a thorough ethnographic study focussing on snuff use by any South American culture is lacking. However, some ethnographers have mentioned the use of psychoactive snuffs. The Yanomamö people of the Amazon rainforest make snuff (*ebene*) from at least three plant species. The most common is *Virola*, but *Justicia* and *Anadenanthera* are also used, sometimes in combination (Chagnon *et al.* 1971; Brewer-Carias and Steyermark 1976). The use of snuff produces an altered state of consciousness that enables Yanomami shamans to contact the spirit world. The snuff is seen as a “food” for the spirits (Jokic 2008). The Baniwa people of the northern Amazon employ snuff as part of shamanistic healing practices. The Baniwa shaman’s main diagnostic and curing procedure involves a snuff-induced trance, the purpose of which is to communicate with the

spirits and deities of the cosmos. Dances with rattles, songs, and especially reading and interpretation of shapes and forms of clouds allow the shaman to contact the deities who dwell in the heavens inform, advise, and assist the shaman regarding the source, character, and treatment of sickness of his community (Wright 1992).

An account of one Baniwa shaman described the concurrent use of snuff (*niopo*) and *caapi* (see section 2.3, this chapter) as a means of contacting ancestors and the spirit world (Wright 2004: 96). Piaroa shamans of the Orinoco basin also use *caapi* and snuff (called *yopo* in Piaroa dialect). A Piaroa shaman stated that “the force of *yopo* and the force of *caapi* work together”. The  $\beta$ -carboline alkaloids in *caapi* may prolong the effect of the snuff containing bufotenine or *N,N*-DMT much like the pharmacology of *ayahuasca* .

S. Henry Wassén identified 43 tribal communities in South America that practiced some form of snuffing. The majority of these tribes were located in the north-western Amazon region around the Rio Negro tributary of the Rio Amazon, and as far south as southern Argentina (Wassén 1967: 264-270).

## **2.3. Banisteriopsis & Psychotria (*Ayahuasca*)**

### **2.3.1. Habitat and distribution**

*Ayahuasca*, Quechua for “vine of the soul” is both the indigenous name for *Banisteriopsis caapi* (Spruce ex Grisebach) Morton and the decoction of *B. caapi* and other plants used by many native peoples of the north-west Amazon. *Banisteriopsis caapi* is widely distributed throughout the tropical zones of northern South America

and the West Indies (Schultes *et al.* 2001: 36). *Psychotria viridis* is primarily distributed through the Amazon lowlands, but as a result of extensive cultivation it has spread into parts of Brazil, Colombia, Brazil and Peru (Rätsch 2005: 456).

### **2.3.2. Preparation of Ayahuasca**

The key component of *ayahuasca* is the *Banisteriopsis* vine (*B. caapi* or *B. inebrians*). *Banisteriopsis quitensis*, *Mascagnia* and *Tetrapteris* species are sometimes used instead of *B. caapi*. All of these plants are closely related large forest lianas of the Malpighiaceae family (see fig. 2.3). However, *B. caapi* and *B. inebrians* are the most commonly cultivated species (Schultes *et al.* 2001: 124). The Malpighiaceae vines contain the  $\beta$ -carboline alkaloids harmine, harmaline and tetrahydroharmaline.

There is some geographical variation as to how *ayahuasca* is prepared. Typically the vine is boiled and other admixture plants are added, with the end result being a thick, brown, bitter tasting liquid. In other regions the vine is soaked in cold water, producing a less concentrated decoction that needs to



**Fig. 2.3** – *Banisteriopsis* vine (Sowewankeri 2012).

be taken in larger quantities (Schultes *et al.* 2001: 126; Rätsch 2005: 703). Usually the leaves of *Psychotria viridis* or *P. carthaginensis* (collectively known as *chacrana*) are added to the mixture. *Psychotria* species contain *N,N*-dimethyltryptamine as a major alkaloid, with the leaves typically containing 0.1-0.6% DMT (Rätsch 2005: 457). *N,N*-DMT is not orally active, as it is broken down by a group of enzymes

known as monoamine oxidases (MAOs) in the liver and gut (Riba *et al.* 2003; McKenna 2004). Harmine and harmaline act as monoamine oxidase inhibitors (MAOIs), whilst tetrahydroharmaline weakly inhibits the uptake of serotonin (Callaway *et al.* 1999). These compounds prevent the oxidative deamination of *N,N*-DMT allowing it to reach the central nervous system, thus rendering *N,N*-DMT orally active (Schultes *et al.* 2001: 127; McKenna 2004; Rättsch 2005: 703; McKenna *et al.* 2008: 351). It takes around 40 minutes before any effect of *ayahuasca* is felt. Effects last around 4 -6 hours, but can last longer (McKenna 2004).

### **2.3.3. Cultural significance**

In Amazon communities, *ayahuasca* is generally used for ritual purposes, such as initiation rites for youths, funeral ceremonies, medicine and divination purposes (Rivier and Lindgren 1972; Luna 1984; Reichel-Dolmatoff 1990: 86; Desmarchelier *et al.* 1996; Schultes *et al.* 2001: 124). It is known by a number of names – *ayahuasca*, *yagé*, *natema*, *nixi pae*, *caapi*, (see table 2.3) and is used throughout the Amazon basin as well as Andean Peru, Colombia, Ecuador and Brazil (McKenna *et al.* 1998).

Brabec de Mori (2011: 28) separates *ayahuasca* use into two mutually exclusive categories. The first, in which a specialist takes *ayahuasca* and uses the powers obtained for curing manipulation of the spirit world or sorcery. The specialist's patients or clients do not take *ayahuasca* under these circumstances. This is what is known as *ayahuasca* shamanism. This type of shamanism is practiced by communities that reside along the bigger rivers in the western Amazon region. Mestizo *médicos* have similar practices, whilst urban shamans differ slightly in that

they sometimes allow their patients/clients to drink *ayahuasca*. In less accessible areas, *ayahuasca* is taken as part of a group. The group are almost exclusively males. This ritual helps form a cohesive group identity, but also allows the group to acquire supernatural abilities, and to summon success for warfare and hunting. These groups do have specialists who perform curing and sorcery, but without the use of *ayahuasca* (Brabec de Mori 2011: 28).

Although customs vary, *ayahuasca* is almost always administered in a ritual context by a shaman amongst a group of people, but not always. For instance, some Shaur peoples take *ayahuasca* individually as a means of dealing with supernatural enemies (Harner 1973: 5). The Shuar call *Banisteriopsis* infusions *natém* (*Banisteriopsis* sp.). The Shuar also use *maikua* (*Datura arborea* or *Brugmansia suaveolens*) and *Nicotiana tabacum* as a means of contacting the spirit world. The use of such plants for recreation is unknown in Shuar groups (Brown 1978; Bennett 1992). In some cases the shaman may be the only person involved who imbibes *ayahuasca*, but often the whole group takes part. The Cashinahua of the Peruvian Amazon are an example of a community where *ayahuasca* is imbibed as part of a group ritual. All Cashinahua men know how to prepare *ayahuasca*, although only one will go into the jungle to collect the plants required for the brew. The plants are boiled to produce a thick, brown beverage that is taken by a group of people in night time *ayahuasca* sessions, usually beginning around 8 in the evening until the early hours of the morning. The men of the group chant individually to communicate with the spirit realm, although being part of the group is still important, as this provides a link to the real world. The spirit world is perceived as

terrifying and contacting this other realm would be overwhelming otherwise (Kensinger 1973: 11).

**Table 2.3**– Indigenous groups from South America that use *Banisteriopsis caapi*

Cultural Group	Area	Name	Reference
Amahuaca (Amenguaca, Sayacu)	S. Peru	nixi; oni xuma	Andritzky (1989)
Ashnanika (Ashaninca, Campa)	S. Peru	kamarampi; hananeroca	Weiss (1973: 44)
Awajún (Aguaruna)	N. Peru	datem	Baud (2009)
Achuar/Shaur	Ecuador/N. Peru	natem; natema	Bennett (1992)
Baniwa	Brazil/ Venezuela	caapi	Wright (2004: 92)
Cashinahua (Huni Kuin)	S. Peru/Brazil	nixi pae	Kensinger (1973: 9)
Desana	S. Colombia/Brazil	gahpi	Reichel-Dolmatoff (1990: 85)
Embera	N. Colombia	pinde, pilde	Reichel-Dolmatoff (1990: 85)
Ese'ejja (Chama)	Bolivia	ayahuasca; jono pase	Desmarchelier <i>et al.</i> (1996)
Guahibo (Sikuani)	N. Colombia / Venezuela	Uipa, kápi	Reichel-Dolmatoff (1990: 85)
Kamsa (Sibundoy)	N. Colombia	biaxii	Langdon and MacLennant (1979)
Kofan	Ecuador / S. Colombia	yaje; cofa; oofa	Duke <i>et al.</i> (2009: 90)
Matsigenka	S. Peru	ka'maranpi,	Rosengren



		kama'rampi; wampu,	(2002)
Sharanahua	S. Peru	shuri; ondi; rambi, rame	Rivier and Lindgren (1972)
Shipibo-Conibo	N. Peru	nishi; oni	Brabec de Mori(2011: 34)
Siona/ Secoya	Ecuador / Colombia / N. Peru	yaje; 'iko	Langdon (1979)
Tsachila	Ecuador	pinde, pilde; napa, nepe, nepi	Ventura i Oller (2005: 155)
Tukano	S. Colombia/Brazil	kaji; kadana, kadana-pira, yage	Reichel-Dolmatoff (1970)
Waorani	Ecuador	mii, miiyagi	Davis and Yost (1983)
Yaminahua	S. Peru / Brazil	shori	Calavia Saéz (2011: 143)

For the Cashinahua, taking *ayahuasca* is an unpleasant event only undertaken to gain knowledge that would be unavailable through regular communication channels that may either affect the whole community or an individual (Kensinger 1973: 13; Grob 2006: 69). *Ayahuasca* is not always used for positive purposes. *Brujos*, (witches), use *ayahuasca* to bewitch and harm others by causing illness and misfortune (Dobkin de Rios 1970a; Lagrou 2004: 261).

The shaman, who is usually male, leads the session with chants. Music is a very important part of *ayahuasca* sessions. Whistling, drumming and singing are thought to guide the group's visions and experiences during the sessions, be it divining

future events or discovering the cause of an illness (Katz and Dobkin de Rios 1971; Dobkin de Rios and Katz 1975; Shanon 2011).

Many authors suggest that *ayahuasca* was established in the Amazon long before the arrival of Europeans. Plutarco Naranjo described an incised stone bowl (see fig. 2.4) from the Pastaza culture of Amazonian Ecuador dating from 500 BC to 500 AD (Naranjo 1986). He suggested that this bowl was used for the consumption of

*ayahuasca*, yet there is no scientific evidence to support this claim. This claim has been uncritically adopted by many authors seeking to make a case for the ritual use of *ayahuasca* being established in antiquity,



despite Naranjo later modifying his claim. In 1995 he published a picture of the bowl in a short review of the archaeological evidence for the use of psychoactive plants in South America with the caption “A vaso or *cocina de brujo* (literally “witches’ cooking pot”). This ceramic container of the Milagro-Quevedo culture (500 BC to 500 AD) perhaps served as a collective vessel so that each participating member of the ceremony could drink part of the liquid” (Naranjo 1995: 398).

Other authors claim that *ayahuasca* preparations have been a part of South American cultures for millennia. Jeremy Narby states “*ayahuasca*-based shamanism is essentially an indigenous phenomenon. It belongs to the people of western Amazonia, who hold the keys to a way of knowing that they have practiced without

interruption for at least five thousand years” (Narby 1998: 154), whilst Dennis McKenna in discussing the prehistory of *ayahuasca* stated “the origins of the use of *ayahuasca* in the Amazon Basin are lost in the mists of prehistory...all that can be stated with certainty is that it was already spread among numerous indigenous tribes throughout the Amazon Basin by the time *ayahuasca* came to the attention of Western ethnographers in the mid-19th century” (McKenna 1999: 207). Benny Shanon asserts “Amerindians have used *ayahuasca* for millennia” (Shanon 2011: 281). Indeed, the purported ancient use of *ayahuasca* was the reason this preparation of plants was included in this research project. Despite this claim, some authors have suggested that *ayahuasca*, that is combination of *Banisteriopsis* and *Psychotria* species, is not as ancient as some suggest.

Peter Gow challenged the often repeated idea that *ayahuasca* shamanism (the use of *ayahuasca* by a trained shaman to diagnose and cure illness) was an “unbroken pre-Columbian tradition...fully integrated within the organic totality of traditional culture” (Gow 1994: 90). Gow argues that *ayahuasca* shamanism has evolved in an urban setting for around three hundred years as a direct response to the “specific colonial history of western Amazonia”. He notes that the more remote forest communities who were buffered from the burgeoning rubber trade and the processes of colonial transformation that accompanied it do not practice *ayahuasca* shamanism (Gow 1994: 91).

Gow argues that *ayahuasca* shamanism has its origins in the formation of *mestizo* (people with mixture of Amazonian and European ancestry) as a social category, resulting from the early Jesuit and Franciscan missions that accompanied the rubber

trade routes in the western Amazon. Many tribal people in the Amazon moved into the missions, as they afforded some protection from inter-tribal violence, starvation and disease. Here they converted to Catholicism and spoke Quechua, the language of trade in the area. The Omagua and Cocama people are examples of communities that joined the mission system. Gow suggests that it was under these conditions that *ayahuasca* shamanism evolved. He noted that *ayahuasca icaros* – healing songs that are sung during healing sessions – are always sung in Quechua (with some input from the Cocama language) even if this is not the language of the community in which the rites are performed (Gow 1994: 107). Bernd Brabec de Mori noted this same phenomenon when he studied the music of the Shipibo. All of their songs were sung in Shipibo, with the exception of the *icaros*, which were sung in Quechua. Brabec de Mori, an ethnomusicologist, noted the similarity in language and structure of *icaros* in different Amazon peoples, yet other songs not associated with *ayahuasca* were markedly different from all other indigenous songs. He cited this as evidence for a more recent common origin rather than millennia old traditions (Brabec de Mori 2011: 36-37).

Gow has also noted that the cosmology of *ayahuasca* shamanism also mirrors that of Catholicism, with particular similarities in the belief that *ayahuasca* is thought to be the body of *ayahuascamama* that is imbibed as part of the ritual, like wine and bread are taken as being the body and blood of Jesus Christ during Christian Eucharist (Gow 1994: 107). Brabec de Mori called this “Christian camouflage” and suggested that rather than being a way for disguising the *ayahuasca* ritual, it suggests that practice evolved entirely within these contexts (Brabec de Mori 2011: 28-29).

Gow postulates that the movement of these new Christian converts up and down the Amazon with the rubber trade helped spread *ayahuasca* shamanism. In his conclusion Gow states “tentative as my historical analysis is [it] explains why *ayahuasca* shamanism is found where it is today...among all those people who were linked to the economy of the western [regions of] Amazonia and is absent among those who were not” (Gow 1994: 110). He noted that other Amazonian peoples who did not have any contact with rubber traders or worked for Brazilian bosses do use *ayahuasca*, but in a completely different context and within a different cosmological framework.

Both Gow and Brabec de Mori agree that *ayahuasca* shamanism as practiced by urban shamans and communities along the major rivers in the Peruvian Amazon is less than 300 years old (Gow 1994: 91; Brabec de Mori 2011: 24). The work of other Amazon ethnographers also supports the suggestion that *ayahuasca* shamanism is a relatively recent development. For example, Glenn Shepard (1998) found that the relatively isolated Matsigenka of the Manu River only started combining *Banisteriopsis* and *Psychotria* during the 1960s, after they were contacted by other Matsigenka from the Urubamba region. The Manu Matsigenka regard *P. viridis* as a dangerous plant, since it is said to cause terrifying visions of fanged bats and snakes (Shepard 1998). Similarly, Alexiades and Peluso (2009: 236) noted that *ayahuasca* was introduced to the Ese Eja of the Madre de Dios region in Peru in the early to mid-20<sup>th</sup> century, whilst Gray (1997: 74) found that it was introduced to the Arakmbut (Madre de Dios region) in the 1970s by neighbouring groups who lived on nearby missions.

*Ayahuasca* shamanism is practiced in urban centres throughout South America, where it is the poorest sections of society with no other access to modern healthcare facilities that seek these kinds of treatments (Dobkin de Rios 1970b; 1973: 75; Brabec de Mori 2011: 44). In recent years traditional Andean medicine has been negatively affected by the *ayahuasca* tourism boom.

In the last two decades there has been an explosion in what has been termed “*ayahuasca* tourism”, in which western tourists seek out *ayahuasca* experiences. This phenomenon has been widely commented on (Dobkin de Rios 1994; Winkelman 2005; Dobkin de Rios 2006; Tupper 2008; Tupper 2009; Davidov 2010), with the general consensus that this form of tourism is detrimental to Amazon communities, as local healers focus more on providing expensive and spectacular experiences for European and American tourists rather than treating their own communities (Brabec de Mori 2011). Marlene Dobkin de Rios (2006) condemned *ayahuasca* tourism in the strongest terms, stating that the “new shamans” do not have the training that is required for this work and are basically charlatans out to make money. She highlighted their lack of knowledge of drug interactions with *ayahuasca* that can cause serious problems in their clients, resulting in hospitalisation and in some cases death. She concluded “...drug tourism in contemporary Peru and the Amazon region is merely a footnote to drug trafficking around the world” (Dobkin de Rios 2006). Other authors have noted the economic inequality in the Amazon directly related to *ayahuasca* tourism as a particular problem. Other problems were noted by Bernd Brabec de Mori (2011), in his work with Shipibo-Conibo communities, he found that they do not agree with the “*ayahuasca*-shaman” identity that has been collectively imposed on them. The

failure by foreign tourists to understand the cultural context of *ayahuasca* and a romanticised view of it being a panacea for all kinds of medical problems (which it is not) were also noted as problems.

The problems associated cultural appropriation and globalisation of *ayahuasca* has been thoroughly discussed by Ken Tupper (2009). Briefly he cites poorly trained “shamans”, particularly non-indigenous “neo-shamans” making large amounts of money and disenfranchising and misrepresenting Amazonian culture as a major problem, given the history of colonialism and oppression by European/North American cultures in the past (Tupper 2009: 125). He states that this kind of cultural appropriation undermines integrity of the community whose culture is appropriated and has an impact on the cultural object itself, in this case the profanation of a sacred practice, as well as inappropriate financial gain. He notes that financial gain for spirituality is anathema to most indigenous Amazon traditions (Tupper 2009: 125).

*Ayahuasca* (*hoasca* in Portuguese) is also used as a sacrament by a number of religious movements, such as the Brazilian União do Vegetal (UDV), Barquinha and Santo Daime churches. These syncretic religions are based on Christianity, but combine aspects of shamanism, animism, various African cosmologies, indigenous botanical knowledge and "folk Catholicism" (Santos *et al.* 2007). The syncretic religious use of *ayahuasca* in a ritual manner has been compared to the Christian practice of Holy Communion (McKenna 2004) and is practiced throughout South America and increasingly in western countries, particularly the United States. Santo Daime has a sizable following in the Netherlands.

## 2.4. Brugmansia & Datura

### 2.4.1. Habitat and distribution

*Brugmansia aurea* (Lagerheim) and *Brugmansia sanguinea* (Ruíz et Pavón), as well as all other *Brugmansia* species, also known as Angel's Trumpets, are members of the Solanaceae (nightshade) family and are native to South America (Rätsch 2005: 94). It is believed that all *Brugmansia* species are cultigens and not wild plants, with the implication that these plants have been used for thousands of years (Vitale *et al.* 1995; Schultes *et al.* 2001: 37; De Feo 2004; Rätsch 2005: 94). The *Brugmansias* are closely related to *Datura* species (see fig. 2.5), which are also believed to have been used for their psychoactive properties for millennia in South America. *Daturas* are smaller plants, whilst the *Brugmansias* tend to be larger shrubs or trees.



**Fig. 2.5** – Left: *Datura stramonium* (Royal Horticultural Society 2011b). Right: *Brugmansia* flowers (Royal Horticultural Society 2011a)

Both *Brugmansia* and *Datura* species are cultivated by shamans in South America, who use the flowers and leaves as additives to *ayahuasca* and other decoctions.



Both species are grown as ornamental plants in Europe as well as the Americas (Royal Horticultural Society 2011a).

#### **2.4.2. Preparation**

As with other psychoactive plants there is wide geographical variation in how both *Brugmansia* and *Datura* are prepared. Ground *Brugmansia* seeds have been added to *chicha*, infusions of leaves and flowers in water or alcohol have been used for medicinal purposes and poultices of ground leaves have also been used as topical anaesthetics (Lockwood 1979; De Feo 2003; De Feo 2004). *Brugmansia* species have also been used as admixture plants in *ayahuasca* and *cimora* (see s2.7) decoctions to modify or strengthen the visions produced by other psychoactive plants in a ritual context (Bristol 1969; Bennett 1992; McKenna *et al.* 1998; Carod-Artal and Vázquez-Cabrera 2006).

#### **2.4.3. Cultural significance**

The Spanish Chronicler Hernández recorded that *Datura* species were particularly important to the Aztec (c AD 1200 -1521) and Maya (c 2000 BC – AD 1500) empires of Mesoamerica (Garcia-Kutzbach 1976; Díaz 1977; Rätsch 2005: 197). Both cultures are thought to have employed *Datura* species, in particular *D. stramonium* as aphrodisiacs (Elferink 2000). Similarly, *Datura stramonium*, known colloquially as *chamico* in Peru, was used as an aphrodisiac for women in the early 19<sup>th</sup> century Peru (Cooper 1949: 555; Elferink 2000).

*Brugmansia* preparations have been recorded as being used by the following communities: Chibcha and Chocó (Colombia); Quechua (Peru, Ecuador and Bolivia); Mapuche-Huilliche (Chile); Záparo, Shuar, Canelo, Inga (upper Amazon); Siona, Pioje and Omagua (Andean montaña); Matsigenka (Amazon basin, Peru, Bolivia) (Cooper 1949; Lockwood 1979; Bennett 1992; Rosengren 2002, see also table 2.4). The *Brugmansias*, also known as *floripondio*, *borrachero* or *misha* by Andean shamans in northern Peru have been used in initiation ceremonies for boys and girls, medicine (*curanderismo*), divination and witchcraft (Lockwood 1979; Bennett 1992; De Feo 2004).

Michael Harner (1984: 90) documented the use of *maikua* juice (made from pressed *Brugmansia* flowers) amongst Shuar communities in the Peruvian/Ecuadorian Amazon region. Wayward children were given *maikua* juice as a punishment. The ensuing visions were believed to have put the child in contact with the supernatural world, in which they learned that their father's wisdom should be respected. The experience may also have put the child in contact with an *arútam*, which was considered a positive experience for personal development.

The most important use of psychoactive plants amongst the Shuar is during initiation rites for young boys. Around the age of eight young boys were accompanied by their father to a local waterfall. Over the course of several days the boy would be given *maikúa* in the hope that he would then see visions (*arútam*) produced by the soul (*wakani*) of a deceased ancestor (Rubenstein 2007).

Alexander von Humboldt and Aimé Bonpland recorded the use of *tonga*, a preparation made from *Brugmansia* at the Temple of the Sun at Sogamoza, north of

Bogota, Colombia in the early 19<sup>th</sup> century (Lockwood 1979), whilst Castellanos (cited in Lockwood 1979) recorded that *Brugmansia* was mixed with *chicha* and infusions of tobacco and administered by the pre-Conquest Chibchas of Colombia to slaves and wives of dead chiefs to induce a state of stupor before being buried alive during the funeral ceremony (Kroeber 1946: 907; Lockwood 1979).

**Table 2.4** – Culturally significant *Brugmansia* and *Datura* species in South America (Bristol 1969; Lockwood 1979; De Feo 2004; Rättsch 2005: 94-111).

Genus	Species	Distribution	Psychoactive material	Medicinal use
<i>Brugmansia</i>	<i>arborea</i> (Linneaus) Lagerheim	Ecuador, Peru, Bolivia, N. Chile	Leaves, fresh flowers, seeds	Anaesthetic, treatment of tumours
<i>Brugmansia</i>	<i>aurea</i> Lagerheim	Columbia to S. Ecuador, Mexico	Stems/stem pith, leaves, flowers, seeds	Headaches, “bone ache”, black magic
<i>Brugmansia</i>	<i>x candida</i> Persoon	Columbia, Ecuador, Mexico	Leaves, flowers	Poultice – placed against ache
<i>Brugmansia</i>	<i>x insignis</i> Lockwood ex Schultes	Western Amazon	Stems, leaves, flowers	Ease traumatic & rheumatic pain
<i>Brugmansia</i>	<i>sanguinea</i> (Ruíz et Pavón)	Andes (Colombia, Ecuador, Peru, Bolivia)	Leaves, fruit/seeds	Ease the pain of arthritis/cramps
<i>Brugmansia</i>	<i>suaveolens</i> (H.B.K) Berchtold et Presl	Andes, cordilleras, central America	Leaves, flowers, stems, juice from pressed stems, seeds	Sores, ulcers, wounds that won’t heal
<i>Datura</i>	<i>innoxia</i> Miller	American southwest, Mexico, Guatemala, Belize,	Leaves, roots, flowers, seeds	Treatment of fever/anaesthetic

Caribbean				
<i>Datura</i>	<i>stramonium</i> Linnaeus	North, central & south America, Europe, Near East, Himalayas, Caribbean	Leaves, seeds, flowers, roots	Anaesthetic, treatment of cancers

Another use of *Brugmansia* by the Chibchas was to “test slaves with the idea that if they wandered out while unconscious, they would sooner or later try to escape” (Kroeber 1946: 906). Before reaching puberty, children were given *Brugmansia* and watched to see if the boys picked up tools or weapons and if girls picked up spindles or grinding *manos*. If so, it was believed that these children would develop into good workers (Kroeber 1946: 906).

## 2.5. Echinopsis (Trichocereus)

### 2.5.1. Habitat and distribution

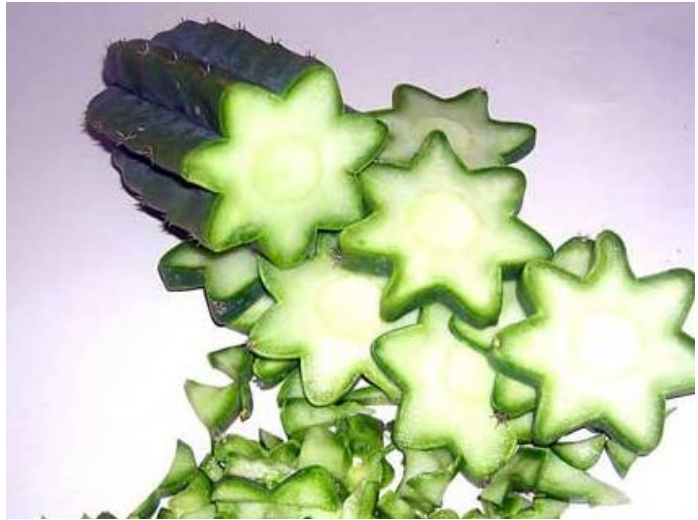
*Echinopsis* species<sup>1</sup> belong to the Cactaceae (cactus) family. *Echinopsis pachanoi* Britton et Rose, also known as the San Pedro cactus, *huachuma* or *aguacolla* is native to the highlands of Peru, but is cultivated in other parts of the Andes, including the coastal regions. It thrives in both dry conditions and moist zones (Rätsch 2005: 505). The San Pedro cactus can reach over five meters in height, and typically has six to eight ribs with small spines. Andean peoples believe that the four-ribbed cactus is especially powerful, as the four ribs represent the four cardinal

<sup>1</sup>Previously *Echinopsis* was known as *Trichocereus*. In botanical literature the term *Echinopsis* is now used, however, in archaeological literature San Pedro is still referred to as *Trichocereus*.

directions (Sharon 1978: 41) however these are rare, and some authors suggest that they have never been observed (e.g. Cordy-Collins 1982).

### **2.5.2. Preparation**

The preparation of San Pedro and other columnar cacti varies according to region. Generally fresh cactus stalks are horizontally sliced into star-shaped piece (see fig. 2.6). These pieces are then boiled in an ample amount of water.



**Fig. 2.6** – Sliced San Pedro cactus (Allen 2005).

Sometimes other plants, such as *Brugmansia* and *Datura* are added to modify the psychoactive properties of the decoction (Schultes 1972). The addition of such plants depends on the preferences of the *curandero* – for instance the Peruvian *curandero* Eduardo Calderón Palomino, who worked extensively with the ethnographer Douglas Sharon, prefers not to add these plants as he feels that they have negative effects on his patients (Sharon 1972: 120; 1978: 40). When other plants are added the drink is known as *cimora* (Schultes *et al.* 2001: 168). After several hours boiling, the juice is poured off and boiled again until around half of the original volume remains (Rätsch 2005: 506). Typically a 25 cm section of San Pedro per person is prepared. In modern preparations lemon or lime juice is occasionally added to facilitate the dissolution of mescaline (Rätsch 2005: 506).

### **2.5.3. Cultural significance**

San Pedro decoctions and *cimora* play a key role in *mesa* rituals by shamans or *curanderos*. The ritual helps the *curandero* diagnose and cure illnesses in his patients. The combination of Christian symbolism and Hispanic cultural traits inherent in San Pedro rituals led some researchers to conclude that these ceremonies were a product of Colonial Peruvian society. However, archaeological discoveries, in particular the depictions of cacti at Chavín de Huántar, has indicated that the ritual use of San Pedro may extend far back into antiquity (Sharon and Donnan 1977; Sharon 2001; Rick 2006), although practices have changed and evolved over time. Like *ayahuasca*, San Pedro and *cimora*-based cures are generally sought out by the urban and rural poor, where other forms of healthcare are unavailable (Krishna *et al.* 2006).

In a traditional healing ceremony the patient and the *curandero* will drink *cimora* or a decoction of San Pedro. During the course of an all-night ceremony involving a *mesa* composed of symbolic and powerful objects, the healer will divine a cause of the illness and prescribe herbs to cure the illness. The symbolism of *mesa* objects is highly complex. The number and type of object varies between each healer, but typical objects include rods, staffs, weapons (particularly swords), pre-Hispanic pottery, crucifixes, shells, rattles, stones, seeds, quartz crystals, top of a San Pedro cactus, tobacco, bells, white flowers, corn flour, sugar (both must be white), spring water, wine, perfume and fruits laid out on a cloth (Sharon 1978: 59; Skillman 1990; Polia 2006).

Traditional beliefs based on pre-Columbian theories fused with western European ideas surrounding the aetiology of diseases are essentially supernatural in nature. A major category of illnesses are known as *mal aires* (Dobkin de Rios 1968). They are thought to be caused by vapours from ancient tombs and ruins. A variety of maladies are associated with *mal aires*, including pneumonia and gastrointestinal problems. General *mal aire* symptoms include nausea, vomiting, headaches, facial tics and chills (Dobkin de Rios 1968; Kail 2008: 117). These illnesses can be treated by a trained *curandero*. *Curanderos* are also involved in solving other problems for their clients, including the use of San Pedro and *mesa* ceremonies for love spells and the solving of money troubles (Millones and León-Llerena 2006: 55).

## 2.6. Erythroxylum

### 2.6.1. Habitat and distribution

*Erythroxylum* species (see fig. 2.7) are native to South America and have been a staple economic crop for thousands of years. As yet it is unclear when domestication occurred, however, recent archaeological discoveries suggest that *Erythroxylum* was domesticated and being used by people in the Nanchoc Valley, on



**Fig. 2.7** – Coca leaves (Cariboni and Chávez 2007)

the northern coast of Peru at least 8,000 years ago, due to the discovery of coca leaves and lime in secure contexts (Dillehay *et al.* 2010).

There are two species of *Erythroxylum* that contain significant amounts of the tropane alkaloid cocaine: *Erythroxylum coca* and *E. novogranatense*. Each species has two distinct subspecies (see table 2.5).

In addition to cocaine, coca leaves also contain other alkaloids, some of which are pharmacologically active. Primarily these compounds are tropanes, pyrrolidines and pyridines, including cinnamoylcocaine, benzoylecgonine (BZE), methylecgonine, pseudotropine, benzoyltropine, tropacocaine,  $\alpha$ - and  $\beta$ -truxilline, hygrine, cuscohygrine and nicotine (Novák *et al.* 1984).

### **2.6.2. Preparation**

Traditional Andean practices such as chewing coca leaves and drinking coca tea provide the consumer with tangible amounts of cocaine, yet the effects experienced are vastly different from users of cocaine, the freebase of cocaine and crack cocaine.

Cocaine hydrochloride is produced by a series of relatively unsophisticated solvent extractions. The process involves three steps: 1). The extraction of crude coca paste from the coca leaf; 2). The purification of coca paste to cocaine base and 3). The conversion of cocaine base to cocaine hydrochloride (Casale and Klein 1993).

The process uses a number of harmful chemicals including kerosene, petroleum, various acids, ammonia and potassium permanganate. The by-products of illicit



cocaine production are having severe environmental consequences in the Amazon areas of Peru, Bolivia and Colombia (Dourojeanni 1992; Henkel 1995).

The freebase form of cocaine is produced by dissolving cocaine hydrochloride in water, then adding a weak base, such as ammonia, which deprotonates the cocaine. This decreases its solubility in water. An organic solvent, such as diethyl ether, is then added, into which the cocaine dissolves. The solvent is then evaporated off, leaving freebase cocaine (Vearrier *et al.* 2010). The production of crack cocaine is similar, except the addition of the organic phase is left out. Usually baking soda is used as a base.

**Table 2.5** – Varieties and distributions of *Erythroxylum* species with significant cocaine content in South America (Plowman 1986b).

Species	Variety	Vernacular name	Distribution
<i>Erythroxylum coca</i>	var. <i>coca</i> (Lamarck)	Huanuco/Bolivian coca	Humid mountain regions; Ecuador to Bolivia
	var. <i>ipadú</i> (Plowman)	Amazonian coca	Tropical lowlands, Amazonia
<i>Erythroxylum novogranatense</i>	var. <i>novogranatense</i> (Morris)	Columbian coca	Dry/hot regions of northern South America
	var. <i>truxillense</i> (Rusby) Plowman	Trujillo coca	Coastal zone of n. Peru

The residual water in crack cocaine produces a crackling noise once heated, giving “crack” its name (Vearrier *et al.* 2010). Both freebase and crack are not suitable for

inhaling or injecting, therefore they are smoked, producing a rapid and intense “high”.

### **2.6.3. Cultural significance**

Coca chewing is a uniquely Andean practice that is integral to both Quechua and Aymara identity. For Quechua people, chewing coca is an unequivocal statement of cultural loyalty that identifies the chewer as *Runa* (Quechua person). Catherine Allen (1981) documented the meaning and significance of coca in a Quechua speaking community in the Province of Paucartambo, Department of Cuzco in central Peru. She found that the act of chewing coca (*hallpay*) is highly ritualised and governed by etiquette involving “elaborate greetings, thank-yous, farewells, and expressions of mutual esteem” (Allen 1981: 159). Only the best leaves are chosen to be chewed. Usually three are picked, and formed into a small pile (*k'intu*). The chewer then blows on the leaves and invokes various spirits and says a prayer before putting the *k'intu* into their mouth. Lime is then added to the quid using a small spoon. The lime helps to extract the alkaloids from the leaves.

Perhaps the most important aspect of *hallpay* is the sharing of coca. The chewer makes a *k'intu* to share with someone else. The same rituals and etiquette are observed. Catherine Allen argues that the adherence to these cultural practices reinforces the chewer’s Quechua identity and helps form and maintain social bonds. In turn this is related to the concept of community and the *ayllu*, an Andean concept of community, rooted in a sense of common origin in, and orientation toward, certain sacred places (Allen 1981). The standardised ritual behaviour

involved in coca chewing “orients the actors spatially, socially, and religiously, and in so doing integrates them into a larger cultural framework” (Allen 1981: 157; 2002). Indeed, to decline an offer of coca is seen as a rejection of an invitation to socialise (Allen 1981; Grisaffi 2010). Coca is also involved in other social and community events, such as initiation into adulthood, marriage, death rites, the dedication of new buildings and the negotiation and sealing of contracts (Carter 1968; Carter *et al.* 1980; Allen 1981; Weil 1995).

As well as being part of everyday life, coca is also involved in divination, sacrifice and healing rituals. Coca is regarded as sacred (there are many creation myths regarding coca) and as a result coca is an important part of ritual offerings and sacrifice, particularly to Pachamama, the female earth spirit, who is often portrayed as the Virgin Mary in the syncretic belief systems of the Andes (Damien 2007: 76). The leaves may be burned or offered as they are (Wiedemann 1979; Rätsch 2005: 249). *Curanderos* may also use coca as part of divination rituals to help diagnose the causes of illness in their patients (Martin 1970). As well as playing a role in diagnosing problems, coca is also used as a medicinal treatment to treat digestive ailments, altitude sickness and mouth ulcers (cocaine acts as a topical anaesthetic) amongst other medical problems (Weil 1978).

Whilst the focus of ethnographic work on coca chewing has been undertaken in Quechua and Aymara communities it should be acknowledged that coca use is more widespread than these groups. *Erythroxylum coca* var. *ipadú* (Plowman) is cultivated in the Amazon regions of Peru, Columbia and Brazil (Schultes 1957; Plowman 1981; Schultes 1981; Plowman 1986a).

It has been suggested that the chewing of coca has helped Andean people to cope with life at altitude (Hanna 1974; Bolton 1976; Weil 1995). Some physiological studies and literature based studies support this idea (Hanna and Hornick 1977; Fuchs 1978; Favier *et al.* 1996; Spielvogel *et al.* 1996) although other authors suggest that coca chewing is a cultural practice that has no effect on aiding life at altitude (Carter *et al.* 1980) or that coca chewing was widespread before the colonial period, so it's health benefits are not altitude specific (Bray and Dollery 1983).

## 2.7. Espingo and Ulluchu

Some putative psychoactive plants in Andean archaeology have yet to be conclusively identified by botanists or lack phytochemical investigation.

Two examples are *ulluchu* (see fig. 2.8) and *espingo*.

Rafael Larco Hoyle first noted *ulluchu* on Moche (AD 100-800) ceramics in his 1938 publication "*Los Mochicas*".



**Fig. 2.8** – Top: A Moche fine line drawing of a figure holding a goblet containing *Ulluchus*. Bottom: An *Ulluchu* tree (McClelland 2003: 51, 54)

He presented the name *ulluchu* without any linguistic explanation, so it is unclear where this name originates (Wassén 1989). This comma-shaped fruit is usually

depicted floating mid-air, in association with messengers, runners and intoxicated priests (Bussmann and Sharon 2009). Larco Hoyle suggested that *ulluchu* was a *Phaseolus* (bean) species



fig. 2.9. *Carica candicans* tree and fruits (see also [http://www.ancient-civilizations.com/ancient-civilizations/ancient-civilizations-2011-2019/](#))

(McClelland 2003). This was refuted by McClelland on the grounds that *Phaseolus* leaves do not resemble the leaves of the *ulluchu* as depicted on Moche ceramics. She also eliminated various *Solanum* (pepino), *Lagenaria* (gourd), *Persea* (avocado) and *Capsicum* (pepper) species for the same reasons (McClelland 1977; McClelland 2003: 43). It has been suggested that *ulluchus* are *Carica candicans* (see fig. 2.9), a wild variety of papaya (Hultin *et al.* 1987). The authors suggest that the unripe papaya, containing the anti-coagulant papain, was used by the Moche priesthood during blood sacrifice ceremonies to keep blood flowing freely. McClelland (2003) disputes this tentative identification, as papaya belong to a group of plants known as cauliflory (“stem-flowery”), in which flowers and fruit grow on the trunk of the tree. *Ulluchus* are depicted hanging from the limbs of the tree. She also noted differences between the depiction of *ulluchu* leaves and the leaves of *C. candicans*, “Papaya leaves do not resemble *ulluchu* leaves, which are triangular, ovoid, or boomerang shapes hanging from limbs. Each large palmate papaya leaf grows on a stem from the top of the tree” (McClelland 2003: 58). Rainier Bussman and Douglas Sharon (2009) also doubt that *ulluchus* are *C. candicans*, however, they note that *C. candicans* is one of the few papayas that are not cauliflorous, that have triangular leaves with entire margins. *C. candicans* fruit

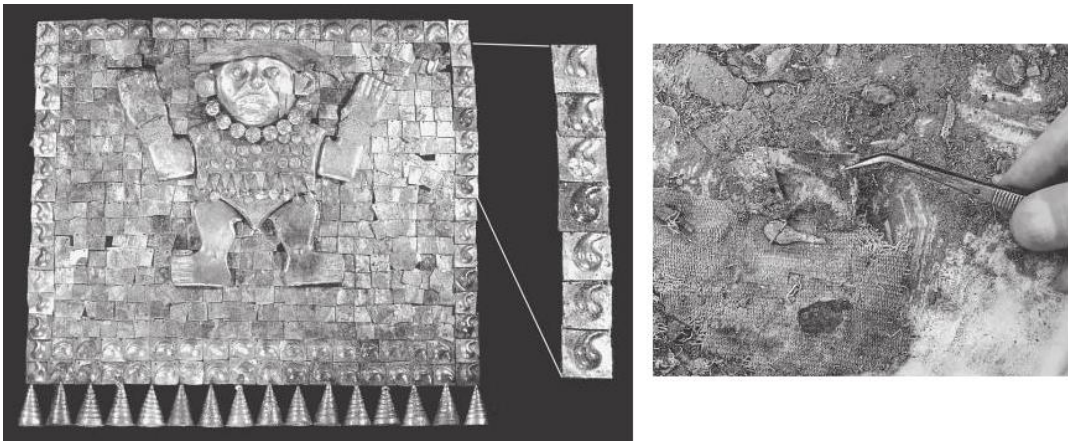
does hang from branches. Based on this alone, the authors suggest that *ulluchu* could be *C. candicans*<sup>2</sup>.

Bussman and Sharon (2009) suggest that *ulluchu* belongs to the genus *Guarea*, based on the similarity of morphological characteristics. They also suggest that when ground and inhaled it may act as a hallucinogen, but there is no phytochemical evidence to support this idea.

Archaeological discoveries have proved that *ulluchu* is a real fruit. However, it is much smaller than depicted in Moche fine-line drawings. Real *ulluchus* have been found in élite burial contexts at Sípan (c AD 100) and Dos Cabezas (AD 150 – 500). At Sípan, *ulluchus* were placed under gold platelets embossed with *ulluchus* that formed the border of a banner (see fig. 2.10). When the platelets were lifted, real fruits were found underneath the hollows (Alva and Donnan 1994: 189). The fruits were extremely fragile and collapsed. At Dos Cabezas, a pile of *ulluchus* were found in the burial of an élite male. The burial dated to c AD 400. In the same pyramid complex, a pile of *ulluchus* were found on a dais (McClelland 2003).

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<sup>2</sup> *C. candicans* has been reclassified under the genus *Vasconcellea*. It is adapted to the highland wet forests in Ecuador and Peru



**Fig. 2.10.** – Left: The banner with embossed *ulluchus* from Sipan (Alva and Donnan 1993: 66). In addition to the discovery of real *ulluchus* in elite Moche burials, beads shaped as *ulluchus* underneath the embossed platelet (McClelland 2003: 60).

*ulluchus* have also been found. Again, these were found in high status burials at San José de Moro (c AD 400-850) and Huaca de la Luna (AD 200-700) (McClelland 2003). The *ulluchu* appears to have been an important part of Moche iconography. It has only been identified in Moche contexts, from the sites discussed above, on the north of Peru (McClelland 2003: 58). *Ulluchu* is also associated in Moche art with another plant thought to have psychoactive properties: *espingo*.

Donna McClelland (2003: 57-58) presented two examples of scenes with copulating couples in which *ulluchus* and *espingo* fruits are depicted. In one scene the male is holding an *ulluchu*, whilst additional *ulluchus* and *espingo* fruits float in the background (see fig. 2.11). The other scene, from a low-relief spout and handle bottle shows an *ulluchu* tree growing from the couple (see fig. 2.11). McClelland suggests the other tree, on the right, is an *espingo* tree. She notes that the *espingo* is the only plant associated with the *ulluchu* in Moche art (McClelland 2003: 58). The complex iconographic scenes are most probably linked to ideas and beliefs regarding fertility and human blood (Donnan 1978: 158-167; Bourget 2006: 169).



**Fig. 2.11** – Top: low-relief scene from a Moche spout and handle bottle and bottom: erotic scene with *ulluchus* and *espingos* (McClelland 2003)

The Spanish chroniclers who wrote about the Inca starting in the 16<sup>th</sup> century recorded the use of *espingo*. In Pablo José Arriaga’s *Extirpation of Idolatries in Peru* (1621), he describes *espingo* as “a little, dry fruit...with a very intense smell...one gets it from the Chachapoyas”. He also noted that it was very highly prized amongst the native people. According to Arriaga, the archbishop of Jaen de Bracamoros forbade the selling of *espingo*, on pain of excommunication, as these seeds were precious offerings to *huacas* (Arriaga 1968 [1621]; Wassén 1973). Arriaga states that those who used *espingo* were “sorcerers”. He states that the powder of this plant was added to beer and the effect of imbibing this beverage drove the drinker “crazy”.



The Jesuit priest Bernabé Cobo also mentions *espingo* in his *History of the New World* (1653), but states its use as medicinal, whilst Antonio Ricardo's Quechua vocabulary (1586) lists *espingo* as a fragrant fruit that was used for various "bewitchments" (Wassén 1973).

S. Henry Wassén reviewed what was known about *espingo* in a review published in 1973. He investigated the idea that *Quararibea* species were *espingo*. An alkaloid test proved negative (Wassén 1973). Prior to Wassén's work, Margaret Towle (1961: 40) noted that Yacofleff and Herrera (1935) identified the seeds depicted on Moche pottery as *Nectandra pichurium*. She noticed that they were strung on a cord (e.g. fig. 2.12) and were very similar to other specimens reported from other archaeological sites in Peru, although she does not specify any in particular (Towle



**Fig. 2.12** – Left: Moche anthropomorphised owl holding a string of *espingos*. Right: a string of *espingos* (McClelland 2003: 57).

1961: 40). Towle also reports other *Nectandra* species from archaeological sites: Wittmack (1888: 327, cited in Towle 1961: 40) reports four pierced cotyledons from

the Acland collection found with a mummified child buried at Ancón in Peru. Wittmack suggests these may be *Nectandra mollis* seeds. A necklace of nine pairs of pierced cotyledons was found at Pachacamac and a similar one found at Chuquitanta was described by Harms (1922: 168, cited in Towle 1961: 40). *Nectandra* seeds were also found on a string in a funerary context in the Chancay valley. Towle notes that this specimen was in the collections of the Botanical Museum at Harvard University (Towle 1961: 40). Peter Eeckhout (2006) reviewed the use of *espingo* at Pachacamac. He states that these seeds are frequently encountered in Late Period archaeological contexts (10<sup>th</sup> century and after) on the north coast of Peru. He suggests that *espingo* probably had a medicinal function, but at Pachacamac these seeds had a role in funerary rites and were often included in burials. Eeckhout also provided a list of examples of *Nectandra* species from archaeological contexts from coastal sites in Peru (see table 2.6).

**Table 2.6** – Examples of *Nectandra* seeds found in archaeological contexts on the Peruvian coast (after Eeckhout 2006).

Culture	Site	Context and association	Reference
Sicán-Lambayeque	Dos Cabezas	Disturbed burials	Montoya (1998: 10)
Chimú	Pacatnamu	Metals, <i>Spondylus</i> shell	Narváez (1995: 103-112)
Chimú	Pacatnamu	Infant burial; two bead necklaces	Hecker and Hecker (1991: 399, 489)
Chimú	Túcume – Templo de la Piedra Sagrada (phases 2-4)	Disturbed burials of adolescents	Narváez 1995 :103-12
Chimú	Chan Chan – Tschudi	Secondary burial	Montoya

			(1998: 9)
Chimú	Chan Chan – Calvario de los Incas	Buried with infants & camelids, <i>Spondylus</i> & <i>Conus</i> shells	Pozorski (1979) Bourget (1997: 113)
Chimú	Chan Chan – SIAR	Camelids & other offerings: feathers, ceramics, textiles and plants material	Topic (1982: 159-160)
Chimú	Huaca del Dragón	Infant burials	Schaedel (1966)
Chimú	Moche – Cerro Blanco	Infants, adolescents and adult females – textiles, feathers, juvenile camelid bones, <i>Spondylus</i>	Bourget (1997: 112-113)
Chimú	Moche – Huaca de la Luna – Platform 1	Disturbed burial of an adolescent	Habetler (1998)
Chimú	Moche – Huaca de la Luna – Platform 1	Adolescents; 19 packets of <i>Nectandra</i> and <i>Ormosia coccinea</i>	Tello (1997: 33) Uceda (1997: 152)
Chimú	Moche – Huaca de la Luna – upper platform	Disturbed burials	Montoya (1997: 23)
Chancay	Ancón	Mummy bundles ( <i>fardos</i> )	Kaulicke (1997)
Ichma	Maranga	Mummy bundles ( <i>fardos</i> )	Jijón y Caamaño (1949: 113, 117, 458)
Ichma (?)	Pachacamac	Feathers, metal, textiles	Baessler (1902-1903: 474)
Inca	Túcume – Huaca Larga	Élite male burial	Narváez (1995: 96)
Inca	Túcume – Templo de Pedro Sagrada (phase 5)	Offerings – metal, <i>Spondylus</i>	Narváez (1995: 103-112)

In a review of plants used by the Moche during sacrificial rituals, Carod-Artal and Vázquez-Cabrera (2007a) came to the conclusion that *espingo* is most likely *Nectandra*. Donna McClelland, in her discussion of *ulluchu* and *espingo* also accepts the identification of *espingo* as *Nectandra* (McClelland 2003: 56). There has been

research that suggests that alkaloids in *Nectandra* may have anti-tumour, analgesic, anti-inflammatory, antimalarial and antileishmanial activity (Moreno *et al.* 1993; da Silva Filho *et al.* 2004; da Silva Filho *et al.* 2008). However, there is no report of any psychoactive alkaloids being present in *Nectandra* species.

## **2.8. Summary**

This chapter has dealt with the most well-known psychoactive plants in the Andes and Amazon regions of South America. There are possibly hundreds more species, but many are lacking a definite botanical classification and phytochemical analyses, for example the *ulluchu*, used by the Moche. Most authors now seem to agree that *espingo* is most likely a species of *Nectandra*, and whilst it may have medicinal uses it is unlikely to produce hallucinations or other alterations in states of consciousness.

The major psychoactive plants from the Andes and Amazon have been discussed in this chapter. *Anadenanthera* and *Virola* snuffs have a very wide distribution throughout the western half of South America. Both of these snuffs have been documented by ethnographers. Although customs vary between communities, generally snuffs are taken to contact the spirit world, as part of healing and other community cohesive purposes.

*Datura* and *Brugmansia* species are widely distributed throughout South America. These species are revered by local *curanderos* and sometimes added to *ayahuasca* and *cimora* to potentiate the psychoactive properties of these preparations.

*Brugmansia* and *Datura* are also used to treat a number of illnesses, including aches and pains, sores and cancers.

The San Pedro cactus (and other *Echinopsis* species) has some of the best evidence to suggest it has been used for a very long time on the Andes. It is still used today by *curanderos* to treat all kinds of illnesses and wider social problems in their communities. The symbolism of the *mesa* used by *curanderos* is a syncretic blend of ancient Andean tradition and Catholic symbolism.

Coca (*Erythroxylum* sp.) is probably the most recognised psychoactive plant, as it contains cocaine. The pharmacology of chewing coca leaves (unrefined product) is very different to the pharmacology of cocaine hydrochloride/freebase/crack (synthetic products), in that coca chewers do not suffer any of the same detrimental health effects or addiction that cocaine users do.

The chewing and sharing of coca leaves amongst Aymara and Quechua people is integral to their communal identity, yet international drug policy implemented by countries outside of South America would see the growing of coca banned and the species eradicated.

## Chapter 3: Chemistry and Pharmacology of alkaloids from South American psychoactive plants

### 3.1. Introduction

This chapter reviews the chemistry and pharmacology of alkaloids found in South American psychoactive plants described in the previous chapter. The chapter focusses on active compounds; that is specific chemicals that are responsible for the psychoactive effects experienced by users of these compounds.

Where information is available, the pharmacology, effective doses and user's experiences are discussed. For some compounds, such as cocaine, there is a vast amount of information available regarding the chemistry and pharmacology of this compound, as it is well known in Western societies as a drug of abuse and so has generated much interest from governments, scientists and sociologists. The difference between the refined, abused drugs cocaine hydrochloride, crack cocaine and cocaine freebase and traditional Andean coca chewing is discussed, as the pharmacokinetics of each of these substances is very different.

For the lesser known compounds, such as *N,N*-dimethyltryptamine and mescaline, there is less available information regarding the chemistry and pharmacology of these compounds. However, it should be noted that there is a considerable corpus of research on these compounds in the psychiatric literature dating from the 1940s to the 1970s, some of which are now seen as unethical in modern clinical frameworks, for example there were a number of studies in which psychiatric

patients were given controlled doses of mescaline (and other drugs such as LSD) to trigger psychotic episodes (Hoch 1951; Pennes 1954; Denber and Merlis 1955).

During the 1940s and 50s a number of authors noted the similarity between the symptoms of psychiatric illness and acute mescaline/*N,N*-DMT intoxication (Stockings 1940; Osmond and Smythies 1952; Gillin *et al.* 1976). The earlier research was prompted by a 1952 article in which the authors proposed that a problem with adrenaline metabolism resulted in the formation of substances that were very similar to mescaline (adrenaline and mescaline have similar chemical structures), which in turn caused the psychoses associated with schizophrenia (Osmond and Smythies 1952). Subsequent clinical research into mescaline-like compounds proved fruitless (Gillin and Wyatt 1976; Barker *et al.* 2012).

Research into urinary indoles proved more successful. In the mid-1950s there were reports of serotonin-like compounds such as *N,N*-DMT and bufotenine in urine of psychiatric patients but not healthy subjects (Bumpus and Page 1955; Rodnight 1956). Subsequently Julius Axelrod reported an enzyme capable of *N*-methylating indole-ethylamines (i.e. serotonin) and producing dimethyltryptamines (Axelrod 1961). Subsequent research into the biochemical causes of schizophrenia and other psychiatric illnesses focussed on the excretion of *N,N*-DMT and bufotenine in patients exhibiting either psychotic or manic symptoms. Some studies found that these patients excreted higher levels of *N,N*-DMT and bufotenine (Tanimukai *et al.* 1970; Rodnight *et al.* 1977; Murray *et al.* 1979), although in some cases the differences between the psychiatric patients and the control group were not statistically significant (e.g. Carpenter *et al.* 1975). Some studies did not detect any

dimethyltryptamines at all (Feldstein *et al.* 1961; Siegel 1965; Perry *et al.* 1966; Kakimoto *et al.* 1967) or reported DMTs in some patients and not others (Perry 1963; Fischer and Spatz 1967; Tanimukai 1967) or reported DMTs in all subjects, including control groups (Spatz *et al.* 1969; Fischer and Spatz 1970; Saavedra and Udabe 1970).

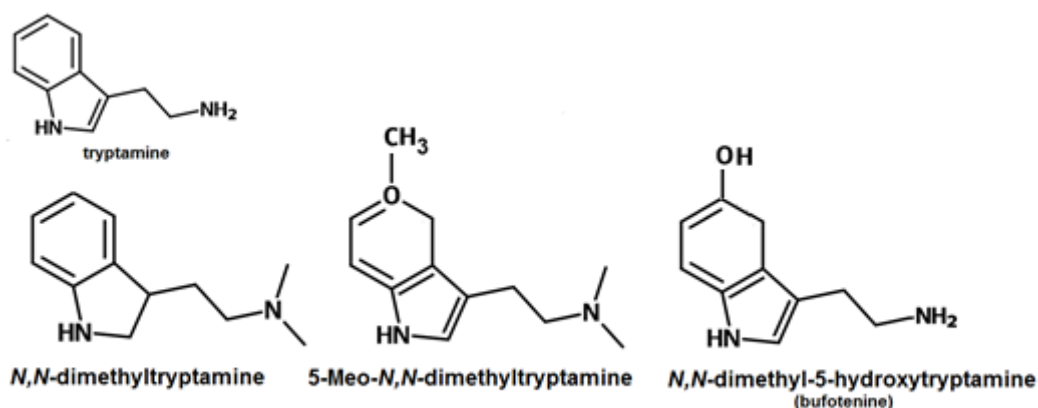
The inconclusive and somewhat contradictory and unethical nature of many of these studies has resulted in researchers looking into other factors that may cause psychiatric conditions, including biochemical processes and environmental factors (Reynolds 2005).

The legal status of each compound under United Kingdom and international law is discussed, as many of the compounds relevant to this thesis are controlled substances. It is necessary to understand how these substances are controlled, as in many cases a Home Office license is required to possess, store and undertake research using these compounds.

### **3.2. Tryptamine alkaloids**

Tryptamine is a naturally occurring compound found in both animals and plants. It is also an endogenous component of the human brain. Tryptamines are a class of compounds containing a tryptamine skeleton modified with additional functional groups (Shulgin and Shulgin 1997: v, see fig. 3.1). Examples of naturally occurring psychoactive tryptamines include *N,N*-dimethyltryptamine and structurally related compounds found in *Anadenanthera* and *Psychotria* species amongst others.





**Fig.3.1** – Tryptamine alkaloids found in psychoactive plants native to South America

### 3.2.1. Pharmacology of *Anadenanthera snuff*

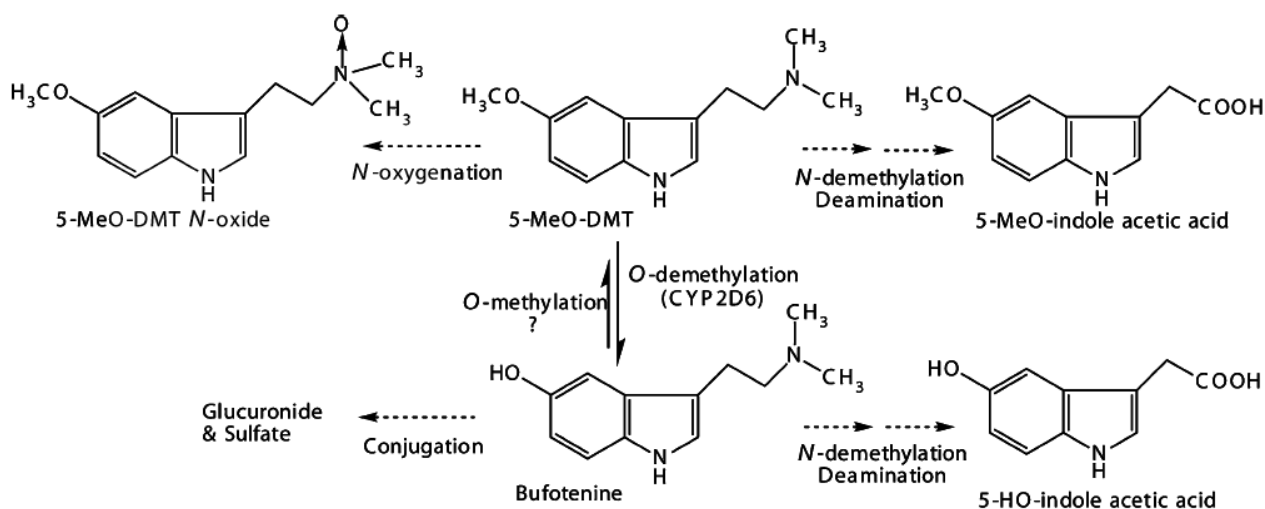
When ingested nasally, as little as 150 mg to 0.5g is an effective dose of *A. colubrina* snuff. Around 1g of powder is the approximate minimum dose for *A. peregrina* (Rätsch 2005: 52-55). The hallucinations triggered by *Anadenanthera* snuffs occur rapidly after ingestion and last around 20-30 minutes. They may take the form of black and white or coloured flowing “worm-like” images and a sensation of bodily heaviness (Schultes *et al.* 2001: 122).

Studies based on animal models have shown that the psychoactive effects produced by tryptamines and other natural alkaloid classes such as phenethylamines (e.g. mescaline, see s.3.3) and ergolines (e.g. Lysergic acid diethylamide, LSD) are a result of the interaction between these compounds and the serotonin receptors in the brain (Nichols 2004; Galanter and Kleber 2008: 183).

The major alkaloid in *Anadenanthera* snuff, *N,N*-dimethyltryptamine, is rapidly taken into and cleared from body tissues (Sitaram *et al.* 1987). The metabolism of

*N,N*-DMT by various *in vivo* pathways include oxidative deamination, *N*-demethylation, *O*-demethylation, and *N*-oxygenation (see fig. 3.2.).

The major metabolite of *N,N*-DMT is 3-indole acetic acid (3-IAA), as well as other minor metabolites such as *N*-methyltryptamine and tryptamine (Szára 1956; Barker *et al.* 1980; Sitaram *et al.* 1987). The same pathways are true for other tryptamines. 5-MeO-DMT is broken down into the pharmacologically active compound bufotenine by *O*-demethylation. *N*-demethylation produces 5-MeO-indole acetic acid, whilst *O*-oxygenation produces the *N*-oxide version of the compound. Bufotenine is broken down by *N*-demethylation into 5-HO-indoleacetic acid (Yu 2008).



**Fig. 3.2** - Deamination of bufotenine and 5-MeO-DMT produces corresponding indole acetic acid derivatives. 5-MeO-DMT can be biotransformed to bufotenine through *O*-demethylation, whereas bufotenine may be methylated to form 5-MeO-DMT (Yu 2008).

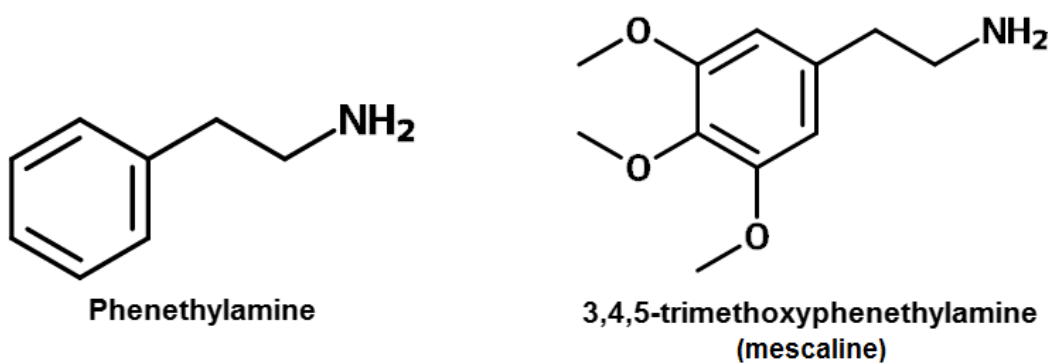
### 3.2.2. Legal status: *N,N*-DMT and related tryptamines

*N,N*-DMT is a schedule 1 drug under the 1971 United Nations Convention on Psychotropic Substances, but the plants that contain DMT are not controlled under

the convention and as such are not subject to international control (Schaepe 2001). Despite this, the *N,N*-DMT containing decoction *ayahuasca* has a somewhat ambiguous legal status, particularly in the United States. However, in 2009 a U.S. District Court Judge found that the Religious Freedom Restoration Act (RFRA) protects the Brazilian-based Santo Daime's use of DMT-containing *ayahuasca* as part of their sincere religious practices (see Church of the Holy Light of the Queen v Muckasey (2009)). Similarly, another Brazilian religious group, the União do Vegetal, won a similar ruling on their use of *ayahuasca* as part of their religious practices (O Centro Espírita Beneficente União do Vegetal v Holder (2010)).

### 3.3. Phenethylamines

Like tryptamines, phenethylamines are naturally occurring compounds found in both animals and plants. Phenethylamines are a series of compounds containing the basic phenethylamine structure modified by additional functional groups (Shulgin and Shulgin 1991: v, see fig 3.3). Mescaline (3,4,5-trimethoxyphenethylamine, see fig. 2.2.) is an example of a naturally occurring phenethylamine. Mescaline has also



**Fig. 3.3** – Phenethylamine and mescaline

been detected in *Opuntia*, *Pereskia*, *Polaskia*, *Pelecypbroa*, *Stenocereus* and *Stenonia* cactus species, but is most well known as being the active alkaloid in *Lophophora williamsii* (Lemaire) Coulter, better known as peyote (Shonle 1925; Kapadia and Fayez 1970; Ma *et al.* 1986; Bruhn *et al.* 2008; Ogunbodede *et al.* 2010).

### **3.3.1. Pharmacology of Mescaline**

The active compound in *Echinopsis pachanoi* is mescaline. Mescaline belongs to the  $\beta$ -phenethylamine family of alkaloids. In addition to *E. Pachanoi*, there are at least 12 other *Echinopsis* species that contain mescaline. Of these, only *E. bridgesii* and *E. peruvianus* (Peruvian Torch cactus) are known to have been used for their psychoactive properties (Rätsch 2005: 847). Mescaline is known to produce a variety of effects after ingestion. Alexander Shulgin, the US-based pharmacologist famous for his self-experimentation with phenethylamines described his experiences with mescaline at various doses (Shulgin and Shulgin 1991). At lower levels he noted deep feelings of empathy and the intensification of colour, as well as hysteria. At higher levels (350 mg) he noted some nausea, as well as the intensification of colour as well as visual hallucinations (Shulgin and Shulgin 1991: 704-705). Other authors describe similar experiences – for instance Aldous Huxley in “*The doors of perception*” describes the intensification of colour of flowers in his garden after taking 400 mg mescaline (Huxley [1954] 2004: 6-7), whilst Arthur Hefter, the scientist responsible for discovering that mescaline was the active compound in peyote (*Lophophora williamsii* (Lemaire ex Salm-Dyck Coulter)) by self-

experimentation experienced minor visual hallucinations, in the form of coloured spots, as well as limb heaviness, nausea and headache (Hefter 1897).

Mescaline is largely excreted as unchanged drug (50-60%) or as the oxidatively deaminated metabolite 3,4,5 trimethoxyphenylacetic acid (27-30%) (Daly *et al.* 1962; Charalampous *et al.* 1966). Other authors have reported various minor metabolites such as *N*-acetyl- $\beta$ -(3,4-dimethoxy-5-hydroxyphenyl)ethylamine and *N*-acetyl-mescaline (e.g. Harley-Mason *et al.* 1958).

### **3.3.2. Legal status: Mescaline and mescaline-containing cacti**

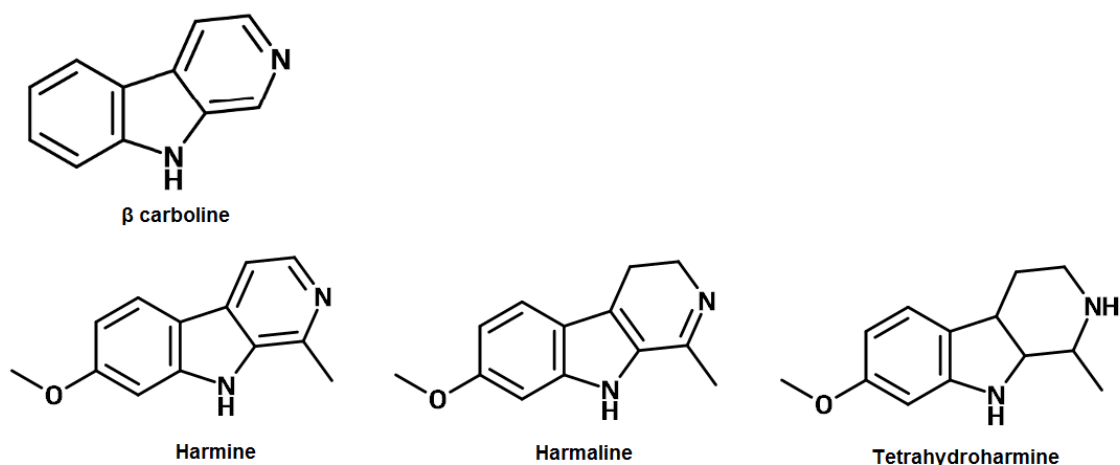
Mescaline is a Class A drug in the United Kingdom under the Misuse of Drugs Act 1971. It also falls under schedule 1, meaning it has no recognised medical use and is thought to be harmful if ingested. It is also a schedule 1 controlled substance under the 1971 UN Convention on Psychotropic Substances. Whilst mescaline is a controlled substance in most western countries, cacti that contain mescaline are not controlled and can be grown as ornamental cacti.

There has been some legal issues in recent years as to whether dried *E. pachanoi* and *E. peruvianus* are “preparations” containing mescaline, a controlled substance, and therefore illegal. In 2006 Saul Sette was arrested after being found in possession of 4.69 kg of dried *Echinopsis peruviana*. He was charged with possessing a Class A drug (mescaline) with intent to supply and possessing prohibited plant specimens (Wood 2007). The Court found that possessing the cacti was not illegal *per se*; yet the prosecution contended that the dried cactus constituted a “preparation or other product” of mescaline and was therefore illegal. In this case

both the Crown and Defence reviewed previous cases in which arguments were made in regard to the meaning of “preparation” and “product” of *Psilocybe* mushrooms. Three cases were cited, and in all the cases were stayed due to lack of legal certainty. The Defendant in the case, Mr. Sette, had letters from Richard Mullins who worked for the Home Office Drug Legislation and Enforcement Unit and from Chris Edwards at the Direct Communications Unit of the Home Office regarding dried mescaline-containing cacti. Both letters suggested that dried cactus specimens were not illegal. Mr Mullins concluded his letter thus: "In itself, drying in order to preserve for purely botanical/horticultural/herbarium purposes- “mere preservation”- does not in law amount to preparation for the production of a controlled drug. Any move beyond “mere preservation” to prepare the cactus/plant/fungus for the unauthorised production of any controlled drug they contain to be considered unlawful under the Misuse of Drugs Act 1971." (Wood 2007). Chris Edwards’ letter from the Direct Communications Unit of the Home Office advised that the cacti are not illegal in their natural state, but concurred that there was a grey area in regards to dried specimens (Wood 2007). The case was rejected in March 2007 before it went to trial. The Judge agreed with the Defence in that there was a lack of legal clarity and that to proceed would be an “abuse of process” (Wood 2007). No species of cacti are mentioned in the Misuse of Drugs Act 1971.

### 3.4. $\beta$ -Carboline alkaloids

Beta-carboline alkaloids (see fig. 3.4) are compounds containing a  $\beta$ -carboline skeleton modified by additional functional groups. Harmine, harmaline and tetrahydroharmine are all  $\beta$ -carboline alkaloids that act as monoamine oxidase inhibitors (MAOIs) (McKenna *et al.* 1984; Edmondson *et al.* 2004). Monoamine oxidases (MAOs) catalyse the breakdown of monoamines by oxidative catabolism (Edmondson *et al.* 2004; Herraiz and Chaparro 2005). Without MAOIs, *ayahuasca* (see section 2.5) would not be orally active, as the MAOs would break down the active compound, *N,N*-DMT in the gastrointestinal tract (Riba *et al.* 2003).



**Fig. 3.4** –  $\beta$  carboline alkaloids and MAOIs: harmine, harmaline and tetrahydroharmine

#### 3.4.1. Pharmacology of *ayahuasca*

Due to the variety of methods and admixture plants used in the preparation of *ayahuasca* the effective dose has not been clearly defined. Table 3.1 summarises the alkaloid levels in various preparations of *ayahuasca*.

The effects of *ayahuasca* vary according to how it is prepared. Admixture plants will alter the effects experienced by the person drinking the decoction. Typical effects

include nausea, dizziness, vomiting, sensations of heaviness, numbness and tingling, euphoria, aggression and vivid hallucinations (Rivier and Lindgren 1972; Schultes *et al.* 2001: 126; Yritia *et al.* 2002; Riba *et al.* 2003). Visions of jaguars and snakes are very common (Schultes *et al.* 2001 126). Both animals have symbolic significance in the Andes. The jaguar in particular is associated with altered states of consciousness and shamanism (Cordy-Collins 1998, see s6.3), whilst the snake appears in many different contexts. The snake has been revered as the earthly embodiment of the creator, Viracocha, by the Inca. The snake was also associated with fertility, agriculture and rebellion, as well as a symbol of wisdom. When the snake appears as *amaru*, a rainbow, the snake metaphor ties the earth to the sky and the underworld (Bolin 1998: 208).

**Table 3.1.** – Alkaloid content of various *ayahuasca* preparations as recorded in peer-reviewed publications

Ayahuasca sample			Alkaloid content mg/ml				Ref
Plant	Origin	<i>n</i>	Harmine	THH	Harmaline	<i>N,N</i> -DMT	
<i>B. caapi</i> <i>P. viridis</i>	Sharanahua, Marcos, Peru	1	0.18	0.10	0.01	0.01	Rivier & Lindgren (1972)
<i>B. caapi</i> <i>P. viridis</i>	Sharanahua, Marcos, Peru	1	0.19	0.07	0.02	0.16	Rivier & Lindgren (1972)
Not stated	Sharanahua, Marcos, Peru	1	0.07	0.03	0.03	0.14	Rivier & Lindgren (1972)
TBC	União Vegetal	do 1	1.70	1.07	0.20	0.24	Callaway <i>et al.</i> (1996)

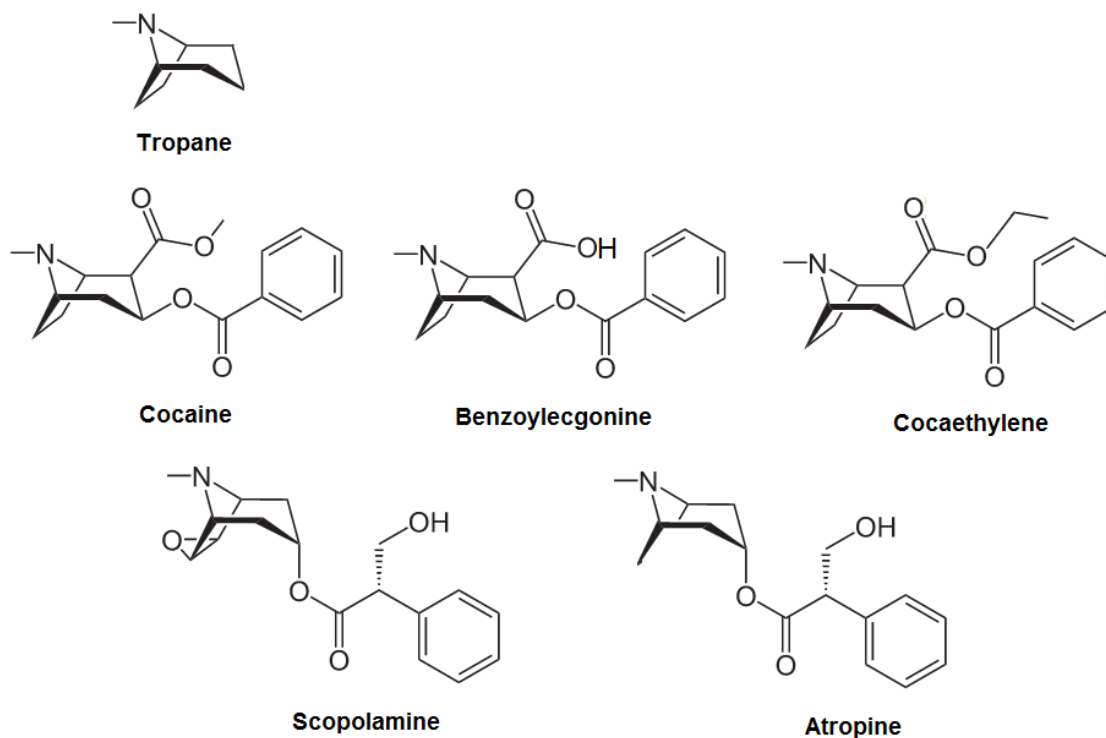


<i>B. caapi</i>	Barquinha	1	5.58	5.42	0.09	12.67	Callaway <i>et al.</i> (2005)
<i>P. viridis</i>							
<i>B. caapi</i>	Santo Daime	3	1.98 – 9.80* (6.84) †	1.68 – 12.79 (7.71)	nd	2.49- 14.15 (6.60)	Callaway <i>et al.</i> (2005)
<i>P. viridis</i>							
<i>B. caapi</i>	Shuar, Ecuador	5	1.80 – 22.85 (14.48)	1.63 – 23.80 (14.32)	nd – 0.66 (0.38)	nd – 4.12 (1.14)	Callaway <i>et al.</i> (2005)
<i>P. viridis</i>							
<i>B. caapi</i>	União do Vegetal	20	0.45 – 6.25 (4.95)	0.45 – 5.26 (4.71)	nd – 0.90 (0.23)	nd – 5.84 (1.12)	Callaway <i>et al.</i> (2005)
<i>P. viridis</i>							
“Brazilian Daime”	Not stated	1	0.53	0.90	0.06	0.72	Riba <i>et al.</i> (2003)

\*Range; † mean (in parentheses)

### 3.5. Tropane alkaloids

Tropane alkaloids (see fig. 3.5) are compounds containing a tropane skeleton with additional functional groups. Tropane alkaloids are found in a wide variety of plant species in South America. Examples of tropane alkaloids of interest for this research project include cocaine, atropine and scopolamine. Scopolamine and atropine are found in various *Brugmansia* and *Datura* species, whilst cocaine is found in *Erythroxylum* species.



**Fig. 3.5** – Tropane alkaloids cocaine, BZE, cocaethylene (formed *in vivo*), scopolamine and atropine

### 3.5.1. Pharmacology of *Brugmansia* and *Datura*

*Brugmansia* and *Datura* species contain a complex mixture of tropane alkaloids, including scopolamine, atropine and the L-isomer of atropine, hyoscyamine, as well as derivatives of these compounds (Griffin and Lin 2000; Rättsch 2005: 94-111). Atropine and scopolamine have recognised medical uses in modern medicine. Atropine is used as a mydriatic (causing pupil dilation) for ophthalmic and optometric treatment and is listed in the World Health Organization’s “Essential Drugs List” (WHO 2010). A therapeutic dose is considered to be around 1 mg. It is possible that 10 mg may be lethal to babies and children, but not adults (Rättsch

2005: 814). Data obtained from animal models (rats) suggests an LD<sub>50</sub><sup>3</sup> value of 500 mg kg<sup>-1</sup> (Sigma-Aldrich 2010). After ingesting atropine one may experience a range of effects, such as agitation, confused speech, hallucinations, spasms, delirium, hot flushes, euphoria and long lasting pupil dilation (mydriasis), as well as irregular heart beat (Rätsch 2005: 814). Atropine is readily absorbed after oral or parenteral administration from the gastrointestinal tract. Metabolism occurs in the liver (Kalser and McLain 1970). The plasma half-life is around 2-3 hours in healthy adults. Around 50% of ingested atropine is excreted unchanged in urine. Noratropine, atropine-*N*-oxide, tropine and tropic acid are major metabolites (Van der Meer *et al.* 1986).

Scopolamine, also known as hyoscine, is structurally similar to atropine and is a characteristic component of plants from the Solanaceae family (Rätsch 2005: 860). Scopolamine has some medical uses, in particular as an antiemetic (preventing nausea) and as a muscle relaxant to relax the muscles of the gastrointestinal tract prior to endoscopy (Hedenbro *et al.* 1991). Experimental data obtained from rats given 55mg/kg oral scopolamine suggests that there may be as many as 18 metabolites of scopolamine including the unchanged parent drug excreted in urine (Chen *et al.* 2005). Studies using animal models indicate that there are marked interspecies differences in scopolamine metabolites. Major metabolites in rats include the three phenolic metabolites, *p*-hydroxy-, *m*-hydroxy- and *p*-hydroxy-*m*-methoxy-scopolamine, whilst in rabbits and guinea pigs the major metabolite is tropic acid. In mice tropic acid was only detected in small quantities, yet

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<sup>3</sup> LD50 – lethal dose that kills 50% of a sample population of a particular test animal under controlled conditions.

gluconuride conjugates were high (Wada *et al.* 1991). The exact metabolic pathway in humans has not been fully elucidated. However, Renner *et al.* (2005) suggest that glucuronide conjugation<sup>4</sup> may be a possibility. They also note that scopolamine undergoes oxidative demethylation in the liver; therefore it is likely that scopolamine has several pathways of metabolism that are not fully understood. As only 2.6% of non-metabolised scopolamine is excreted in urine, a first-pass metabolism (where the drug is absorbed in the digestive system and metabolised by the liver before it can enter the bloodstream and cross the blood-brain barrier) is suggested to occur after oral administration of scopolamine (Renner *et al.* 2005).

### **3.5.2. Pharmacology of cocaine**

Cocaine inhibits the re-uptake of the neurotransmitters noradrenaline, dopamine (DA) and serotonin (5-HT) (Ashley and Hitzemann 1990: 117; Isenschmid 2003: 209). The excess noradrenaline in the brain after cocaine use is thought to be responsible for the classic side effects of cocaine use: mydriasis, vasoconstriction, hypertension, tachycardia and tachypnea (Isenschmid 2003: 209). The behavioural effects of cocaine are thought to be a result of dopaminergic actions (Isenschmid 2003: 209). Cocaine produces an intense sense of euphoria, increased mental awareness, increased energy levels and sense of self confidence. However, there are also undesirable effects such as paranoia, hallucinations and dysphoria. Symptoms of cocaine toxicity include profound central nervous system stimulation with psychosis and grand-mal convulsions, and occasionally respiratory paralysis. The ability of cocaine to produce

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<sup>4</sup> The addition of glucuronic acid via a glycosidic bond to any substance that cannot be used as energy by an organism. The glucuronic acid functional group increases the water solubility of the compound, facilitating the excretion of the substance by the kidneys.

increased muscular activity may result in involuntary muscle spasms and hyperthermia (Isenschmid 2003: 210). Acute myocardial infarctions and other heart disease have been reported in people without prior experience of heart disease after cocaine use (Ascher *et al.* 1988; Amin *et al.* 1990; Karch *et al.* 1995; Mittleman *et al.* 1999). Cocaine users report an initial 'rush', in which brain concentrations of noradrenaline and dopamine are briefly elevated followed by a 'crash', in which the levels of these neurotransmitters are reduced to below normal levels. The positive reinforcement of the rush versus the negative reinforcement of the crash is believed to be the principal reason for chronic cocaine abuse (Isenschmid 2003: 209).

The least effective route of ingestion is the oral route (i.e. drinking coca tea/chewing coca). Due to significant first pass effects, approximately 80% of orally ingested cocaine is lost before it can pass the blood-brain barrier (Isenschmid 2003: 211). Therefore the levels of cocaine that enter the brain are much lower than other routes of administration. Coca chewers (that is the combination of unprocessed coca leaves with an alkaline catalysts such as lime or powdered shells) do not become addicted to coca leaves and there appear to be none of the adverse side effects that cocaine or crack addicts experience, although some reports suggest that coca chewers suffer from poorer health. However, these studies do not address poverty and the lack of access to medical care and blame coca chewing as the cause of poor nutritional states and increased prevalence in diseases and parasites such as hookworm (e.g. Buck *et al.* 1968; Buck *et al.* 1970). However, many authors believe that coca chewing is beneficial to Andean people, helping them deal with the

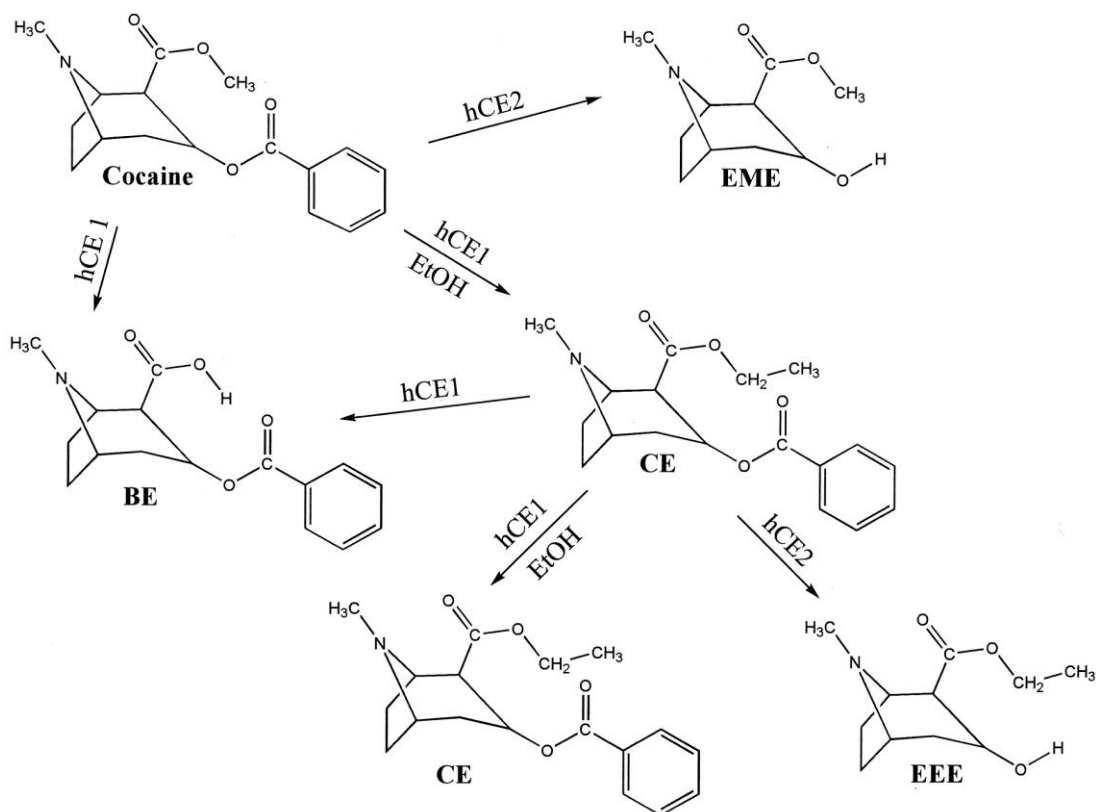
effects of living at altitude, by increasing oxygen saturation and reducing sensitivity to cold conditions (Martin 1970; Hanna 1974; Weil 1978; Weil 1995).

There is a large corpus of work looking into the chemistry and pharmacology of cocaine hydrochloride and crack in modern populations. Edward Cone (1995) compared the pharmacokinetics of intranasal and smoking routes in six male volunteers with a history of cocaine use. Levels of cocaine, BZE and other metabolites were determined in plasma using GC/MS. 'Crack' smoking resulted in rapid penetration into the bloodstream, with a peak plasma concentration of cocaine after 5 minutes. The concentrations ranged from 154 - 345 ng/mL after smoking 42 mg cocaine base. Intranasal cocaine produced lower values over a more prolonged period of time – peak plasma concentrations ranged from 40 - 88 ng/mL after 0.39-0.85 hours. The average bioavailability for the smoking route was 70.1% and 93.7% for the intranasal route. Cone (1995) determined the half-life of cocaine to be between 40 minutes and 4 hours, depending on initial dose. Whilst the smoking route has lower bioavailability the effect of the drug is almost instant and produces higher peak plasma concentrations compared to the intranasal route. This may explain why those who smoke crack are more likely to develop chronic cocaine addiction than those who administer the drug in other ways.

Cocaine from oral administration takes longer to appear in plasma compared to smoking or insufflations. However, the pharmacokinetics of coca chewing is not fully understood (Karch 2008: 30-31). Habitual users typically chew 12-15 g leaf three or four times a day. The alkaloid content varies, but is generally under 0.5%; therefore it is unlikely that the amount of cocaine at any one time is over 75 mg

(Karch 2008: 31). Experimental evidence has shown that there is a difference in peak blood concentrations of novice and habitual chewers. Novice chewers who spit out their saliva had a mean concentration of 38 ng/mL after 1 hour, however habitual chewers who swallowed their saliva had a mean value of 249 ng/mL (range 130-859 ng/mL) (Holmstedt 1979; Paly *et al.* 1979).

Cocaine is metabolised by enzyme and non-enzyme mediated reactions into a number of related compounds. The major metabolite is benzoylecgonine (BZE or BE) and ecgonine methyl ester (EME). In the presence of alcohol, cocaethylene (CE) is formed (see fig. 3.6).



**Fig. 3.6** – Cocaine metabolism producing BE (benzoylecgonine), ecgonine methyl ester (EME) and CE (cocaethylene) (Laizure *et al.* 2003).

### **3.5.3. Legal status: *Erythroxylum* and cocaine**

Coca leaves (*Erythroxylum* spp.) are Class A controlled substances in the UK. They are included in Schedule 1. The definition for Schedule 1 substances under the Misuse of Drugs Regulations 2001 is that there is no recognised medical use for the substance and is thought to be harmful if ingested (although there is overwhelming evidence to the contrary in the case of coca leaves). Under the Misuse of Drugs Act 1971 the penalty for possession is up to seven years in prison or an unlimited fine or both and for dealing, up to life in prison or an unlimited fine or both. Cocaine, ecgonine (and any derivative of ecgonine that can be converted to ecgonine or cocaine), anhydroecgonine, benzoylecgonine isopropyl ester are Class A drugs under the Misuse of Drugs Act, but are categorised into Schedule 2, meaning that the drug can be administered by a clinician. There are strict storage guidelines and requirements for retaining Schedule 2 drugs, including the requirement for record keeping and storage in a locked safe with limited access. The penalties for possession and dealing are the same for coca leaves, as these are also Class A drugs.

On an international level, coca leaf is prohibited in any form. This stems from the inclusion of coca leaf under Schedule 1 of the UN Single Convention of Narcotic Drugs of 1961, which established that “The parties shall so far as possible enforce the uprooting of all coca bushes which grow wild. They shall destroy the coca bushes if illegally cultivated” (Article 26, and that “Coca leaf chewing must be abolished within twenty-five years from the coming into force of the Convention” (Article 49, 2.e) (Metaal *et al.* 2006). This decision was based on a commission that investigated coca chewing in Peru and Bolivia in 1949. The conclusions were that coca chewing was not drug addiction, but due to coca’s ability to suppress hunger it



was part of propagating a constant state of malnutrition and “reduces the economic yield of productive work, and therefore maintains a low economic standard of life” (ECOSOC 1950). The report has since been shown to lack a solid scientific basis and has been criticised for its methodological approach and racist undertones (Metaal *et al.* 2006). The criminalisation of coca has had devastating effects on local Andean economies, particularly the livelihoods of farmers. The issue of coca chewing has been a political hot topic for decades, with many groups petitioning for the removal of coca from the UN Convention of 1961 and recognition as part of traditional Andean cultural heritage. In January 2011, members of The Washington Office on Latin America (WOLA), the Andean Information Network (AIN) wrote to the US secretary of state Hillary Clinton calling for the Obama administration to immediately withdraw its objection to Bolivia’s proposed amendment to the 1961 United Nations Single Convention on Narcotic Drugs that would effectively remove coca from the list of illegal drugs, citing the long history of coca chewing in Andean cultures as well as scientific evidence supporting the notion that chewing coca does not appear to have negative health implications. The authors of the letter also cited the UN Declaration on the Rights of Indigenous Peoples 2007 that states “Indigenous peoples have the right to maintain, control, protect and develop their cultural heritage, traditional knowledge and traditional cultural expressions.”

### **3.6 Summary**

This chapter has introduced and discussed the major alkaloids found in psychoactive plants native to South America. These compounds fall into four

categories: tropane alkaloids (cocaine, BZE, atropine, scopolamine), phenethylamines (mescaline),  $\beta$ -carbolines (harmine, harmaline) and tryptamines (*N,N*-dimethyltryptamine, bufotenine).

Most of these compounds are subject to control under United Kingdom and international laws. In some cases these laws directly affect the customs and culture of Andean people, putting them in contravention of international law, for example the chewing of coca. In some cases the law is not clear as to what is legal or not, as in the case of *R v Sette*, in which it was found that dried San Pedro cactus did not constitute a preparation of mescaline, and was therefore not illegal.

To import, retain and use these compounds for research, the institution in which this work will take place must have a Home Office Drugs Licence (as directed by the Misuse of Drugs Act 1971). Records must be kept detailing the receipt and use of every controlled substance.

# Chapter 4: The Biology of Human Scalp Hair

## 4.1. Introduction

Hair is extremely robust, and under the right conditions can survive for long periods in the archaeological record. Bioarchaeologists have utilised hair to investigate ancient lifeways, including diet and dietary change (White 1993; Macko *et al.* 1999a; Williams and Katzenberg 2012), migration (Ehleringer *et al.* 2008) , stress levels (Webb *et al.* 2010), genetics (Bonnichsen *et al.* 2001b; Gilbert *et al.* 2007a) and drug use (Cartmell *et al.* 1991b; Springfield *et al.* 1993; Ogalde *et al.* 2009). Although hair may survive in the archaeological record it does not necessarily mean it is well preserved due to the action of keratinolytic fungi (Gilbert *et al.* 2006; Wilson 2008: 131; Wilson *et al.* 2010).

This chapter discusses the structure and growth of scalp hair, because this is fundamental to understanding how toxins are incorporated into the growing hair. The premise that drugs, hormones, metal ions and other compounds are incorporated into hair is the basis of this research project. At present there are a number of theories as to how drugs enter hair and what factors affect this process. However, many of these mechanisms have not been fully elucidated. It is certain that drugs interact with melanin (Larsson and Tjälve 1979; Gygi *et al.* 1996; Joseph *et al.* 1996; Joseph *et al.* 1997; Bourges *et al.* 2003), and this is probably the major pathway by which drugs are bound to hair. However, it is likely that other processes are also involved. All of the drug incorporation into hair research is done using

modern clinical and forensic specimens. There is a large body of literature on the effects of cosmetic treatments of hair (bleaching, perming, relaxing) and how this affects drug incorporation into hair (Pötsch and Skopp 1996; Jurado *et al.* 1997; Nakahara 1999b; Wennig 2000; Yegles *et al.* 2000; Morini *et al.* 2010; Sporkert *et al.* 2012). Modern cosmetic treatments will not be discussed as they are not relevant to ancient hair, but weathering (i.e. mechanical change) from grooming practices such as brushing will be considered, as these processes affect the structure of hair (Bottoms *et al.* 1972; Robinson 1976; Kelly and Robinson 1982; Schramm and Kuhnel 1992; Gamez 1998).

The final section of this chapter discusses the fairly limited research into the effect of the burial environment on the survival of drugs in hair, as well as the survival of hair in arid environments, specifically desert conditions, as the hair specimens that form the basis of this research project were recovered from individuals buried in hyper-arid sites in river valleys along the Pacific coast of South America. Some hypotheses regarding the survival of drug compounds in hair over archaeological timescales are presented.

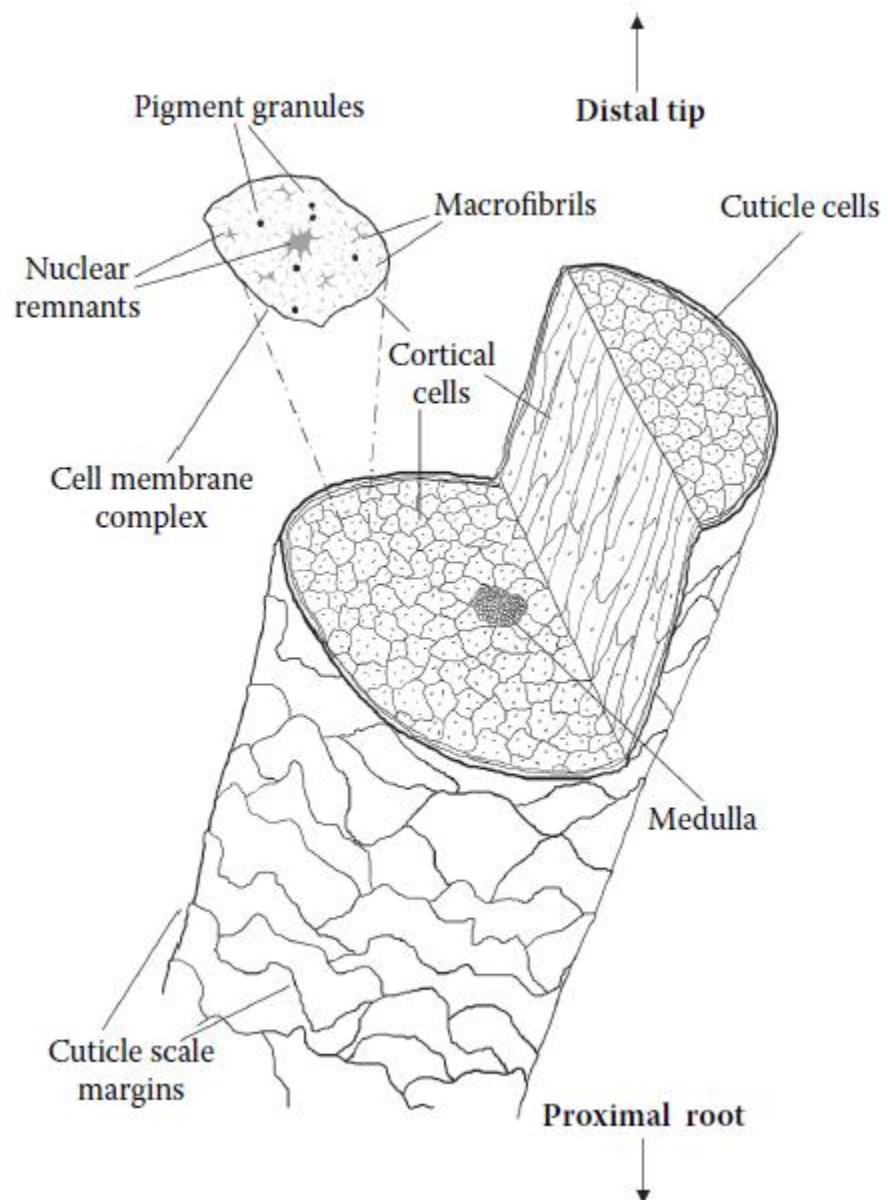
## **4.2. Hair Biology**

### **4.2.1. Hair Structure**

Hair is synthesised in the hair follicle. It is composed of 65-95% protein, 1-9% lipid and 0.1-5% melanin with small amounts of trace elements, polysaccharides and water (Kronstrand and Scott 2007: 1). Hair is not a homogenous structure; it is in fact composed of three distinct cellular structures: the cortex, cuticle and medulla,

as well as melanin granules (see fig. 4.1). The cuticle is a chemically resistant layer that surrounds the cortex and consists of flattened, imbricated scales 7-10 layers thick in humans (Wolfram and Lindemann 1971) that overlap in such a way as to hold the cortex together (Harding and Rogers 1999: 22). The cuticle itself is composed of three layers: the endocuticle (the innermost portion), bounded to the exocuticle by an irregular margin, and the 'A' layer, which forms the outermost margin of the exocuticle (Harding and Rogers 1999: 24). The cuticle has a lipid outer surface that is covalently bonded to an underlying protein matrix (the A-layer). This gives hair a hydrophobic quality and provides a tough outer layer to protect the hair (Jones 2001). Beneath the cuticle is the cortex, the main bulk of the fibre. In a mature hair fibre, the cortical cells retain some nuclear remnants and some pigment granules, however the majority of the cortex is filled with keratin macrofibrils around 0.1-0.2  $\mu\text{m}$  in diameter. The macrofibrils are oriented longitudinally and are composed of keratin microfibrils embedded in a matrix of proteins with a high sulphur content (these are known as keratin-associated proteins or KAP). The KAP proteins are amorphous and comprise about 40% of the protein content of the cell (Harding and Rogers 1999: 18 ).

The medulla is located at the centre of the hair fibre. This structure is formed from a column of cells that are composed of trichohyalin that is chemically distinct from other hair proteins in that it contains citrulline (an amino acid) (Harding and Rogers 1999: 16). The medulla in human hair can be either continuous or discontinuous and may be either air or fluid filled (Robbins 1979: 16). Fine hair tends not to have a medulla (Robbins 1979: 16), but greying hair has a greater tendency for medullation and wider fiber diameter than other types of hair (Longia 1966).



**Fig. 4.1** – Anatomy of the hair shaft (Wilson and Gilbert 2007: 149)

Melanins are formed in specialised cells called melanocytes, which are enclosed in cytoplasmic organelles known as melanosomes. The hair bulb is the site of melanin formation, and most pigment is transferred to the cortex and to a lesser extent the medulla (Kronstrand and Scott 2007: 3). The colour of hair depends on the size, type, shape and distribution of melanin granules.

Hair pigments fall into two general categories: eumelanins, which are dark brown to black in colour and phaeomelanins which are red/yellow (Harding and Rogers 1999: 56). All pigmented hair contains a mixture of both melanins, although the relative amounts vary between individuals (Wilson *et al.* 2001). The different types of pigment can be distinguished by their chemical properties: eumelanins are insoluble in acid and alkali and do not contain sulphur, whilst phaeomelanins are soluble in dilute alkali and contain sulphur (Harding and Rogers 1999: 57).

#### **4.2.2. Hair Growth**

The hair follicle is a complex organ in which division, differentiation and migration of cells occur, resulting in the production and growth of the hair fibre. Each fibre is formed as the result of the biosynthesis and hardening of the contents of the medulla, cortex and cuticle cells of the hair shaft (Harding and Rogers 1999: 5). Human hair growth is cyclic, going through three distinct stages: anagen, catagen and telogen.

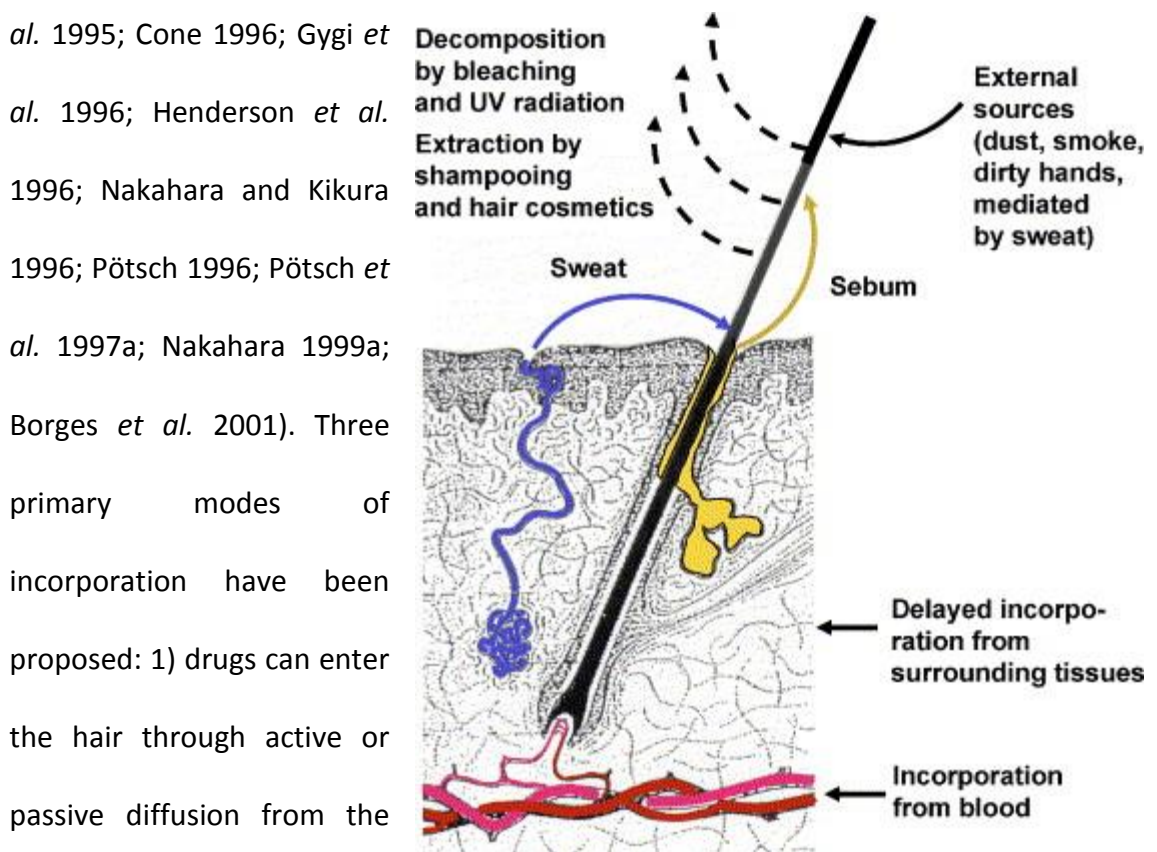
Hair is produced in the hair follicle during anagen, a period of high cellular activity in which the hair is actively growing. This process takes approximately three years, but can last as long as ten years (Harding and Rogers 1999: 40; Tobin 2005: 311; Kronstrand and Scott 2007: 3). The next phase, catagen, is the regression phase, lasting approximately three weeks, during which the lower 70% of the anagen hair follicle is resorbed (Wilson 2008: 126). Telogen is the resting phase, lasting on average three months, during which no significant tissue remodelling occurs.

Typically telogen hairs do not have any root sheath attached and very little pigment at the root, and only require a small force to dislodge them.

### 4.3. Drug incorporation into hair

#### 4.3.1. Mechanisms for incorporation

The mechanisms by which drugs are incorporated into hair have been widely discussed in the scientific literature (Henderson 1993; Gygi *et al.* 1995; Nakahara *et al.* 1995; Cone 1996; Gygi *et al.* 1996; Henderson *et al.* 1996; Nakahara and Kikura 1996; Pötsch 1996; Pötsch *et al.* 1997a; Nakahara 1999a; Borges *et al.* 2001). Three primary modes of incorporation have been proposed: 1) drugs can enter the hair through active or passive diffusion from the



**Fig. 4.2** Incorporation and elimination of drugs from hair (Pragst and Balikova 2006).

bloodstream 2) diffusion from sweat (from eccrine glands), sebum (from sebaceous glands attached to the hair follicle) and other secretions, or 3) external contamination from vapours or powders from the environment (Kronstrand and Scott 2007: 5). Whilst incorporation from the bloodstream is most likely the major route for drugs to enter hair, a combination of all three mechanisms is probably



involved in the deposition of drug molecules in hair fibres (see figure 4.3). This combined mechanism has been demonstrated by Henderson (1993) and is generally accepted in practice (Pragst and Balikova 2006).

#### **4.3.2. Melanin-drug interactions**

A number of binding sites in hair for drugs have been investigated. The majority of this work has focussed on melanin as a binding site for a wide range of toxins with variable physiochemical properties (Larsson and Tjälve 1979; Gygi *et al.* 1996; Joseph *et al.* 1996; Joseph *et al.* 1997; Pötsch *et al.* 1997b). Studies comparing pigmented and non-pigmented hair have shown that pigmented hair accumulates and retains drug compounds more readily. Joseph *et al.* (1996) compared hair from people of African (black hair) and European (brown, blonde hair) ancestry. They removed the lipid component of the hair and bleached some specimens to remove melanin. Experiments with radiolabelled cocaine were performed on untreated, lipid-extracted, and bleached portions of hair from the different groups. Cocaine binding was significantly higher ( $p < .01$ ) in hair from African males (i.e. highly pigmented hair). The amount of drug binding was similar among African females and all European specimens. The lowest amount of binding was observed with blonde European females. Binding experiments also revealed that specific cocaine binding generally did not differ significantly between lipid-extracted hair and untreated hair, suggesting that lipids are not a major site of binding for drugs. However, bleaching of most hair specimens resulted in significant ( $p < .01$ ) decreases in specific binding compared with untreated hair (Joseph *et al.* 1996). Other studies

have confirmed that black hair absorbs drugs more readily than brown and blonde hair fibres (Reid *et al.* 1994). Chad Bourges *et al.* (2003) performed *in vitro* binding experiments with cocaine, amphetamine, BZE and *N*-acetylamphetamine and synthetic melanins. Results indicate that basic drugs (cocaine and amphetamine) bind to eumelanins (i.e. dark pigments) and mixed eu-/phaeomelanins to varying degrees, but not to pure phaeomelanins. BZE and *N*-acetylamphetamine, net neutral molecules, did not bind to any type of melanin. Other research groups have found that drugs preferentially bind to darker hair pigments, for example, Uematsu *et al.* (1990) found that haloperidol (an antipsychotic drug used to treat schizophrenia) preferentially bound to dark hair but not white hair, whilst Gygi *et al.* (1996) found that pigmented hair had a greater capacity to bind and incorporate codeine and its metabolites compared to non-pigmented hair.

Melanin affinity is a measure of the binding of drugs and toxins to melanin. It has been found that organic amines and metal ions have high melanin affinity (Larsson and Tjälve 1979; Kronstrand and Scott 2007: 9). Yuji Nakahara *et al.* (1995; 1999a) investigated the melanin affinity of 20 common drugs of abuse. The 20 drugs were incubated with melanin at 36°C in the dark for 2 hours. After incubation and centrifugation, the drug concentration in the filtrate was determined by gas or liquid chromatography. Of the 20 drugs, cocaine had the greatest melanin affinity, 11-nortetrahydrocannabinol-9-carboxylic acid (THCA) the least. There was a 3600 fold difference between cocaine and THCA.

Melanin has been shown to be the key binding site for drugs in hair, yet other research shows keratin may also be a binding site for drugs. Applegren *et al.* (1997)

detected clenbuterol (a bronchodilator) in pigmented and non-pigmented hair from cattle. They performed a comparison of the binding of <sup>3</sup>H-clenbuterol to eumelanin and keratin using Scatchard analysis (an equation calculating the affinity constant of a ligand with a protein) and found more than one binding class for melanin, but only one for keratin, and that the association constant for keratin and the second class of melanin was comparable. This shows that keratin is also a binding site for drugs. This may well explain why drugs have been detected in grey and white hair in various studies (e.g. Rothe *et al.* 1997; Lee *et al.* 2010).

#### **4.3.3. Lipophilicity and basicity**

Experimental data has indicated that the levels of neutral and acidic drugs, such as steroids, cholesterol, barbituric acids and cannabinoids in hair are generally very low compared to basic drugs, in particular cocaine. This is particularly evident in pigmented hair (Uematsu *et al.* 1990; Reid *et al.* 1994; Henderson *et al.* 1998).

An explanation for the preferential binding of basic drugs to pigmented hair may be the result of two additional physicochemical factors: lipophilicity and basicity. The pH of hair is around 3.67 (Wilkerson 1935) whilst the pH of blood is 7.4. There is a biomembrane composed of phospholipids between the bloodstream and hair follicle. Diamond and Katz (1974) proposed a lipid bilayer model in which the passage of molecules is dependent on their lipophilicity (lipid soluble drugs being able to pass the lipid bilayer more readily) and pH on each side. Nakahara deduced that basic drugs, such as cocaine, various opiates and amphetamines, with high pH values, will accumulate in the cortex, as the drugs will diffuse from an area of high

pH via a pH gradient to an area of lower pH (i.e. into the hair matrix). Nakahara (1999a) suggests that the pH of hair is largely dependent on melanin pH. Non-pigmented hair is less acidic than pigmented hair. Nakahara surmises that this would affect the pH gradient, by making the hair have a lower pH and therefore creating a greater gradient between the bloodstream and hair follicle. Once the drug enters the hair, it will be protonated and unable to diffuse back into blood plasma (Kronstrand and Scott, 2007: 7).

Yuki Nakahara (1999: 59) also noted that cationic drugs, such as cocaine, opiates and amphetamines are found in higher concentrations in hair, as opposed to anionic drugs, such as cannabinoids. He stated that cationic drugs have a higher tendency to be attracted by the negative charge of hair, especially if the pH of the drug is higher (i.e. more basic). De Lauder and Kidwell (2000) demonstrated the preferential binding of cationic compounds to hair. Eleven hair samples from various ethnic groups were exposed to rhodamine 6G, a cationic dye, or fluorescein, an anionic dye and viewed under fluorescence microscopy. It was found that the rhodamine 6G was most easily incorporated into African-American hair. By comparison, Caucasian brown hair required 10 times the concentration and four times as much time for the same amount of permeation, whilst the anionic dye was not readily incorporated into any of the hair specimens. A pH effect was observed in all specimens studied. For untreated African hair, the rate of rhodamine 6G diffusion increased from pH 3.0 to 7.0, and then decreased slightly at pH 9.0. The researchers suggest that at this pH the dye became a zwitterion, therefore removing its overall positive charge of the molecule and as a result reducing its ability to diffuse into the hair.

#### **4.4. Effect on the depositional environment on drug preservation in hair**

Drugs have been detected in hair from forensic and short-term experimental contexts. The reports from the forensic literature are mainly case reports (Table 4.1 summarises these). Morphine was found in the hair of two drowning victims with known histories of heroin use seven months after death (Mari and Bertol 1997). Another case report found cocaine in scalp and pubic hair of a 17 year old man exhumed seven months after death and cocaine and lidocaine in the scalp hair of a 25 year old female exhumed 2 months after death (Arado *et al.* 2001). Segmental hair analysis of an 18 year old woman with a known history of drug use was performed after her body was exhumed several months after death. The analysis confirmed that the woman used heroin and cannabis before she died, but use was lower in the months immediately prior to death. It was suggested that loss of tolerance prior to taking a regular dose contributed to her death (Tsatsakis *et al.* 2001). Another case report involving the death of a 26 year old woman from an acute cerebral infarction after thrombosis of the left internal carotid artery with no medical history of heart disease was investigated twenty months after death (De Giorgio *et al.* 2007). A 2 cm segment of hair closest to the scalp was spiked with a deuterated internal standard then subject to liquid-liquid extraction and GC-MS analysis. Cocaine and traces of BZE were detected, confirming she had used cocaine before her death. Cocaine has been reported to cause heart problems and sudden deaths in young adults; the authors suggest cocaine use probably contributed to the woman's death (De Giorgio *et al.* 2007). In another case study, amitriptyline (a tricyclic antidepressant with sedative action), its metabolite nortriptyline, and

bromazepam (a benzodiazepine sedative) were detected in the hair of a one month old baby who had died eight months previously. The hair analysis was crucial in prosecuting the mother for deliberately poisoning her child (Gaillard *et al.* 2011). Other drugs, such as buprenorphine (semi-synthetic opioid used to treat heroin addiction/ painkiller), cocaine and opiates have been detected in body fluids (urine, bile, blood) and tissues (liver) in a state of advanced decomposition some 20 days after death (Borriello *et al.* 2008).

The case reports described above indicate a wide range of drugs can be detected in hair in decomposed human remains, and in many cases analysis of hair has aided medico-legal investigations into drug-related deaths. Pascal Kintz *et al.* (2008) reported on case studies in which they had analysed hair from decomposed remains 18 months (advanced decomposition with large amounts of putrefactive fluid) and 20 years postmortem (skeletonised, but with some adherent hair).

Discussion of postmortem artefacts resulting from external contamination during the postmortem process found that hair that had been in contact with decomposition products produced homogenous positive hair tests in people with no histories of chronic drug use. The case studies involved people who had died due to drug poisoning or, in one case, a suspected murder by a serial rapist who administered Rohypnol (flunitrazepam) to the victim.

**Table 4.1** – Summary and comparison of case studies involving the analysis of hair for drugs in decomposed human remains.

Age/Sex	Hair	Analytical	Drugs detected	Postmortem	Reference
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	sample	technique		interval	
18/F	2-3 cm	n/a	Morphine	7 months	Mari and Bertol (1997)
20/F	segments			7 months	
17/M	n/a	DCM/H <sub>2</sub> O, SPE, GC-MS	Cocaine	7 months	Arado <i>et al.</i> (2001)
25/F			Cocaine, lidocaine	2 months	
18/F	1 – 1.5 cm segments	GC-MS	Opiates, cannabinoids	7 months	Tsatsakis <i>et al.</i> (2001)
26/F	2 cm proximal segment	n/a	Cocaine, BZE	20 months	De Giorgio <i>et al.</i> (2007)
?/M	100 mg hair	0.1M HCl @ 55°C, LLE, GC-MS	Buprenorphine, cocaine, opiates	20 days	Borriello <i>et al.</i> (2008)
n/a	6 cm	DCM, LC-MS/MS	cyamemazine	18 months	Kintz <i>et al.</i> (2008)
17/F	4 cm	MS/MS	flunitrazepam	20 years	
1 month/F	50 mg	Acid hydrolysis, SPE, LC-MS/MS	Amitriptyline + metabolites	8 months	Gaillard <i>et al.</i> (2011)

DCM = dichloromethane, LLE = liquid/liquid extraction, SPE = solid phase extraction

In this case the authors concluded the positive hair tests were due entirely to contact between putrefactive fluids containing drug compounds and hair during the postmortem period (Kintz *et al.* 2008). The authors suggested that the discovery of homogenous hair test results from segmental analysis may be the result of postmortem contamination, and this should be taken into account when dealing with heavily decomposed remains.

One issue that has been rarely addressed in these forensic cases is the effect of the depositional environment on the preservation of drug molecules in the hair. One would expect there to be some degradation of the hair, by either microbial or chemical action, particularly in environments not conducive to preservation of organic materials – for instance warm, wet conditions. It is also likely that the

original amount of drug in the hair will have lowered due to degradation of drug molecules in the hair fibre. Experimental work by Skopp *et al.* (2000) into the stability of cannabinoids in hair reported that hair that had tested positive for cannabinoids using GC-MS generally no longer tested positive for the same compounds after 10 weeks exposed to sunlight (3 of 11 samples had quantifiable amounts of THC after 10 weeks). Tests of control samples kept at ambient temperature in the dark did not reveal any change from the levels detected at the beginning of the experiment. Ultra violet and visible light are known to affect the physico-chemical properties of hair keratins and melanin, with the most noticeable change being bleaching as a result of free radical formation resulting in photo-modification of the redox properties of melanin (Wilson 2008: 129). Pötsch *et al.* (1995) obtained hair from drug users at postmortem. Natural and bleached hair samples from these individuals were exposed to the following conditions: submerged in water in a laboratory in tap water for four weeks and six months; soil for 6 months. The bleached hair gave negative results after exposure to water or to soil for 6 months as well as after immersion in tap water at room temperature for 4 weeks. Natural hair exposed to the same conditions showed dramatically reduced levels of opiates. These results are not surprising, as bleaching is known to damage the condition of hair, thereby facilitating the loss of drug molecules from the hair. The same research group (Skopp *et al.* 1997) exposed hair samples from drug users to sunlight, rain and wind for a three month period over summer in a temperate climate. They found that there was a considerable decrease in morphine and 6-acetylmorphine (heroin metabolites) concentrations and only small changes in cocaine and BZE concentrations.



Other unpublished studies also suggest that drug levels in hair decrease due to environmental exposure. Estelle Conlon (2006) obtained hair samples from drug rehabilitation patients in Bradford. The samples were subdivided, with one portion buried for three months, and then analysed against the undegraded (control) portion. Each volunteer had taken either opiates, cocaine, benzodiazepines or a combination of these. The masses of both samples were recorded. The samples and controls for experimental degradation were sewn into nylon mesh bags and subject to burial at one of four sites (see table 4.2).

Hair samples were recovered after 12 weeks and prepared for analysis. The samples were air dried and brushed to remove adherent dirt. The masses before and after burial were compared. The samples buried in peat lost around a quarter of their original mass, whilst the others lost 10-15% of their original mass on average. In terms of preservation (loss of original mass of hair was used as a way of quantifying this) Conlon found that hair buried in moor peat had the least mass loss, and lab soil the most (Conlon 2006: 298).

Moor peat > Marine > Woodland > Surface exposure > Lab soil

**Table 4.2** – Depositional environments for Conlon’s (2006) experimental work.

Sample numbers	Deposition site
1-5	Oxenhope Moor (peat), 30 cm depth

Control 2	Anaerobic, acidic, waterlogged conditions, pH 3.1
6-10	Oxenhope Moor, exposed to elements
Control 2	Alternately wet/dry conditions
11-15	Marine environment, Key Haven Nature Reserve, Hampshire
Control 4	Submerged in water
15-20	Peat-free compost, laboratory environment
Control 5	Dry, pH 8.2
21-25	Deciduous woodland, Otley, 30 cm depth
Control 6	Dry, pH 4.4

As a method of obtaining some results from the degraded hair, samples were sent to Dr. Larry Cartmell, who tested the hair for cocaine and nicotine using RIA. Cartmell tested 9 samples - 4 non-degraded, 4 degraded and one unknown sample. Samples 2 (peat soil), 6 (surface exposure), 19 (peat-free lab soil), 23 (deciduous woodland), their non-degraded counterparts and a Medidrug hair standard (certified reference material) sample were sent for analysis. The RIA analyses revealed cocaine and nicotine in all non-degraded samples (see table 4.3). The samples from the marine environment were not tested, but one could expect there to be a significant decrease in the drug levels in hair brought about by continual movement/ solvent action of the water (Pötsch *et al.* 1995; Conlon 2006: 296).

**Table 4.3** – Results of RIA analyses of experimentally degraded hair (Conlon 2006).

Sample	Cocaine	% decrease	Nicotine	% decrease
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	(ng/mg hair)	cocaine	(ng/mg hair)	nicotine
D-2 (Peat soil)	516.90		0.68	
U-2	1203.30	<b>-57.04%</b>	3.11	<b>-78.14%</b>
D-6 (surface exposure)	658.87		0.98	
U-6	1318.98	<b>-50.11%</b>	3.94	<b>-75.13%</b>
D-19 (peat-free lab soil)	336.53		0	
U-19	664.02	<b>-49.32%</b>	1.87	<b>-100%</b>
D-23 (deciduous woodland)	168.95		0	
U-23	1508.53	<b>-88.80%</b>	1.18	<b>-100%</b>

Five of Conlon's experimentally-degraded hair with their non-degraded counterparts (see table 4.4) were analysed as part of the pilot study for the current PhD (Brown 2007). The analytical method used was LC-MS/MS, in contrast to RIA utilised for Conlon's work. Specimens were chosen on the basis that they were from self-reporting cocaine users rather than on depositional environment. As Conlon (2006: 296) suggested, the marine environment was not conducive to the survival of drug molecules within hair fibres. Both samples from the marine environment lost over 98% of their original cocaine content. The decrease in BZE levels was also large (76% and 92% respectively). The samples from the deciduous woodland also lost around 72% of the cocaine and BZE levels (although sample D-21 only decreased around 5%). Although only nine samples were analysed from each of these studies, both Conlon's work and the pilot study show that the deciduous woodland environment was not favourable for the preservation of drug molecules in hair.

**Table 4.4** – Results of LC-MS/MS analysis of experimentally degraded hair from self-reporting cocaine users (Brown 2007).

Sample	Cocaine (ng/mg hair)	% decrease cocaine	BZE (ng/mg hair)	% decrease BZE
D-12 (marine)	156.4		3.3	
U-12	2.2	<b>-98.63%</b>	13.6	<b>-75.71%</b>
D-14 (marine)	465.2		2.2	
U-14	3.0	<b>-99.35%</b>	28.5	<b>-92.35%</b>
D-20 (peat free lab soil)	216.8		2.0	
U-20	79.4	<b>-63.40%</b>	19.8	<b>-90.07%</b>
D-21 (deciduous woodland)	1161.1		49.7	
U-21	318.5	<b>-72.57%</b>	52.2	<b>-4.73%</b>
D -25 (deciduous woodland)	829.6		18.8	
U-25	227.7	<b>-72.56%</b>	59.1	<b>-73.28</b>

#### 4.5. Summary

Although hair may appear well preserved it is likely there will have been degradative change to the structure of the hair. Within the cortex, the component most resistant to microbial degradation is the melanin granules. Drug compounds such as cocaine do bind to melanin pigment granules, which may explain the detection of compounds such as cocaine, BZE and other drugs in archaeological human remains from arid and frozen environments (see the next chapter for a detailed discussion of drug compounds in ancient hair).

Experimental work has indicated that drug compounds in hair can degrade within weeks depending on the depositional environment, with acidic and marine environments being the least favourable conditions for preservation of drug molecules in hair, based on the limited parameters studied thus far. There has been no experimental work to see the effect of desert environments on the preservation of drugs in hair. Even though organic preservation is often very good in these conditions it is still very likely that the amount of drug detected in hair is probably a

fraction of the original amount. Temperature is most likely the key variable – high temperatures have been identified as being detrimental to other biomolecules such as collagen and DNA (Poinar and Stankiewicz 1999; Collins *et al.* 2002) and cocaine *in vitro* (Skopp *et al.* 2001). The effect of contamination of hair from body fluids containing drug compounds is also an issue, as contact with these fluids can contaminate hair, resulting in what Kintz *et al.* (2008) calls the “external postmortem artefact” Clearly more experimental work is required in this area to expand what is already known, for example laboratory and field based experiments modelling a desert environment may help understand some of the environmental variables affecting the preservation of drug molecules in hair.

# Chapter 5: Drug Compounds in South American Mummy Hair

## 5.1. Introduction

Testing human hair for drug compounds is now fairly routine in forensic and clinical medicine, as evidenced by the large numbers of papers published in scientific journals, the establishment of international standards for validation of methods and books on the subject (Baumgartner and Hill 1996; Kintz 2006; 2007; Musshoff and Madea 2007; Peters *et al.* 2007; Kintz 2008).

Cocaine has been one of the most studied drugs in hair testing, as it is readily incorporated into the growing hair and is widely used all over the world. Valente *et al.* (1981) were the first to detect cocaine in hair. They used commercially available radioimmunoassay (RIA) kits to demonstrate the presence of cocaine and opiates in the hair of drug users. Svetla Balabanova and Jamos Homoki published a gas chromatography method six years later (Balabanova and Homoki 1987). Not long after this, a short report published in a well-regarded German journal caused considerable controversy. In 1992 a Munich based team headed by Balabanova reported finding nicotine and benzoylecgonine (BZE) in mummies from Egypt. The findings were immediately dismissed by the scientific community (Bisset and Zenk 1993; Björn 1993; Brothwell and Spigelman 1993; Hertting 1993; McIntosh 1993; Schäfer 1993; Wilson 2005).

A number of explanations have been put forward to explain the findings of New World drugs in Old World human remains. Rather than there being long distance trade between Peru and Egypt in antiquity, it is more likely the mummies were contaminated at some point prior to their analysis, either by the use of nicotine based pesticides used by 19<sup>th</sup> century curators to prevent insect damage in museum collections (Buckland and Panagiotakopulu 2001) or by contamination either by people using cocaine around the mummies. Cocaine used to be a legal stimulant available in pharmacies (Jay 2010: 92) or in the laboratory. The samples were analysed in a forensic laboratory that routinely dealt with cocaine.

These findings highlight the need for rigorous methods including the use of control samples as well as knowledge of the curation history of human remains, particularly those in museum collections, to avoid further controversies.

The discovery of BZE, a metabolite of cocaine in South American mummies was far less controversial (Cartmell *et al.* 1991b). Coca is native to the Andes and is thought to have been used for thousands of years. In recent years other researchers have attempted to detect other less well known compounds in Andean mummies (Cartmell *et al.* 2005; Ogalde *et al.* 2009). This chapter is a review of hair drug tests carried out by a limited number of researchers on Andean mummies. The methods and interpretations are reviewed critically in terms of scientific rigour and archaeological context.

## 5.2. Coca chewing in the Andes

It has long been held by various scholars that during the Inca period (c AD 1450 – 1532) access to coca was controlled by the state (Rowe, 1946; Garcilaso de la Vega, 1987 [1609]). It is thought that imperial drive to restrict coca began around AD 1230, in the reign of Inca Roca, but did not become a monopoly until the early 15<sup>th</sup> century (Rowe 1946). Coca acquired a divine status and its association with ritual is clear (Cortella *et al.* 2001; Ceruti 2004; Bray *et al.* 2005). Various authors suggest that as the Inca Empire expanded, coca was shared with lesser and lesser ranks of nobility, until almost everyone had access to it (Mortimer 1974; Phillips and Wynne 1980; Cartmell *et al.* 1994).

As a means of investigating the purported Inca coca monopoly of the late 15<sup>th</sup> century, Larry Cartmell *et al.* (1991b) utilised commercially available radioimmunoassay kits normally used to test urine for drug metabolites and used them to test ancient hair for the main metabolite of cocaine: benzoylecgonine (BZE). In a pilot study, eight individuals from southern Peru and northern Chile were tested. These eight individuals dated to the Chinchorro culture (c 2000 BC) and the latest were “Inca” (c AD 1400). Results greater than 5 ng metabolite/10 mg hair was accepted as a positive result. Both negative and positive controls were included and results were confirmed by gas chromatography mass spectrometry (GC-MS), with only the “Inca” (c AD 1400-1532) samples testing positive for BZE. These individuals came from an unnamed site in the Camarones Valley in northern Chile. The individual that tested positive were a female aged around 25 and an infant male, aged around 3 years. Both were found buried with grave goods characteristic of the Inca time period. Subsequent radiocarbon dates placed them in the mid to late 15<sup>th</sup>



century, consistent with the middle of the Inca period. Cartmell *et al.* acknowledged that their sample size was extremely small, but suggested that early coastal populations did not chew coca, as they “employed a maritime subsistence strategy and probably had little access to coca leaf” (Cartmell *et al.* 1991b: 12).

*Erythroxylum coca* grows in the moist montane tropical forests (*yungas*) on the eastern Andean slopes around 400 – 1800 meters above sea level. There is evidence that coca was being cultivated and used around 8,000 years ago (Dillehay *et al.* 2010). By the Middle Horizon (c AD 550 - 1000) coca was a key economic crop in the fertile river valleys of the southern coast (Kolata 1993b: 213) and was a common inclusion in funerary offerings from sites along the southern coasts of Peru and northern Chile (Aufderheide 2003: 157).

Early coastal dwellers may not have had access to this variety, but a variety of coca known as Trujillo coca (*Erythroxylum novogranatense* var. *truxillense*) grew on the northern Peruvian coast from c. 2,000 BC onwards and it is in this area that coca chewing is believed to have originated (Plowman 1986b). However, populations further south, such as the Chinchorro (7000-2000 BC), who possibly predate the arrival of coca on the northern coast may have only had access to coca via trade. Cartmell *et al.* suggest this is very unlikely, as the Chinchorro and the Quiani culture that followed, were primarily marine-oriented (Cartmell *et al.* 1994). It should be noted that there is no archaeological evidence that the Chinchorro had access to coca (Aufderheide 2003 158).

Using the same methods (RIA followed by GC-MS) for a follow-up of 163 individuals from coastal and lowland sites in Chile, similar results were observed (Cartmell *et al.* 1991a, see table 5.1). The earliest samples from Chinchorro (7000 BC-2000 BC) and Quiani (2000 BC – 1500 BC) individuals did not test positive for BZE. However, the authors did not consider that possibility that any compounds that may have been present in the hair had degraded. This is particularly pertinent given the long post mortem period (possibly up to 9,000 years), body processing and extreme environment in which these individuals were buried. High temperatures increase the rate of degradation of biomolecules. This temperature effect is well documented for DNA (Hofreiter *et al.* 2001; Pääbo *et al.* 2004; Marchant 2011) and collagen (Nielsen-Marsh *et al.* 2000: 442; Collins *et al.* 2002; Roberts *et al.* 2002).

GC-MS analysis of some of the positive results was undertaken. The findings indicated that there was at least one false positive from the initial RIA analysis (Cartmell *et al.* 1991a). Thirteen Inca individuals were tested. Of these, nine of these tested positive for BZE. The authors suggest that the grave goods and heavily worn clothing are indicative that these “Inca” individuals were of a low socioeconomic group. Cartmell *et al.* (1994) doubted that these individuals were highland-related Cuzco Inca, as their diets, reconstructed by trace element (strontium and calcium) analysis, suggest long term residence on the coast due to a large component of the diet being derived from marine sources. It should be noted that trace elements are particularly susceptible to diagenesis within the depositional environment, making interpretation of results problematic (Lambert *et al.* 1985; Klepinger *et al.* 1986; Price *et al.* 1992; Elliott and Grime 1993; Burton and Price 2002; Fabig and Herrmann 2002).

Both the Cabuza (AD 400 - 1000) and Maitas Chiribaya (AD 1000 – 1250) individuals also had a number of positive hair tests (Cabuza 10/16 and Maitas Chiribaya 54/97), indicating that significant proportions of these pre-Inca cultures has access to coca. This is not surprising, particularly for the Cabuza and Maitas Chiribaya populations, as coca quids are found *in situ* in the cheeks of some individuals (Cartmell *et al.* 1994; Aufderheide 2003: 344).

**Table 5.1** - BZE test results in hair samples from mummies recovered from Northern Chile (Cartmell *et al.* 1991a).

Culture	Number positive	Number tested
Chinchorro (7000 BC-2000 BC)	0	23
Quiani (2000 BC – 1500 BC)	0	3
Alto Ramirez (1000 BC – AD 300)	1	3
Cabuza (AD 400 – AD 1000)	10	16
Maitas Chiribaya (AD 1000 – AD 1250)	54	97
San Miguel ( AD 1050 – AD 1300)	2	8
Inca ( AD 1400 – AD 1500)	9	13

In another study, again by Cartmell *et al.* (1994) consisting of 254 individuals (including the 163 from the previous study) from seven cultures located in the Azapa, Osmore and Camarones valleys, found significant positive hair tests using RIA as with previous studies. Of the 254 samples tested by RIA, 114 (45%) had positive reactions. Sixty-one of the positive samples were tested again using GC-MS. One sample discoloured, so could not be tested. The remaining 60 samples all

tested positive for BZE. In addition, twenty samples that were negative using RIA were also negative when tested using GC-MS.

As with previous findings (Cartmell *et al.* 1991a; Cartmell *et al.* 1991b), the earliest individuals (Chinchorro and Quiani) did not test positive, however two individuals from the Alto Ramírez culture (c 1000 BC – AD 300, see s7.2.2) did test positive.

Based on radiocarbon dates (not stated in the paper), one of these individuals was from one of the earliest Alto Ramírez groups. This suggests that coca chewing was practiced on this part of the coast around 3,000 years ago.

Larry Cartmell *et al.* (1994) were the first to use a segmental approach to hair analysis. As hair grows around 1 cm per month, it is possible to reconstruct patterns of coca use by determining the presence or absence of BZE in each segment. This method was used to determine coca use in the months before death in two individuals. A 9 cm length of hair from female aged approximately 25 (Inca period) and a 14 cm length of hair from a female aged approximately 50 (Chiribaya) were divided into 13mm segments, each of which were analysed independently (Cartmell *et al.* 1994). The Chiribaya female appears to have used coca continually. However the Inca female appears to have abstained from coca for around 5 months, but used coca in the 3 months prior to death (see table 5.2).

**Table 5.2** – Benzoylecgonine (BZE) RIA test reactions in successive 13mm hair segments from two individuals: a female aged ≈25 (“Inca”) and a female aged ≈50 (Chiribaya) (Cartmell *et al.* 1994).

	<i>Months prior to death</i>													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
“Inca”	+	-	-	-	-	-	+	+	+					
Chiribaya	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The same analytical technique employed by Cartmell’s research group (RIA) was used to determine the levels of cocaine, nicotine and xanthine derivatives in the cranial hair of an adult female Peruvian mummy, known as “Copara 662” (Balabanova *et al.* 1992b, see table 5.3). This individual dates from the Inca period, c1500, and was found at Rio Tunga, near Nazca, Peru. Xanthine derivatives (caffeine, theobromine) are found in a number of plants native to South America, including *Theobroma cacao* (cocoa), *Erythroxylum* sp. (coca) and *Ilex* sp. (*yerba maté*). The authors suggest the finding of xanthine derivatives may indicate the inclusion of some of these plants in the diet. Whilst only a class of compounds were detected, this kind of analysis may provide complementary data to dietary stable light isotope analyses.

**Table 5.3** – Xanthine derivatives, cocaine and nicotine levels in the hair of Copara 662 as detected by radio and enzyme immunoassay (Balabanova *et al.* 1992b).

<b>Compound</b>	<b>Amount detected</b>
Xanthine derivatives (caffeine, theobromine)	50.0 ng/mg
Cocaine	2.4 ng/mg
Nicotine	1.4 ng/mg

In the discussion of these results, Balabanova *et al.* (1992b) state that the levels of drug detected may not reflect the amount present at death, as it is likely that some degradation has occurred over the intervening years, making comparison with modern hair difficult.

Balabanova's research group also tested hair, bone, teeth and soft tissue of 62 mummies from the Munich Peruvian Mummy Collection for cocaine, nicotine and tetrahydrocannabinol using RIA and GC-MS (Parsche *et al.* 1994). The mummies were collected by Heinrich Ubbelohde-Doering between 1932 and 1954 from sites in the Chicama and Jequetepeque valleys on the north coast and sites along the Rio Grande de Nazca on the south coast (Parsche *et al.* 1994). A number of individuals tested positive for cocaine, nicotine and THC in all tissues tested. The positive tests for cocaine and nicotine in hair and soft tissue and bone is not surprising. Various drugs of forensic relevance have been detected in bone and teeth (McIntyre *et al.* 2000; Raikos *et al.* 2001; Cengiz *et al.* 2006; Pellegrini *et al.* 2006; Schloegl *et al.* 2006; Watterson 2006; Marchei *et al.* 2008; Lafrenière and Watterson 2009; McGrath and Jenkins 2009; Lafreniere and Watterson 2010; Watterson *et al.* 2012).

More surprising are the positive tests for THC (Balabanova *et al.* 1992a; Parsche *et al.* 1994), the active compound in *Cannabis sativa*. Cannabis is not native to the New World, only being introduced by the Spanish in the 16<sup>th</sup> century (Mignoni 1997). THC was detected in all tissues, but not all individuals. It is not clear by which method the data presented was generated.

Rivera *et al.* (2005) analysed hair samples from 11 individuals using the same methods (RIA followed by GC-MS) as Springfield *et al.* (1993) for traces of cocaine and BZE. The individuals were from the Alto Ramirez culture. The site, Pisagua-7, is situated on the northern Chilean coast. Of the 11 tested, two tested positive. These were 725-A, C2 (calibrated C14 date 1140-900 BC) a female aged 40-45, and 741, a female aged 3-4 (calibrated C14 date 940-810 BC). Two other individuals present evidence of cocaine and BZE, but fell under the RIA limit of detection (3 ng drug/10mg hair). Despite these results, Rivera *et al.* (2005) reiterated the importance of detecting drug metabolites in hair as a means of determining ancient coca chewing practices. They state that: “only demonstration of the chemical presence of cocaine and its metabolites in the body tissue of ancient human remains can establish that the deceased had chewed coca leaves or imbibed their cocaine content as tea” (Rivera *et al.* 2005: 456). This is crucial, as it has been suggested that some individuals were buried with coca, yet their hair and tissues did not test positive for cocaine or BZE (Cartmell *et al.* 1991a; Aufderheide *et al.* 1994). There may be a number of reasons for this. It may be that the drug compounds have degraded during the long postmortem period or it may be that the analytical method used to test the hair did not have the sensitivity to detect very low levels of BZE/cocaine. It may be that the inclusion of coca leaves in their

funerary assemblage was symbolic and not necessarily something they practiced during life.

A slightly different approach, in that a wider range of target compounds were selected, was taken by Báez *et al.* (2000). Nineteen individuals from the Formative cemeteries at Topater (100 BC) and Chiu-Chiu 273 (10 BC to 140 AD) were analysed using GC-MS for cocaine and cocaine metabolites (BZE, EME, cocaethylene), opiates (morphine, codeine, 6-MAM, heroin) and cannabinoids (cannabidiol,  $\Delta^9$ -THC, cannabitol). All samples were negative for all compounds. This is not particularly surprising for the opiates and cannabinoids, as *Papaver* sp. (from which opiates are extracted) and *Cannabis sativa* (botanical source of cannabinoids) were unknown in South America during this period.

The choice of these drugs as target compounds is somewhat surprising, given that Chiu Chiu, a site situated in the confluence of the Loa and Salado Rivers, has the second highest frequency of snuff kits in the Middle Loa River area (Torres 1995; 1998). Snuff kits are the characteristic artefacts associated with the inhalation of snuffs made from the bark and seeds of *Anadenanthera*. These items are typically a wooden tray and a hollow bone or wood tube, often contained in a small bag. There are numerous examples from the arid regions of northern Chile, in particular the areas around San Pedro de Atacama (Llagostera *et al.* 1988; Torres 1995; 1998). *Anadenanthera* sp. contains the hallucinogens 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine), *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine, amongst others (Rätsch 2005; Torres and Repke 2006).



The uncritical application of standard forensic techniques to archaeological material is problematic, as these procedures are designed for testing hair for commonly abused drugs from modern western societies. This approach also highlights the lack of defined research questions informed by the assemblage or by the known or suspected cultural use of psychoactive plants. Furthermore it demonstrates the lack of consideration for the age and condition of the sample, potentially compromising data from subsequent analyses.

### **5.3. Hallucinogens**

In recent years there has been an interest in the analysis of ancient hair for hallucinogens (Castro *et al.* 2002; Ogalde *et al.* 2009). Archaeological evidence suggests hallucinogenic plants have been employed over a considerable time depth in the Andes, and a number of these practices still exist today – for example the use of San Pedro cactus in *curanderismo*, or folk healing, in Peru (Sharon 1978; Bussman and Sharon 2006).

The first study of archaeological hair for hallucinogens utilised GC-MS to test 31 hair samples from Topater and Chiu-Chiu 273 for harmine, harmaline, tetrahydroharmine and *N,N*-dimethyltryptamine, the active compounds in *ayahuasca* (Castro *et al.* 2002). The authors commented that due to the porosity and fragile nature of the hair, solvent washing prior to analysis was not performed, suggesting that the hair was in a poor state of preservation which may have implications for the preservation of drug compounds. None of the samples tested positive. There may be a number of reasons for this. Firstly, the sample size was

small and the individuals represented were not those who ingested *ayahuasca*. It may be that it was used by a minority, possibly élite males, as has been suggested for snuffing practices based on contextual evidence (Torres 1995; Torres Rouff 2002). There have also been compelling arguments by a number of authors that *ayahuasca* is a relatively recent cultural practice rather than an ancient tradition (Gow 1994; Brabec de Mori 2011).

They also raise the issue of differential/poor preservation of hallucinogenic alkaloids in hair. There is a paucity of research into the analysis of hair for hallucinogens and the long term stability of these compounds in hair. It is possible that these compounds are not stable in the long term and may degrade, leaving only trace amounts or breakdown products.

In a similar study of 32 individuals from the Azapa Valley in northern Chile, two individuals tested positive for harmine (Ogalde *et al.* 2009). These individuals date from the Tiwanaku Horizon (500-1000 AD). In this period there is abundant evidence, in the form of snuffing artefacts, for the consumption of *Anadenanthera* snuffs as well as evidence for coca chewing. One of the individuals who tested positive for harmine, an adult male (AZ-141, tomb 33) was buried with snuffing artefacts as well as a four pointed hat and pan pipes – all elements of social prestige in the Tiwanaku Horizon (Berenguer and Daulsberg 1989; Goldstein 1996; Ogalde *et al.* 2009). None of the samples tested positive for 5-methoxy-*N,N*-dimethyltryptamine. The authors state that “this information is extremely useful because it shows the snuffing kits used in Azapa Valley were not related to *Anadenanthera* consumption” (Ogalde *et al.* 2009: 469). Based on a sample of only

32 it is not possible to rule out the consumption of *Anadenanthera* in the Azapa Valley during the Tiwanaku Horizon.

#### **5.4. Alcohol**

Alcohol is the most widely used psychoactive substance in the world. It has been fundamentally important in social, economic, political, and religious contexts for millennia (Dietler 2006; Hayashida 2008). In the Andes, *chicha*, corn beer (although other plants have been used to ferment *chicha* and others have been added to enhance the effect of alcohol) was an important part of commensal politics and ritual feasting (Hastorf and Johannessen 1993; Goldstein 2003; Jennings 2005).

Larry Cartmell *et al.* (2005) developed a method of detecting markers of alcohol in ancient hair as a means of investigating drinking behaviour in archaeological populations. These markers, known as fatty acid ethyl esters (FAEEs), could help resolve “the issue of timing of widespread introduction of an alcoholic drink and also the patterns of use in these ancient populations” (Cartmell *et al.* 2005: 115). In a pilot study, seven spontaneously mummified individuals from coastal and valley sites around Arica were tested for FAEEs. All individuals belonged to the Maitas Chiribaya culture of the Azapa Valley (c AD 1000 - 1250).

Of the seven tested, three had quantifiable limits of FAEEs (two males, aged around 20 and 50 and a female, also aged around 50, see table 5.4). The results suggest that fatty acid ethyl esters are very stable over a long period of time and may provide a useful marker to determine alcohol consumption. The use of FAEEs is limited. FAEEs are deposited at the proximal end of the hair shaft from sebum, so

they are largely exogenous surface lipids, as opposed to being incorporated into the growing hair from the bloodstream (Auwärter *et al.* 2001; Wurst *et al.* 2004) making them unsuitable to use in conjunction with segmental analysis of hair to provide time resolved information on drinking habits.

**Table 5.4** – Fatty acid ethyl esters in Andean mummy hair (Cartmell *et al.* 2005).

Age	Sex	FAEEs ng/mg hair
15	M	<LOD
55+	M	<LOD
17	M	<LOD
20	M	0.010
50+	M	0.057
47	F	0.598
50+	F	<LOD

When cocaine and alcohol are simultaneously ingested, an enzyme mediated reaction in the liver produces cocaethylene, a pharmacologically active compound that is incorporated into hair (Jatlow *et al.* 1991; Politi *et al.* 2007). Cocaethylene was detected in the hair of the three child mummies from Lulllaillaco using liquid chromatography tandem mass spectrometry (LC-MS/MS) (Brown *et al.* 2008). This confirms that all individuals consumed a combination of coca and alcohol in the last months before their deaths.

## 5.5. Nicotine

Nicotine is an alkaloid found in the plants belonging to the Solanaceae family. Genera that contain nicotine include *Solanum*, *Datura* and *Nicotiana*. The tobacco plant (*Nicotiana tabacum*) is native to Mesoamerica and South America. The use of tobacco in antiquity as a component of pre-Columbian ritual is not disputed (Janiger and Dobkin de Rios 1976; Wilbert 1990; Rättsch 2005 384). As a result, there have been a number of methods developed to detect nicotine and its metabolite cotinine in naturally mummified human remains.

As part of scientific investigations of a number of mummies in the Reiss-Engelhorn-Museen, Mannheim, Germany, the hair of eight pre-Columbian mummies were tested for cannabinoids, opiates, nicotine and cocaine. No cannabinoids, cocaine or opiates were detected, but three mummies tested positive for nicotine (Musshoff *et al.* 2009; Musshoff and Madea 2010). Cotinine was not detected, and without detecting cotinine it is not possible to imply that nicotine was ingested in some form. The authors suggest that the analytical detection limits may not have been sufficiently low to detect this metabolite. In addition, they suggest that results from these analyses should be viewed with caution, as external contamination from cosmetic or conservation treatments cannot be ruled out. Nicotine solutions are an effective insecticide and have been known to have been used by 18<sup>th</sup>/19<sup>th</sup> century conservators to prevent insect damage to mummies in their collections (Buckland and Panagiotakopulu 2001). Nicotine is also a component of cigarette smoke and can cause non-smokers to test positive for nicotine in hair tests (Al-Delaimy 2002; Kim *et al.* 2009). In the days before smoking bans human remains may have been exposed to smoke and as a result have been contaminated by nicotine. The use of

inappropriate storage for samples is also an issue, with containers that have been used to store tobacco products known to have been re-used to store bioarchaeological samples.

Cartmell *et al.* (2001) have also analysed a large number of mummies for nicotine and cotinine. In a paper presented at the World Mummy Congress in Cartagena, Columbia in 1995, Cartmell and his team presented their study of 144 individuals from a number of archaeological populations. All 144 individuals were tested for cotinine using RIA (see table 5.5.).

**Table 5.5** – Tobacco exposure levels by culture group after Cartmell *et al.* (2001).

<b>Culture</b>	<b>Number tested</b>	<b>Number &gt; 2 ng/mg</b>
Chinchorro	23	11
Alto Ramírez	14	11
Cabuza	14	2
Chiribaya	33	6
San Miguel	4	1
Cam-9 “Inca”	13	1
San Pedro	43	35

Of the 144 tested, 140 (97%) had positive results. For quality control purposes, 20 positive and 3 negative samples were analysed by GC/MS. All negative samples were confirmed as negative, and 19/20 of the positive samples were confirmed. They suggest that the one sample that did not test positive using GC/MS had a cotinine level below the limit of detection of the instrument rather than being a

false positive. Nicotine levels were determined in 35 samples that tested positive for cotinine. Thirty-three (94%) had detectable nicotine levels. Again, the authors suggest that the negative results by GC/MS were a result of the two samples having nicotine levels below the limit of detection of the instrument.

Many food plants native to the Americas, in particular the *Solanaceae* family (tomatoes, aubergines, potatoes, bell peppers), contain nicotine (Sheen 1988; Domino *et al.* 1993; Siegmund *et al.* 1999). *Nicotiana tabacum*, the tobacco plant, is also a member of the Solanaceae family. Cartmell *et al.* (2001) proposed a cut-off limit of 2 ng/mg or more to confirm tobacco use. This limit factored in dietary nicotine and differences between modern cigarette tobacco and the ancient cultivar (although the criteria employed or the data this was based on is not stated in the original publication). Using these criteria, 67/144 (47%) fulfilled the criteria for tobacco use.

Although the sample sizes were not big enough to investigate age and gender difference within cultures, it was noted that infants under the age of two had higher levels than the 3-14 age group. The authors suggest that this could be due to transplacental transfer *in utero*, or the transfer of nicotine in breast milk. As the curation history of the mummies is known, and the hair collected from the mummies was taken during an anatomical dissection, it is unlikely that the nicotine and cotinine detected in these mummies is a result of contamination, as has been suggested for other mummies in museum collections (Buckland and Panagiotakopulu 2001).

## 5.6. Critique of methods: Sample selection and preparation

The washing of hair to remove contamination is a contentious issue in forensic toxicology. This step is generally included as it is essential to remove contamination resulting from environmental exposure that may result in a false positive result, yet a number of studies have shown that it is impossible to completely remove contamination from hair (Blank and Kidwell 1995; Baumgartner and Hill 1996; Kidwell and Blank 1996). However, these washing procedures have been applied to archaeological hair to remove possible contamination, adherent decomposition products and other material from the depositional environment. Springfield *et al.* (1993) used a standard methanol rinse as a decontamination procedure then analysed it alongside the ancient hair samples. It was found that the washing procedure acted more as an extraction rather than a decontamination procedure, removing a substantial amount of drug and metabolites from the hair. Springfield *et al.* (1993) demonstrated that BZE was lost from hair during the washing process with their work on ancient Peruvian individuals. This is perhaps not surprising, as hair buried for an extended period is often degraded and porous (Chang *et al.* 2005; Wilson *et al.* 2007a; Wilson *et al.* 2010). The condition of the hair varies greatly depending on the depositional environment, for example hair from the frozen Inca child sacrifices from Lullacayo was extremely well preserved, as it was possible to obtain stable light isotope data, mtDNA profiles and data regarding coca and alcohol use from hair specimens from these individuals (Wilson *et al.* 2007b; Brown *et al.* 2008). The Maiden, a 15 year old young woman yielded the highest BZE hair tests from any South American mummy ever tested (Wilson *et al.* 2007b). In contrast, the positive hair tests from spontaneously mummified individuals from



desert environments have much lower values, suggesting that some environments are more conducive to the preservation of drugs in hair than others.

Clearly taphonomic factors that influence the survival of hair are an important consideration and should be taken into account when interpreting data from hair drug tests. Some authors have flagged the lack of understanding of how drugs are preserved in hair (Balabanova *et al.* 1992b; Castro *et al.* 2002), however, forensic case reports and experimental work suggests that moist and acidic conditions are not conducive to the preservation of drug molecules in hair (Conlon 2006; Brown 2007). As yet there has been no investigation into the effects of arid conditions on the preservation of drug molecules in hair.

### **5.7. Critique: Analytical methods**

Radioimmunoassay is defined by IUPAC (1997) as “An assay based on the reversible and non-covalent binding of an antigen by a specific antibody employing radioactivity labelled antigen to measure the fraction of the antigen bound to a substoichiometric amount of antibody”. RIA has successfully been used to detect picogram levels of compounds in complex biological matrices, yet due to the fact that there can be cross-reactivity of some antigens, resulting in false positive tests. False positives have been reported on archaeological material (Cartmell *et al.* 1991a). Poor precision is a problem unless experimental conditions are carefully controlled to ensure reproducible binding reactions (Fifield and Kealey 2000: 472). Another disadvantage is that a proportion of immunoassays can only detect classes of drugs, not specific compounds (Pragst and Balikova 2006). Immunoassay testing

kits developed for urine (like the ones utilised by Cartmell's research group) are of insufficient sensitivity to be used on hair extracts. Due to the problems with precision and cross reactivity, RIA is generally used as a screening tool, as they are fast, easy to handle and can be automated, which saves both time and money, although there are issues with handling and disposing of radioactive compounds. The standard protocol in forensic toxicology is to then confirm and quantify any positive results with a different analytical method, typically GC-MS, although liquid chromatography (LC) is increasingly being used, due to the fact that LC does not require compounds to be derivatised prior to analysis, has shorter analysis times and lower limits of detection. The relative lack of sensitivity may be an issue where there may be much lower levels of analyte in the sample due to degradation over an extended period of time. This possibility that the analytical method lacked the sensitivity to detect very low amounts of target analytes has been acknowledged by some authors (e.g. Musshoff *et al.* 2009).

### **5.8. Interpretation**

A general critique of many of these studies is the lack of integration of the chemical data with archaeological and biological data. The hair tests alone may tell us that a percentage of individuals chewed coca or used snuff, but without integration with the other data there is a limit to our understanding of these practices. However, the study by Ogalde *et al.* (2009) did integrate their chemical data with age/sex data of each individual and some cultural traits (i.e. "*Orejones*", males with elongated ears

for wearing ear ornaments, significant grave goods such as snuff kits). This approach is useful in that it can help reconstruct short term life histories from chemical data obtained from hair as well as develop a picture of the social identity of the individual. On a wider scale it may help us understand certain social conventions regarding the use of psychoactive substance in the past - for example it has been suggested that 20% of adult males used snuff between the 3<sup>rd</sup> and 10<sup>th</sup> centuries AD based on the inclusion of snuff trays in funerary offerings (Torres 1995). Hair analysis may be able to provide an insight into who used snuffs and what this may say about the cultural and social identities during this time period.

### **5.9. Summary**

The application of forensic hair testing techniques to ancient hair has helped bioarchaeologists look at patterns of coca chewing in Ancient Andean populations, proving beyond doubt that the practice was ubiquitous prior to the arrival of the Spanish in the 16<sup>th</sup> century. Other investigations have been less successful. The major problem seems to be the uncritical application of methods developed for forensic work without consideration for the age, condition or archaeological context of ancient assemblages. For example, *Papaver sp.* and *Cannabis sp.* are not native to the Americas, so testing ancient hair for opiates and cannabinoids serves no purpose and only wastes a precious, finite resource. The lack of a research question informed by the archaeological assemblage also hinders meaningful interpretation in the wider biocultural context. However, authors such as Ogalde *et al.* (2009) have

made some progress by integrating anthropological data and contextual information with the results from their analyses.

# Chapter 6: Psychoactive Plants in Andean Material Culture

## 6.1. Introduction

Chapters 2 and 3 examined the botany and chemistry of psychoactive plants native to the various ecozones in the Andes. Many of them are still used today and it is suspected that their use has a very long history. A number of these plants, for example coca, have been identified in archaeological contexts and there is a documented material culture associated with their use. Tom Dillehay *et al.* (2010) recovered the earliest examples of coca leaves<sup>5</sup> from a secure context from house floors in the Nanchoc valley of northern Peru. These leaves were radiocarbon dated to the end of the Las Pircas phase (c. 8000 – 7800 cal BP). It was also noted that lime pressed into spheroid shapes was associated with the leaves. The authors conclude that coca chewing accompanied by lime production technologies accompanied the rise of agriculture and social complexity in this region. However, not all psychoactive plants have such a visible presence in the archaeological record. Many plants have been identified from iconographic representations in textiles, ceramics and sculpture – from San Pedro cacti on Chavín textiles to stylised *Anadenanthera* imagery on Wari ceramics and Tiwanaku sculpture.

The archaeological evidence for the use of various psychoactive plants in the past is varied. This chapter aims to bring together examples of the variety of artefacts described in the literature. This section is broken down into time periods (horizons)

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<sup>5</sup> These leaves were identified by Botanists based on macroscopic and microscopic morphological traits and the archaeological specimens and modern coca leaves

devised by John Howland Rowe (1962, see s1.3). Artefacts, architecture, botanical remains and chemical analyses of archaeological material are discussed in the context of more general events from the time period, starting with the Late Preceramic.

## **6.2. Preceramic (9500 – 1800 BC)**

The Preceramic dates from around 9500 to 1800 BC. The latter half, known as the Late Preceramic period or “Cotton Preceramic”, is associated with stable ecological zones and sea levels after the end of the Pleistocene. Michael E. Moseley suggests that the contemporary marine and meteorological conditions were in place roughly 5,000 years before present (Moseley 2001: 107).

The Late Preceramic in Peru is identified in the archaeological record by Jeffrey Quilter (1991) as being associated with large formal architectural spaces, distinctive ceremonial architecture and mortuary practices, domesticated plant remains and small artefacts such as beads and snuff trays. He also characterised this time period as an era of increasing social complexity, including the beginnings of social differentiation (as inferred from burials) and the spread and influence of ceremonial systems over large areas, as well as the establishment of trade networks and exchanges between different ecological zones as a means of obtaining crops and other plant based items that grow in specific ecozones (Quilter 1991: 387).

During the Late Preceramic there is extensive evidence of the early cultivation of plants. Deborah Pearsall provides an excellent summary in her paper entitled “*The origins of plant cultivation in South America*” (Pearsall 1992). Coastal peoples grew

cotton (*Gossypium barbadense*), gourd (*Lagenaria siceraria*), squash (*Cucurbita* sp.) and various beans (*Phaseolus* sp.) (Moseley 2001: 108). As well as the cultivation of food plants there has been some indication that coca, (*Erythroxylum* sp.) was being cultivated. What is believed to be the remains of coca were found at Ayacucho cave, suggesting early peoples recognised the stimulating effect of chewing coca as far back as 5,300 – 3,900 cal BC (Pearsall 1992 181; Owen 2006). Some authors, in particular botanists, have questioned whether coca was present at these sites. Timothy Plowman noted that specimens from the Ayacucho region were recovered from human coprolites (MacNeish *et al.* 1975). Plowman suggests that it would be difficult to identify coca in this context, as it is generally chewed and not swallowed (Plowman 1986b: 136). However, nutritional research has shown that coca provides many components otherwise lacking in an Andean diet. Experiments have shown that 100g of Bolivian coca (*E. Coca*) would more than satisfy the recommended daily allowance of calcium, iron, phosphorus, vitamin A, vitamin B2 and vitamin E (Duke *et al.* 1975). The nutritional value of coca may have been recognised by ancient people and may have been considered a foodstuff rather than a stimulant. The material is no longer available for re-examination, as it has been lost (Plowman 1986b: 136).

Whether or not coca was being cultivated and used during the Late Preceramic in the Ayacucho region is debatable, yet there is evidence coca was cultivated and used by earlier populations in the Nanchoc valley in northern Peru (Dillehay *et al.* 2010). There is some evidence that other psychoactive plants were being utilised. Snuff trays and other items suggestive of the use of psychoactive plants have been

found at a number of Preceramic sites. Junius B. Bird found what he describes as snuff trays and bird bone snuff tubes at Huaca Prieta<sup>6</sup> (see fig. 6.1) in the Chicama Valley on the Peruvian coast in 1946 (Bird 1948: 27). Bird does not specify how many of these items he recovered, however at least one of these is now housed in the American Museum of Natural History in New York. One of the trays found was associated with the remains of an adult male, buried lying prone in a fully extended position. Other associated finds include tubular bird bone beads, jet mirror fragments, textile fragments and two bone snuff tubes (Bird 1948: 27). Another snuff tray from Huaca Prieta was found in a storage vault around 25 meters from the burial that included the previous snuff tray. Pottery from the initial ceramic period was found in the same context, dating the tray to c 1200 BC (Torres 1987b: 24).

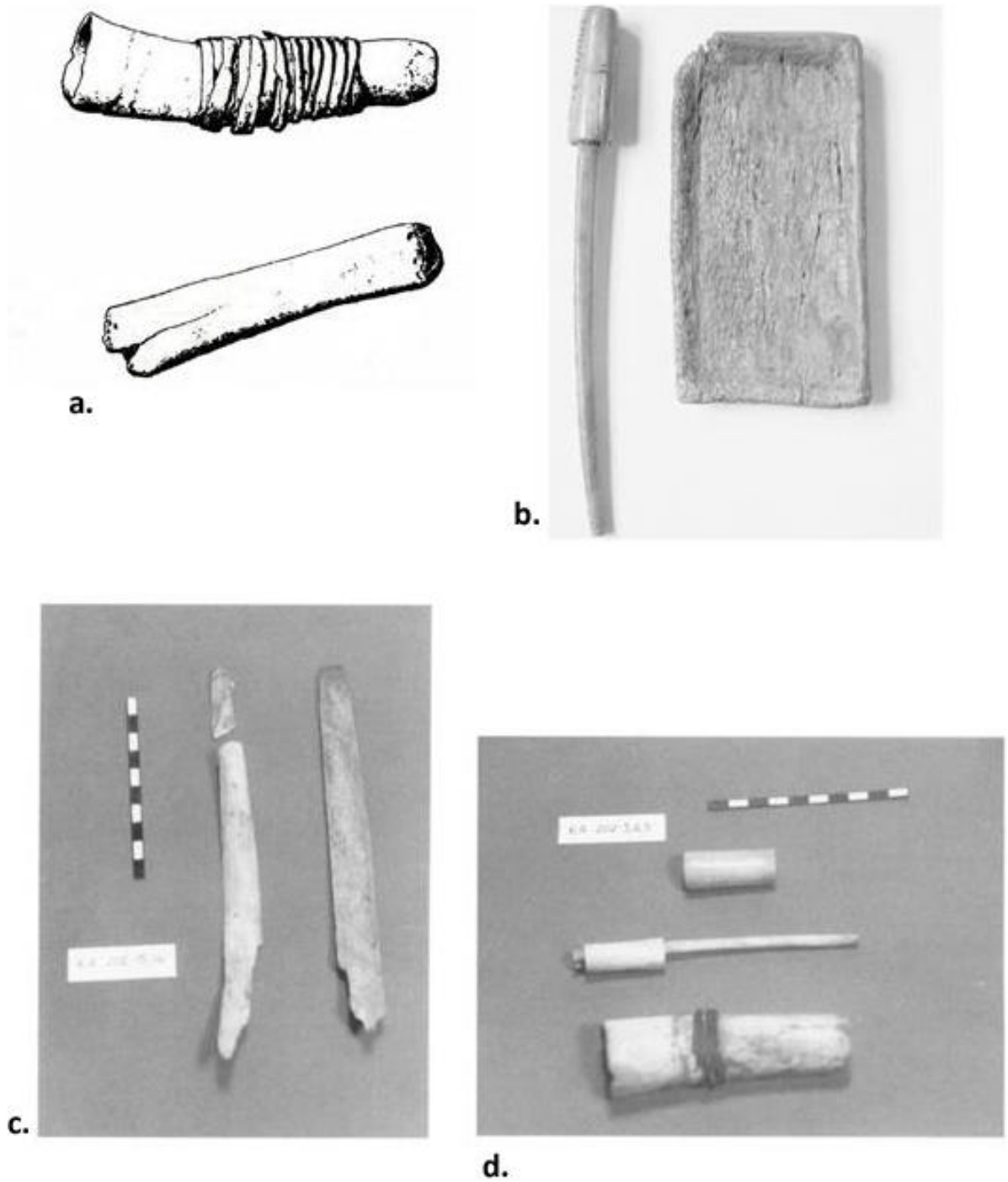
Frédéric Engel excavated two snuff trays from Unit 1, a mound at Asia, a site in the Omás River basin on the central coast of Peru. The mound dates to the last phase of the Preceramic period in this area (Engel 1963: 12). Fifty-two pits were excavated within Unit 1. Those with human remains were described as “graves”, those without were deemed “pits”.

Trays and tubes were found in graves 17 and 47 and pits 4 and 36. Radiocarbon dates from material found at Unit 1 yielded a date of 1225 ± 25 BC (Engel 1963: 12). Both trays found in graves were associated with adults, although no data on the sex of the individuals in the graves is given.

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<sup>6</sup> Bird calculates an estimate of 3750 BP (c 1800 BC) for the introduction of Cupisnique pottery in this area based on stratigraphy and the formation of ground cover/debris (Bird 1948: 28).





**Fig. 6.1** – Snuffing artefacts; a. Bone tubes from Inca Cueva, NW Argentina c 2130 BC (Torres 1998); b. Whale bone snuff tray, bird and fox bone snuff tube. Huaca Prieta, Chicama Valley, Peru c1200 BC (Bird 1948); c & d: Spatulae and bone tubes, Kilometer 4, Ilo, Peru (c 3000 – 1000 BC) (Wise *et al.* 1994).

In both cases the tray was found alongside wooden tube and bottle-shaped gourds (Torres 1987b: 24). The discovery of utilitarian objects within the grave is suggestive of a belief in the afterlife, in that the inclusion of snuffing equipment in the funerary assemblage may be a way of assisting the deceased into the next world. Engel also found leaves he thought were coca along with deposits of burnt lime. Rury and Plowman (1983) believe that the identification of coca leaves at Asia 1 is dubious, particularly as the specimens no longer exist.

Other early evidence for the use of psychoactive plants in the Andean region comes from the sites of Inca Cueva and Huachichocana in the province of Puna de Jujuy in northwest Argentina (Aguerre *et al.* 1973). Inca Cueva is a cave with no stratification or associated human remains. Two bone pipes (see fig 6.1), *Anadenanthera* and *Prosopis* seeds, amongst other items were recovered from the rear of the cave. Radiocarbon dates indicate an age of 4080±80 B.P. Huachichocana is also a cave, but with clear stratigraphy. The remains of a male aged approximately 15 years old were found in a layer radiocarbon dated to 3400±130 B.P. Four stone pipes were found associated with this individual. The pipes were covered with a red pigment. Presumptive tests on the pigment yielded a positive result for alkaloids; however a particular compound is not specified (Fernandez-Distel 1980: 75; Torres 1995; 2001).

Karen Wise *et al.* (1994) reported the discovery of snuffing equipment (see fig. 6.1) in a burial from Kilometer 4, a site dating to c 3,000-1,000 BC located just north of Ilo on the Peruvian coast. The burial contained the remains of an adult male aged approximately 45-50. This individual was buried in a flexed position on his right side,

facing southwards toward the ocean. The authors mention that this individual was buried with an unusual assortment of artefacts including bone and shell implements, textiles, unidentified plant material as well as bone spatulas, tubes and what appears to be a bone enema tube. Due to the rich assortment of textiles and other items compared with other burials in the area, the authors suggest this man may have been a local élite or possibly a shaman (Wise *et al.* 1994).

Seeds (not identified botanically) containing harmine were found in a shell heap at Quebrada las Conchas near Antofagasta on the Chilean coast (Llagostera 1979). The seeds were analysed at the Karolinska Institutet of Stockholm. The analytical procedure was not specified in the original publication (Llagostera 1979). The heap also contained stone tools, abalone shells (*Concholepas concholepas*), fish, bird and marine mammal bones as well as burned human bones. It was inferred by Wise *et al.* (1994) that the psychoactive properties of these seeds were known to the local population as they were found in a context associated with human activity.

Further south, on what is now the coast of northern Chile, snuff kits have been found buried with Chinchorro individuals from sites around Arica. These burials date from the late Chinchorro period (c 2500 BC), but are not discussed in detail (Rivera 1991; Arriaza 1995a: 144).

### **6.3. Initial Period (1800 – 900 BC)**

The Initial Period is so called as it was the first time Andean populations developed and used ceramic technologies. During this period the peoples of the coast largely subsisted by exploiting abundant maritime resources, whilst the people of the

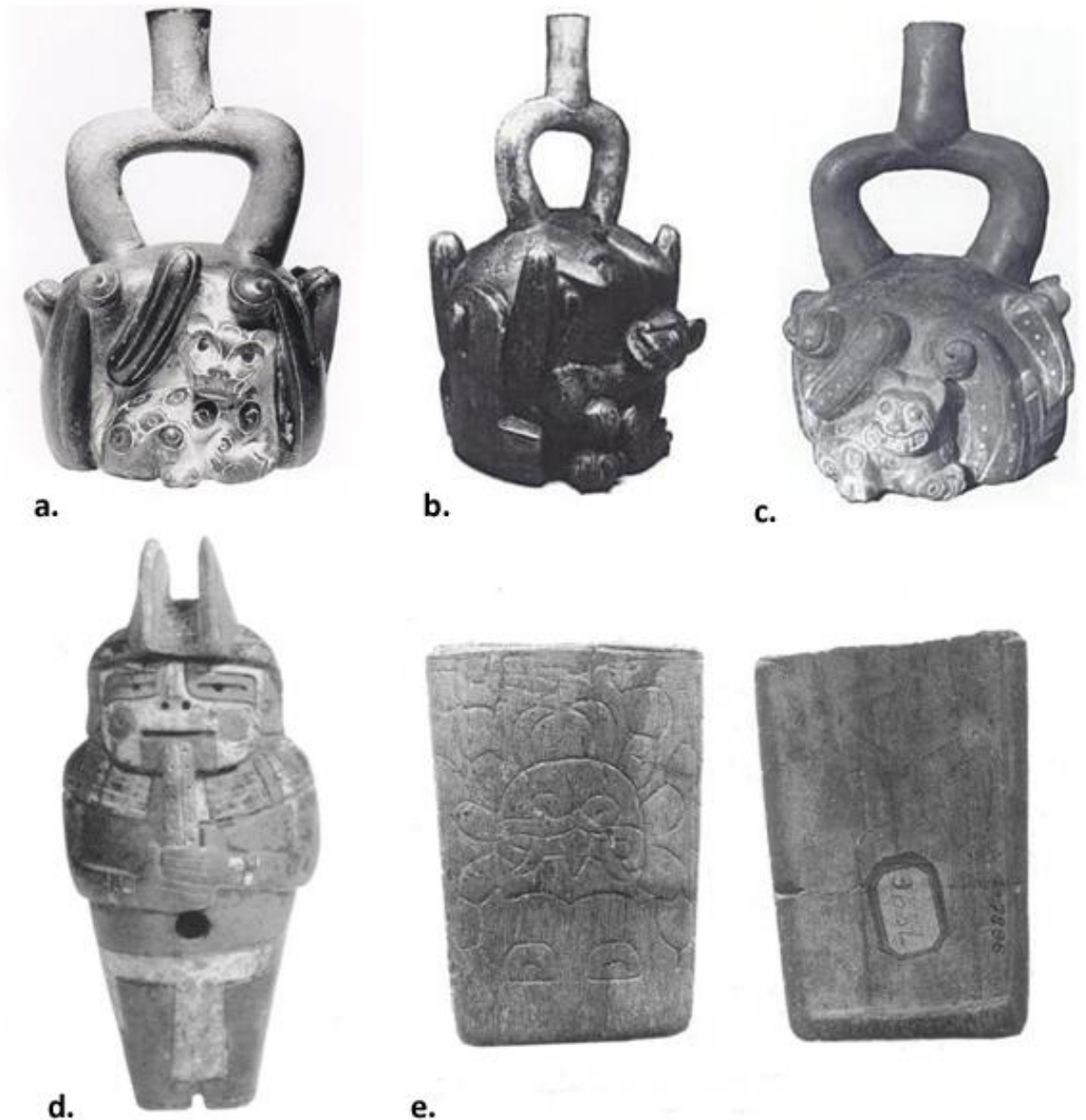
fertile river valleys were practicing agriculture, herding and pastoralism (Fung Pineda 1988: 67; Pozorski and Pozorski 2002: 79). The variety of cultivars appear to have increased during this time period as more appear in refuse contexts – for example avocado (*Persea americana*), guava (*Psidium guajava*), pacai (*Inga feuillei*) and peppers (*Capsicum* sp), as well as cotton, squash, gourds and beans (Fung Pineda 1988: 82).

The Initial Period also saw the construction of monumental architecture along the north and central coast of current day Peru, helped by the population increase as a result of the mastery of irrigation and other agricultural techniques (Moseley 2001: 133-136; Kembel and Rick 2004: 53). With this came the establishment of power structures (Fung-Pineda 1988: 94 suggests a politico-religious system) associated with temple pyramid complexes (e.g. Garagay in the Rímac Valley and Sechín Alto in the Casma Valley, amongst many others) that were erected along the coast (Fung Pineda 1988: 94; Pozorski and Pozorski 2002: 78-97).

These complexes were presumably to house large audiences to witness rituals and ceremonies. It is these coastal temple sites that provide some evidence for the use of psychoactive cacti, most probably as part of rituals enacted at these ceremonial centres.

At Garagay, a ceremonial complex near Lima, spines from the San Pedro cactus spines were found as part of “doll-like figurines” thought to have been votive offerings at the temple complex. These figures are made of clay, with painted faces and dressed in textiles. One of these figurines carries San Pedro cactus spines on its back, suggestive of the “staffs of power” associated with the principal deity at

Garagay (Burger 1995: 64). The fact that they are from a psychoactive cactus is perhaps not coincidental (Burger 1992: 64; Torres 1995). Similar cactus spines were also found embedded in adobes left in the fill of a Pyramid, part of the middle temple at Garagay (Burger 1995: 64).



**Fig. 6.2** - Psychoactive symbolism on stirrup spout vessels and an early snuff tray; a-c. Tembladera/Cupisnique stirrup spout vessels ornamented with cacti, jaguars and volutes (Cordy-Collins 1998: 160-161); d. Example of a male figure holding a “San Pedro” stalk (Sharon 2000: 47); e. Incised bone snuff tray found at Supe in 1905 by Max Uhle (Burger 1995: 97).

The votive figurines were found associated with polychrome friezes representing spiders with feline attributes and warrior figurines (Pozorski and Pozorski 2002: 89), symbols typically associated with shamanistic transformation in later traditions. The combination of botanical material with psychoactive properties in a ceremonial context possibly hints at the use of these plants for ritual purposes – possibly as a means of contacting spirits or ancestors (Dobkin de Rios 1982; Burger 1992).

Douglas Sharon (Sharon and Donnan 1977; Sharon 2000; 2001) has extensively reviewed the ethnoarchaeological evidence for the use of San Pedro on the northern Peruvian coast. He reports that 32 vessels depicting the San Pedro cactus have been found dating to the late Initial Period. All of the vessels he describes have been attributed to the Cupisnique culture (also known as Tembladera, c 1200-900 BC). The Cupisnique/Tembladera tradition represents a coastal pre-Chavín style found in the Jequetepeque, Zaña and Lambayeque river valleys on the northern coast of Peru (see fig 1.1) (Cordy-Collins 1998: 156).

The San Pedro cactus is depicted in stirrup-spout vessels with felines, serpents and volutes (spirals, see fig. 6.2a, b and c), themes that appear to have persisted from Cupisnique through to the Moche period (Cordy-Collins 1998). Douglas Sharon (2000: 4) describes a stirrup vessel with a feline and volute design found in a middle Cupisnique burial at Puémape, a site situated on the coast halfway between Chiclayo and Trujillo. The individual was female, and buried in a flexed position on her right side. Her grave goods included weaving equipment, an incised gourd, stone objects with red pigment and an anthracite mirror, an object associated with high status during this period.

Three stylised male figures holding what Sharon (2000: 3, see fig 6.2d) believes to be San Pedro to their mouths have also been found at sites in the Jequetepeque valley. Other authors believe these items are musical instruments (Quilter 2005: 42). These figures are thought to be associated with the Cupisnique tradition (Sharon 2000: 3; Quilter 2005: 42). Other archaeological evidence to support the use of hallucinogens during this time period is scarce. However, a bone snuff tray incised with an anthropomorphic crab was found by Max Uhle in 1905 at Supe, which is possibly indicative of *Anadenanthera* use (Burger 1988: 103). Burger also illustrates a small spoon decorated with a stylised feline face (felines are associated with shamanic transformation) found by Rosas and Shady at the highland site of Pacopampa. Burger believes that it would have been used in the preparation and ingestion of snuff (Burger 1995: 107).

Coca leaves have been tentatively identified at Las Gaviotas near Ancón and Ancón itself. These sites are located about 40 km north of Lima. The early phase of the site has been dated to c 1700-1900 BC, whilst the leaves at Las Gaviotas date to 1400-1800 BC. As with other archaeological specimens of coca, these samples were not botanically identified and no longer exist (Rury and Plowman 1983).

#### **6.4. Early Horizon (900 – 200 BC)**

The Early Horizon saw the rapid spread of civic-ceremonial architecture in the highlands as the earlier coastal sites were abandoned. This era also saw changes in beliefs and ritual practices. Moseley (2001: 131-132) suggests that the increased concerns with the cosmos and religion accompanied social and economic

transformations. The increasing dependency on agriculture (the foundation of wealth) may have set the precedence for the worship of Pachamama and powerful mountain spirits (*apus*) (Moseley 2001: 131-132; Kembel and Rick 2004). There also appears to have been a shift in societal structures, with a certain section having significant wealth and power differences in positions of control (Kembel and Rick 2004: 53). It has been suggested that those in power were the architects of the new artistic styles and beliefs.

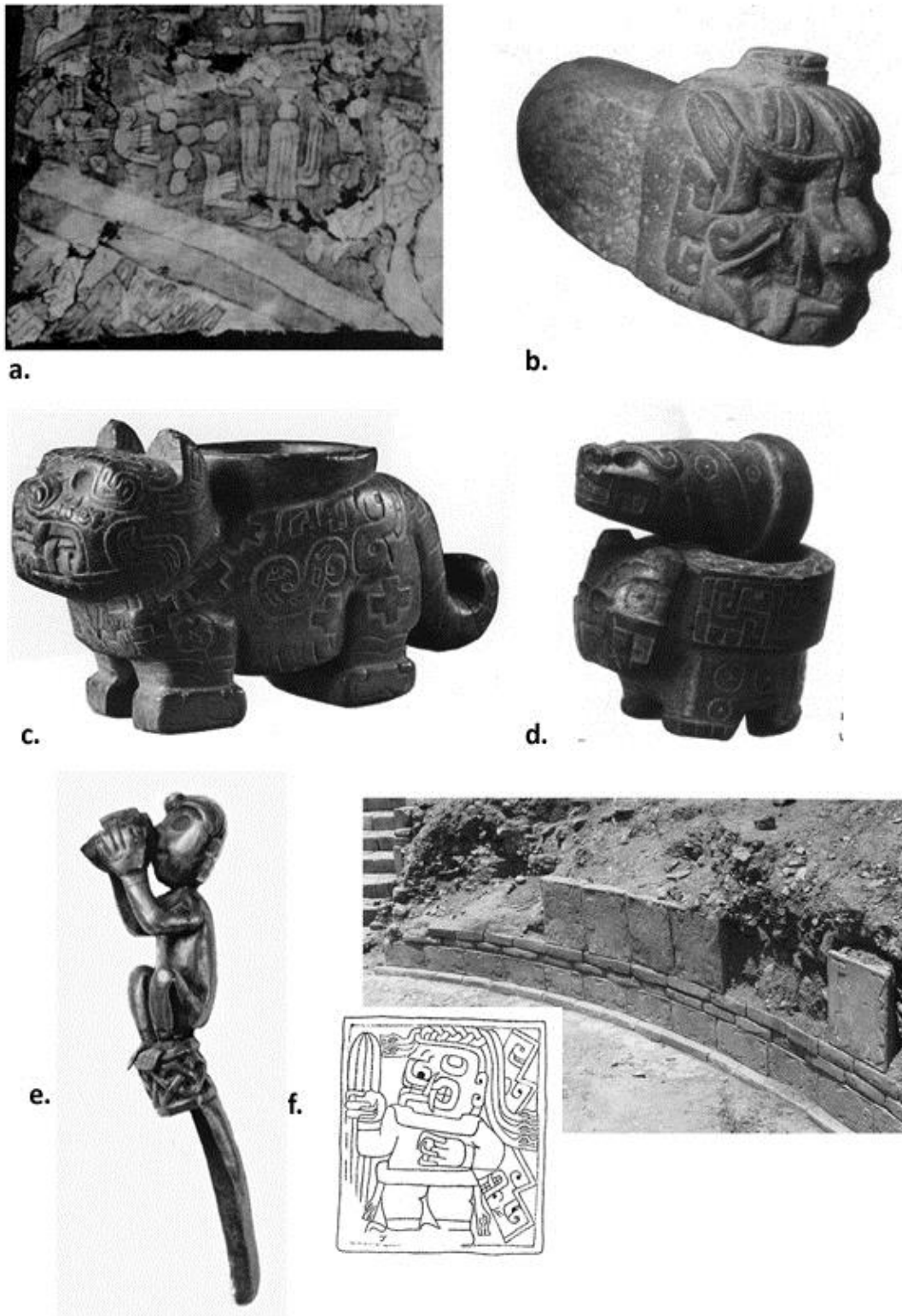
It would seem ritual was the main function at some of the major Early Horizon sites. By far the most important was Chavín de Huántar, centre of the Chavín culture. There is evidence that Chavín had a very wide sphere of influence. Other ceremonial sites such as Kuntur Wasi in the Jequetepeque valley and Pacopampa near Chota are contemporaneous with Chavín de Huántar. Burger (2008: 698) suggests that there is ample evidence of the exchange of commodities and emulation of style between these centres. There is evidence of Chavín in the high puna grasslands around Junin and Pasco, further south around Huancavelica and Vilcashuaman and at sites such as Cerro Blanco in the Nepeña Valley and Carhua (Karwa) in the Ica Valley (Church 2002: 46-47; Burger 2008: 697).

Situated at the confluence of the Huacheqsa and Mosna rivers at an altitude of 3,150 metres above sea level, Chavín de Huántar consists of plazas, terraces and stone platform buildings. They form two U shaped complexes, both of which surround a sunken courtyard. Stone-lined galleries and passages extend throughout the entire complex (Moseley 2001: 163; Kembel and Rick 2004: 59).



There is extensive evidence that the San Pedro cactus, *Echinopsis pachanoi*, was an important part of Chavín ritual. There are numerous examples of cactus representations at Chavín de Huántar. Perhaps the most well-known is the depiction of a ritually-attired anthropomorphic male holding what is described as a San Pedro cactus in a frieze located in the sunken courtyard (Burger 1992: 273 see Fig. 6.3f). The sunken courtyard also has numerous depictions of felines, most probably jaguars. The San Pedro cactus is also shown in the tenoned heads found throughout the temple complex (Sharon 2000: 5; 2001). One example shows a face in the stages of transformation with cacti behind the eyes (see fig 6.3b). This seems to strongly indicate that the San Pedro cactus was used to produce visions, probably for ritual purposes, given the context of the heads. Sharon (2001) stated that the locations of the cacti are appropriate; given the cactus is the catalyst for “second sight”. Additionally, remains of San Pedro cactus rolled into “cigars” were found at the Chavín site of Las Haldas on the north coast of Peru. These finds are thought to date to c 800 BC (Sharon and Donnan 1977).

The San Pedro cactus is also depicted in an example of Chavín textile known as the “Shamanism textile” (Cordy-Collins 1982). The textile was reportedly found in 1969 at Carhua, in the Ica Valley on the south coast of Peru. It was part of a cache of two hundred Chavín textiles found by looters. Of the 200 textiles in the cache, one was painted with “images of transformation and transcendence” (Cordy-Collins 1982).



**Fig. 6.3** - Snuffing symbolism in stone, textile and metals. a. The “shamanism textile” (Cordy-Collins 1982); b. Tenon head, Chavín de Huántar (Burger 1995 176); c&d. Stone mortars (Burger 1995 157, 200); e. Gold and silver snuff spoon, possibly Chavín (Burger 1995 201); f. Sunken court at Chavín de Huántar. Insert image shows a male figure holding a cactus (Burger 1992 273).

The textile is interesting, in that a number of representations of hallucinogenic flora are represented alongside common symbols of shamanism, such as jaguars, deer, hummingbirds and a Chavín “staff god”. Cordy-Collins (1982) positively identifies the cacti represented in the textile as San Pedro (*E. pachanoi*). However, there are other plant species depicted in the textile, including a plant tentatively identified as *Anadenanthera peregrina*.

The plants represented bear a vague resemblance. It has been noted that Chavín art occasionally makes use of “simultaneous Picasso-like views” which makes identification difficult. However, given the other elements represented in the textile, it is likely that the representation is of a hallucinogenic plant. Alana Cordy-Collins (1982) also mentions the perspective of plants and other elements in the textile and notes that “true scale was not of a particular concern to the Chavín artist”, however, the unusual scale may be of significance. Macropsia and micropsia (seeing things larger or smaller) are common aspects of the psychoactive experience (Dobkin de Rios 1996: 132; Pearson 2002: 119). It may be that the artist was trying to convey these perspectives in the textile.

As well as San Pedro, some authors believe that other psychoactive substances were used by the Chavín cult. Julio C. Tello, the Peruvian archaeologist who discovered and excavated Chavín de Huántar argued that the role of hallucinogenic snuffs in shamanistic transformation is clearly expressed in the monumental sculpture at the site (Tello 1960). Shamanic transformations appear to be a central feature of Chavín ideology. Through the use of hallucinogenic snuffs and decoctions, shamans believed they transformed into an animal intermediary, such as a jaguar or

eagle, which enabled them to contact the spirit realm. Felines are common representations in ceramics and stone sculpture in shamanistic traditions throughout South America (cf Benson 1972).

Richard Burger (1992: 157) concurs with Julio C. Tello (1960) that snuffing was practiced at Chavín de Huántar. He believes that the Chavín tenon heads are very suggestive of the use of hallucinogenic snuff. Burger's (1992) typological analysis suggests that the heads represent different stages of transformation – from human to jaguar. Some of the heads represent naturalistic faces, while others show contorted faces – “as if they were experiencing the onset of nausea” (Burger, 1992: 157). Burger also discusses another key feature of these heads; the depiction of nasal mucus running down the faces of a number of statues. When hallucinogenic snuffs are taken, the mucus membranes in the nasal passage become inflamed, causing mucus to flow down the face. The 17th century friar Pedro Simon witnessed this first hand in the Muisca people of Colombia:

“they take these powders and put them in their noses and which, because they are pungent, make the mucus flow until it hangs down to the mouth, which they observe in the mirror, and when it runs straight down it is a good sign” (Simon [1882-1892] cited in Burger 1995: 157).

The flow of mucus depicted in these heads, Burger argues, is the most conspicuous external index of an altered mental state. Likewise, facial contortions and bulging eyes are also suggestive of the use of psychoactive substances. Ethnographic accounts by witnesses of these practices support Burger's argument (Chagnon 1968: 5; Cordy-Collins 1980; Burger 1995: 157; Torres 1995; Berenguer 2001). The

flow of nasal mucus appears to be part of the theatricality of the ritual, and therefore has been depicted in the monumental architecture of the Chavín culture (Berenguer 2001).

In a later article, Burger (2011) describes a low relief stone carving from Chavín de Huántar that was publicly displayed for the first time in 2008. The image is of a fanged anthropomorphic figure surrounded by botanical elements, which Burger identifies as *Anadenanthera*, as these elements look like *Anadenanthera* seed pods. Although *Anadenanthera* seed pods usually contain 8-16 seeds, only 2 or 3 seeds are depicted in the seed pods in the image.

Burger (2011) suggests that the plant represented is *Anadenanthera colubrina* var. *cebil*, as this species has a wide distribution in the Andes, as far north as the Upper Ucayali and the Marañón drainages. Although this area is not in the immediate area of Chavín de Huántar, Burger suggests *Anadenanthera* seeds may have been a trade item, given the wide sphere of influence of Chavín. He notes that *Anadenanthera* seeds have been traded by later populations in the Orinoco basin (Chagnon *et al.* 1970). He suggests that the inaccurate depiction of the number of seeds in the seed pods could be due to the artist being familiar with the seeds and not the whole plant, or stylistic/artistic conventions.

Numerous small objects bearing Chavín iconography have been found on the coast and in the highlands of Peru. The most common objects are elaborately carved mortars that are too small to adequately grind corn or other cereals, therefore it has been suggested that they were used to grind snuff powder. Burger (1988: 124) describes the finest examples of these mortars found by Rafael Larco Hoyle at

Pacopampa. One mortar is carved in the form of a bird, whilst the other is a feline, possibly a jaguar. The pestles are serpentine in form (see fig 6.3 c&d). Similar mortars have been found at Chavín de Huántar and nearby sites associated with Chavín influence, such as Olayán and Matibamba. Similar mortars were reported by Cristóbal de Albórnos during the 16<sup>th</sup> century to have been used by native priests to prepare *vilca*, a hallucinogenic snuff made from *Anadenanthera* bark (Burger 1992: 272). Small elaborately decorated spoons thought to have been used for the inhalation of snuff have also been found at Chavín sites, including Chavín de Huántar (Burger 1995: 200-201).

On the northern coast of Peru there are depictions of San Pedro in the ceramics from the Salinar culture (c. 400-200 BC). Salinar influence is evident on the northern coast from the Lambayeque to the Nepeña valleys (see fig. 1.1). A number of Salinar vessels realistically depict San Pedro cacti, including several vessels that have a cactus as a handle (Sharon & Donnan 1977). Further north, dental calculus on the teeth of individuals from a late Chorrera cemetery (c 840 BC) in Ecuador was analysed by X-ray fluorescence (Klepinger *et al.* 1977). The elemental composition was consistent with dental calculus. There were higher than normal magnesium levels, which the authors suggest were a result of chewing plant material (magnesium is a component of chlorophyll), possibly coca. Further analysis of these specimens using scanning electron microscopy to examine plant microfossils such as pollen and starch may help confirm this hypothesis (Henry and Piperno 2008; Hardy *et al.* 2009; Henry *et al.* 2011).

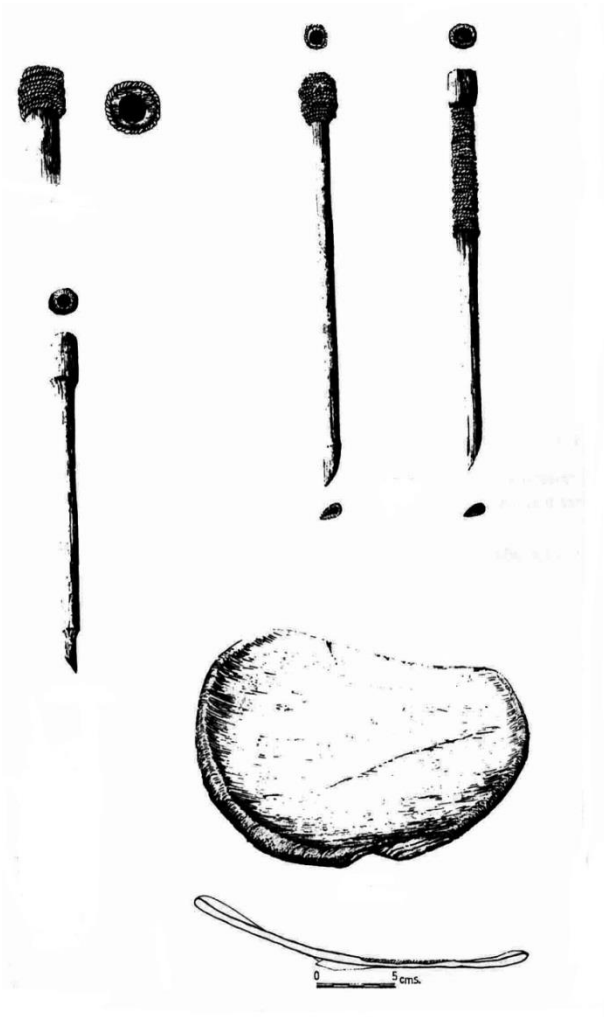
Further north, small ceramic lime pots and figurines depicting *in situ* coca quids from the Valdivia period (3500–1500 BC.) suggest coca has been used for at least 3,500 years in coastal Ecuador (Lathrap *et al.* 1975).

Whilst the Chavín culture flourished in the northern highlands and along the northern coast, the southern coastal valleys of Pisco, Chincha and Cañete and the Paracas peninsula were home to the Paracas culture. Most of what is known about Paracas has come from two major sites: Wari Kayan necropolis and Paracas cavernas, both of which are cemetery sites. Both have yielded exquisite woven textiles that were used to wrap mummy bundles (Pozorski and Pozorski 2002: 226; Cáceres-Macedo 2004: 57). A recurring theme on Paracas textiles is “the ecstatic shaman” – a human figure with a sharply arched back with the head thrown back and the arms stretched out (Paul and Turpin 1986). Paul and Turpin (1986) have suggested that this shaman is in a trance-like state as a result of “the monotonous sounds of music, sensory deprivation, hallucinogens or powerful intoxicants”. Further discussion of items associated with the shaman point to the possible ritual use of hallucinogens. The shaman is often seen holding a long tube, which Paul and Turpin interpreted as a flute or a tube for ingesting hallucinogens (Paul and Turpin 1986).

Definite archaeological evidence of the use of hallucinogens by the Paracas people is somewhat tenuous. However, based on the amount of evidence from both earlier and later traditions it would seem highly likely that San Pedro or other mescaline-bearing cacti were used as part of ritual and healing practices. It is possible that the people of the Paracas peninsula may have had access to coca. Margaret Towle

examined a mummy bundle (*fardo*) found at Paracas Necropolis in 1927. She identified leaves rolled into a quid and carefully wrapped, however the leaves were very fragmented and too small and brittle to positively identify (Towle 1952).

Further south, on the coast of modern Chile, Percy Dauksberg (1985) reported a snuff tray and three snuff tubes were from a selection of seven burials excavated by the Museo Regional de Arica between 1959 and 1961. The tombs were located on the coast of Arica in northern Chile, proximate to the Chinchorro sites of Morro-1, Morro-3 and Morro-4. These inhumations date to around 800 BC, coinciding with the early Faldas del Morro phase (c 700 BC – AD 400). The



**Fig. 6.4** – Bone tubes and snuff trays from the Faldas del Morro burials (Dauksberg 1985).

burials consisted of a single body buried in a flexed position with funerary offerings and covered in reed mats (Dauksberg 1985). This is a significant departure from the earlier Chinchorro burials that were buried in an extended position. In some cases the bodies were artificially mummified (Arriaza 1995b; Guillén 2004). The snuffing equipment was found in tombs 3, 4 and 5. These are detailed in table 6.1. In addition, three tubes were found but lack contextual information (Dauksberg 1985).



Junius B. Bird found snuffing equipment at Playa de los Gringos, a site roughly contemporaneous with Faldas del Morro (c 800 BC). These items include a soft wood model snuff tray and tube that was probably specially made for the grave. These items are associated with a young male (aged around 15-20).

**Table 6.1.** – Three burials with snuffing equipment from the Arica coast, c 800 BC (Daulsberg 1985).

<b>Tomb</b>	<b>Dimensions</b>	<b>Age/Sex</b>	<b>Snuffing Equipment</b>	<b>Other grave goods</b>
<b>3</b>	80 x 150 cm	“adult”	Snuff tray	Bird bone necklace, 2 lithic points, vegetable fibre brush, two sticks, harpoon, decorative belt, leather pouch and a piece of leather
<b>4</b>	100 x 160 cm	Adult male	Snuff tube	Basketry, scallop shell, vegetable fibre brush, bird bone necklace, harpoon, fishing line, bone tools and 3 spindle whorls
<b>5</b>	80 x 150	Adult male	Snuff tube	Buried wearing a turban with cactus spine needles, basketry, 2 shells, bone tools, bag of wool, vegetable fibre brush, bird bone necklace

The grave of the young male from Playa de los Gringos was very disturbed and the body and objects damaged and scattered. Bird notes that it was impossible to know if all the items found in the grave of the young male belonged to this particular individual (Bird 1943: 225). Bird also noted numerous coca bags (in graves 3 and 6) as well as possible preserved coca leaves in grave 1. In addition, a workman found a

snuff tray and tube wrapped in animal skin in an eroded grave at Quiani in 1940, however fuller contextual information is lacking (Bird 1943: 248).

### **6.5. Early Intermediate Period (200 BC – AD 600)**

The end of the Early Horizon saw the abandonment of the large temple sites, including Chavín de Huántar. In very broad terms, the abandonment of these sites appears to correlate with a lengthy transformation in Andean societies in which new technologies and innovations in art and architecture emerged, as well as economic and political changes resulting in new social arrangements (DeLeonardis and Lau 2004: 77). During the Early Intermediate period residential communities vastly outnumbered ceremonial centres for the first time. There also seems to have been a shift in ideology that was reflected in art and iconography. Rather than the “other-worldly” emphasis of earlier art styles, both art and architecture gave way to more earthly concerns (Moseley 2001: 173). This is particularly evident in the erotic and conflict-themed vessels of the Moche (cf Bourget 2006).

Perhaps the most important culture of the Early Intermediate Period was the Moche (or Mochica) culture of the northern coast of Peru (c 1-800 cal AD). The centre of the Moche culture was at Cerro Blanco, at the mouth of the River Moche. Moche influence is evident from the Piura River in extreme northern Peru to the Huarmey river valley on the central coast (see fig. 1.1).

Psychoactive plants with possible ritual connotations are portrayed in the ceramics of the Moche. Bonnie Glass-Coffin *et al.* (2004) provide several examples of ceramics found at the temple complexes of Huaca de la Luna and the Huaca del Sol

at Cerro Blanco. The ceramics depict female healers (*curanderas*) holding stalks of the San Pedro cactus. Some of the ceramics portray expressions of serenity, interpreted by Glass-Coffin as one of shamanic ecstasy or of the *mocha* (an act similar to blowing a kiss), a Pre-Columbian gesture of reverence. This gesture is still used by *curanderos* today (Glass-Coffin 1999).

As with the earlier Cupisnique ceramic tradition, the San Pedro cactus is often depicted alongside jaguars in Moche ceramics with “volutes”. These swirling patterns resemble visual forms similar to those experienced during the early stages of hallucination (Cordy-Collins 1998: 166). The jaguar is an ancient symbol of the shaman and shamanic power in the Americas, and Cordy-Collins argues that “these three...images form a triad whose symbolic meaning cannot be misinterpreted: the feline symbolizes the shaman, the four-ribbed cactus...his ability to transcend ordinary reality, and the volutes bespeak of the actual transcended state” (Cordy-Collins 1998: 166-167).

Marlene Dobkin de Rios (1977) discussed the depiction of hallucinogenic plants in Moche ceramics in light of contemporary psychoactive plant use in folk healing sessions in Peru. She argued that the use of psychoactive plants were pivotal in Moche tradition to “achieve contact with supernatural realms...to permit the magical manipulation of forces...to serve social goals”, such as the curing of illness and resolution of social problems. Dobkin de Rios specifically mentions the use of *E. pachanoi*, but also *Datura* species, which are often added to San Pedro brews to enhance the psychoactive experience. Several authors have suggested the use of *Datura*, and the closely related *Brugmansia* species have considerable antiquity

(Lockwood 1979; Elferink 2000; De Feo 2003), however depictions of it in artefacts are rare.

Coca chewing is depicted in the ceramics of the Early Intermediate Period. The figures, with a distinctive bulge in the cheek indicating a coca quid, are known as *coqueros*. An example from the Moche culture shows a seated man with a small container of lime and a spoon, typical objects associated with coca chewing (see fig. 6.5b). This artefact was found amongst a number of ritually broken items found in the fill of tomb B at Dos Cabezas in the Jequetepeque valley on the northern Peruvian coast (Donnan 2007: 31). Similar depictions of coca chewing are found in the ceramics of the Nasca culture on the southern coast. Both men and women are depicted with a characteristic bulge in the cheek, indicative of a coca quid (Silverman 2002: 152). Coca leaves have been botanically identified in the grave goods of an individual from a necropolis at Nazca. The leaves were removed from a feathered *chuspa* (a small bag or pouch) and examined by microscopy and were found to be similar to modern Peruvian coca and distinctly different from Bolivian coca, based on leaf morphology (Griffiths 1930).

The San Pedro cactus also appears in the ceramics of the Nasca culture. Donald Proulx describes an example of a Nasca vessel that depicts a scene of a musician/shaman playing panpipes, surrounded by images of cacti and storage vessels, with participants drinking from small cups. He states: "it seems clear that the cacti are deliberately displayed to indicate their role in providing the connection to the spirit world by means of the mind-altering drug they contain" (Proulx 2001: 130).

Additionally, star-shaped iconographic elements in Nasca ceramics have been argued to be slices of San Pedro cactus by Fernando Cabieses as well as Donald Proulx (2006: 167). San Pedro cactus may be associated with death and rebirth. A number of funerary urns from the Nasca period show a mummy bundle with what has been interpreted as San Pedro cacti growing out of the shoulders. Sharon and Donnan (1977) suggest that San Pedro depiction in these funerary urns (see fig. 6.5) may be symbols of regeneration. The Quechua word *mallqui* has two meanings. It means both “mummy of an ancestor” and “tree” or “seed”. Douglas Sharon suggested that this formed a conceptual analogy in the Nasca cult of the dead. He suggested that the message of these urns was that they were buried – like seeds – for germination in the afterlife. Furthermore, he suggests that the San Pedro stalks symbolise the capacity of the carefully buried “seed-person” to be reborn out of darkness – “like the night-blooming San Pedro cactus does every spring” (Sharon 1978: 41).

The remains of psychoactive species have been found at the Nasca ceremonial site of Cahuachi. The species identified as *Echinopsis pachanoi* and *Prosopis chilensis*, a fruit sometimes used to make *chicha* (Valdez 1994).

Given the context and the nature of the plants discovered it could be inferred that San Pedro was used by the Nasca for ritual purposes. It should be noted that there has yet to be any definitive proof that the Nasca actually used San Pedro (Proulx 2006: 62). Based on the importance of San Pedro in earlier cultures, in particular Chavín, Dobkin de Rios and Cardenas (1980) argue that San Pedro was available, and most likely used by the Nasca to contact the spirit world and ancestors.

## **6.6. Middle Horizon (AD 600 – AD 1000)**

The Middle Horizon saw the decline of the Moche culture, probably due in part to drastic environmental changes during the middle of the 6<sup>th</sup> century that resulted in famine that particularly affected coastal communities (Moseley 2001: 223). There is evidence of widespread regional droughts in AD 563-564, AD 636 and AD 645 from ice cores from Quelccaya ice cap in southern Peru (Thompson *et al.* 1985; Dillehay and Kolata 2004). It appears that fluctuating environmental conditions including flooding and drought, as well as tectonic activity caused considerable social and economic problems for ancient inhabitants of Peru (Moseley 1983; Craig and Shimada 1986; Sandweiss *et al.* 2001; Dillehay and Kolata 2004). Tom Dillehay and Alan Kolata concluded that human communities responded differently to short and long term environmental changes. For instance – during El Niño events communities moved to less flood prone areas and during droughts they changed which cultigens were grown or relied on other means of subsistence – such as maritime resources (Dillehay and Kolata 2004).

The same authors also suggest protracted environmental instability may have undermined the control of the ruling class leading to state collapse and migration of populations. This model has been suggested for the collapse of both the Moche and Tiwanaku cultures (Moseley 2001; Dillehay and Kolata 2004: 223).

Therefore, the Middle Horizon is associated with radical change and re-organisation as people adjusted to new environmental and economic conditions, as well as new ideologies (Moseley 2001: 225). During the middle horizon two major polities co-existed in Peru: Wari (Huari, (500 AD-800 AD), principally located in the southern

Huamanga basin with important sites at Huari, Conchopata, Pachacamac, Cerro Baul (Knobloch 2002: 138) and Tiwanaku (AD 500 - 1000) located in the Lake Titicaca basin with its core region extending throughout the Peruvian and Bolivian *altiplano*, with important sites at Tiwanaku (Tiahuanaco), Omo, Azapa and San



**Fig. 6.9** Depictions of Gods in the Wari and Tiwanaku. a. Female figure (coca quid) with both hands on her cheeks (Latin American Studies.org); b. Male figure (coca quid) with both hands on his cheeks (Covis Images); and possibly even with cacti growing from the shoulders (Sharon 2000: 75); c. Moche *curandera* – hooded female figure holding the top of a cactus in her hand. Her expression is one of serenity (Glass-Coffin *et al.* 2004: 85).

It seems that Wari and Tiwanaku also shared iconography that was spread from the centres of both polities, albeit expressed in different media (Isbell 2008: 736). Isbell uses the example of the Staff God, which appears identical in both cultures – carved in stone on the Ponce Monolith at Tiwanaku and painted on giant offering jars at Conchopata, a major Wari site located near the modern city of Ayacucho (Isbell 2008: 736). Alongside shared iconography, Wari and Tiwanaku material culture was spread throughout Peru, northern Chile and the Bolivian *altiplano*. These items included striped tunics woven on looms with stylised religious imagery, drinking vessels known as *keras*, polychrome ceramics, four pointed hats, *tupu* pins and *Anadenanthera colubrina* symbols (Knobloch 2000; Isbell 2008: 738).

The centre of the Tiwanaku culture was a city of the same name (sometimes spelled Tiahuanaco). Situated at 3800 metres above sea level in the southern Lake Titicaca region some 30 km from La Paz, the site covers 420 hectares. At the centre there was a ceremonial site composed of sunken courtyards and stepped pyramids that possibly functioned as a place of pilgrimage (Goldstein 2002: 325). The surrounding area was residential and was thought to house a population of around 20 - 40,000 during the 6<sup>th</sup> century (Goldstein 2002: 320).

Tiwanaku material culture, in particular textiles, snuff kits and ceramics, were spread throughout the South Central Andes during the Middle Horizon. Snuff kits are part of the *complejo de rapé* (snuffing complex) which is overwhelmingly associated with Tiwanaku and is considered a marker of highland influence (Berenguer 2001).



Constantino Manuel Torres has researched Tiwanaku snuff trays extensively. He states that 85 snuff trays and 23 tubes with Tiwanaku iconography have been found. They are made from stone, wood and bone. Only one wooden tray has been recovered. Torres believes that wooden trays were manufactured and used at Tiwanaku, but the site's climate and soil are not conducive to preservation of organic artefacts, hence very few wood or bone snuff trays exist (Torres 2004b: 114).

Tiwanaku influence spread into what is now northern Chile, including the area around San Pedro de Atacama. Indeed, the highest concentration of snuff kits (see fig. 6.6) have been reported from this area, with some 612 kits being recorded in the literature, due in part to favourable conditions for preservation (hyper-arid, low precipitation) (Torres 1995). Generally snuff kits were found with adult males; however there are some exceptions. An example is tomb 113 at Solcor 3, a cemetery site. An adult female was found with a bag and bone snuffing tube. The burial was dated to  $1,380 \pm 60$  BP and displayed Tiwanaku iconographic elements (Torres *et al.* 1991; Torres Rouff 2002). Agustín Llagostera *et al.* (Llagostera *et al.* 1988) recorded snuffing equipment associated with older females at Solcor 3. However, like previous authors, they noted that most snuff kits were associated with young adult males. Llagostera *et al.* (1988) commented on the fact that only two of 13 secure<sup>7</sup> contexts in which the individuals were definitely associated with snuffing equipment were older females (see table 6.2).

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<sup>7</sup> Snuff trays and tubes were found in other graves that contained multiple individuals. Often these graves had been disturbed and it was not possible to associate items with specific individuals.

They suggest that snuffing may have been associated with shamanism. It is assumed that shamans were males. However, older women were thought to possess knowledge of medicinal herbs, spells and dances. It is perhaps unsurprising that the older women were buried with simple snuffing equipment due to their involvement with the health and wellbeing of their communities (Llagostera *et al.* 1988).

**Table 6.2** – Biological and contextual information of the two adult females buried with snuffing equipment at Solcor 3, San Pedro de Atacama (Llagostera *et al.* 1988).

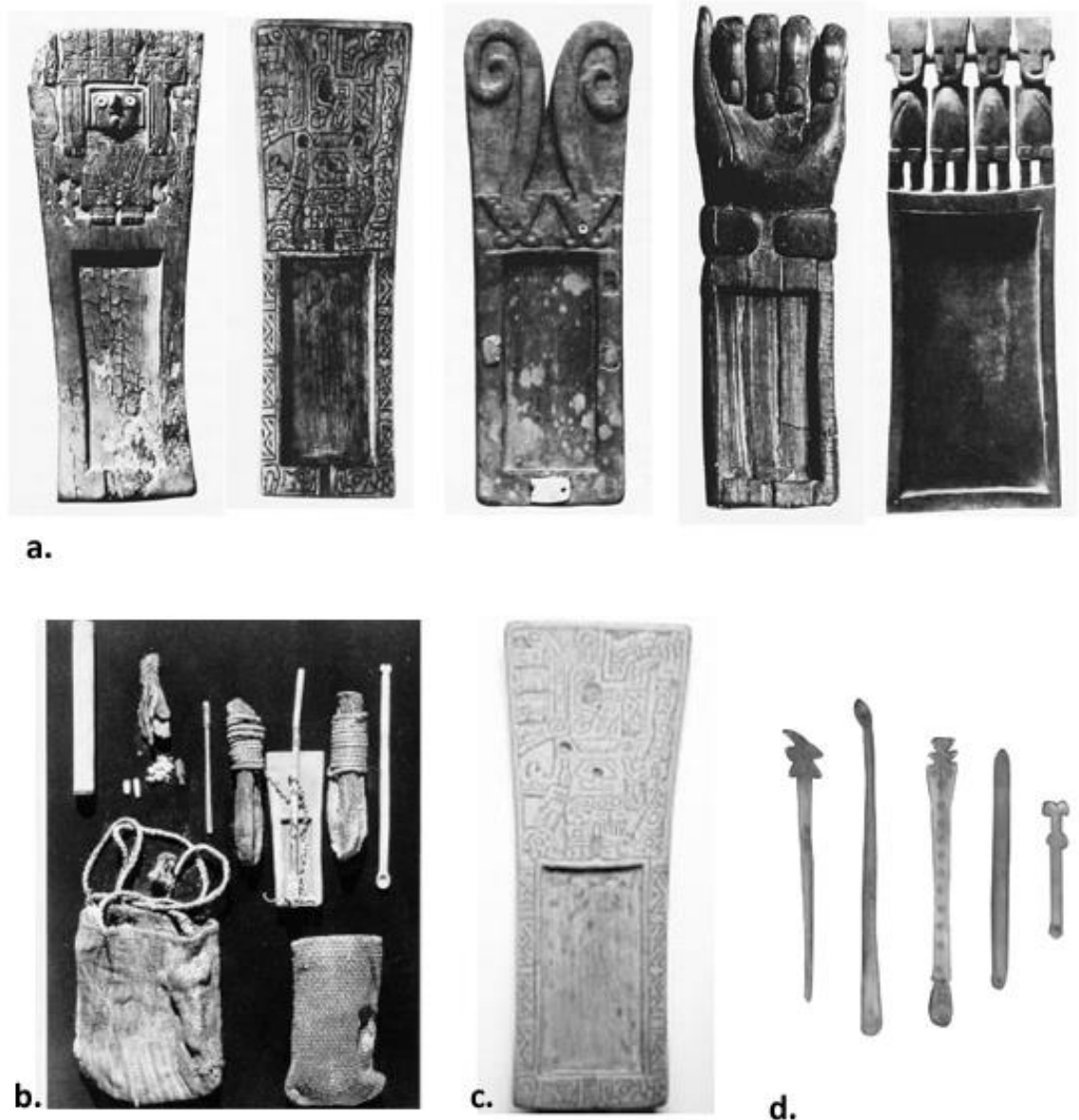
<b>Cemetery phase</b>	<b>Tomb number</b>	<b>Age at death</b>	<b>Cranial modification</b>	<b>Snuffing equipment</b>	<b>Other grave goods</b>
A	8	45-50	no	Bone tube	Ceramics, baskets, vegetal fibre brush
B	1	45-50	no	Decorated bone tube, plain rectangular tray	Wooden spoon, gourd, basket, yarn, cactus needles, ceramics

Snuffing equipment is generally associated with relatively “high status” male burials (Goldstein 1996; Torres Rouff 2002), however this is not exclusively the case. Copper snuff spoons and bone snuff tubes have been found associated with high status female burials at the élite residential complex of Qoripata at the Wari complex of Huaro, in the Huaro Valley near Cuzco. The burials date to Middle Horizon (AD 540-900) (Glowacki 2002: 282; Janusek 2008: 268).

Constantino Manuel Torres *et al.* (1991) analysed snuff samples from funerary contexts from Solcor 3, in the San Pedro de Atacama region of northern Chile. There were 118 graves at Solcor 3, in which 205 individuals were interred. Of these, 39

individuals were found with snuffing artefacts. Thirty-seven snuff kits were associated with adult males, whilst the other two were found with a mature female and an infant. The material analysed by Torres *et al.* came from tomb 112, which has been dated to c 780 AD. The tomb contains the remains of an adult male, aged approximately 45, buried in a flexed position, facing east, towards the Licanbur volcano. Amongst his grave goods there were two sets of snuffing equipment, including lumps of “snuff”.

The alkaloid fraction of the snuff samples from Solcor 3 was extracted by solvent extraction, then analysed using gas chromatography-mass spectrometry (GC-MS). A number of psychoactive alkaloids were present in the material, including dimethyltryptamine, 5-methoxydimethyltryptamine and 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine). Torres *et al.* (1991) suggest that the botanical origin of the material is *Anadenanthera*, as this is the only genus implicated in snuffing that includes bufotenine as a major alkaloid. The same authors also report that small pouches containing seeds were found in burials at Solcor 3, dating from the same period as burial 112.



**Fig. 6.6.** - Snuffing equipment a. Snuff trays from Bolivia, Argentina and Chile c AD 600 - 1000 (Torres 1986); b. Typical snuff kit from Solcor 3, San Pedro de Atacama (Torres 1995); c. The snuff tray from Niña Koni, Bolivia (Torres 1995). The snuff spoons are of unprovenanced, but probably from southern Peru and northern Chile (ICOM 2003). The snuffing equipment is made of wood. The snuff spoons belong to the genus *Anadenanthera*, confirming the premise that *Anadenanthera* snuffs were used in the San Pedro de Atacama area. Torres (1995) states that the size and chronology of the sample suggests that 20-22% of the adult male population used snuffs in the period c. 200-900 AD. It is important to bear in mind that the inclusion of a snuff kit as a grave offering does not prove that the individual used snuff during

their lifetime; rather it may be that the inclusion of snuffing equipment may be a symbol of status or a symbolic offering to aid the deceased into the spirit world.

A site known as Callichu near the town of Niño Korin just east of Lake Titicaca yielded a number of artefacts associated with snuffing and the consumption of hallucinogens. The site was investigated by S. Henry Wassén in 1970. The site itself consisted of a long passage with niches that contained burials in a seated, flexed position. Each individual was dressed in a sleeveless tunic, pointed cap and sandals. These individuals were buried with a number of “ritual” artefacts. It is unclear whether or not all the artefacts were found together or were distributed along the passage. The finds include five wooden snuff trays, wooden tubes and containers, enema syringes, a number of spoons and spatulas, baskets, fur and skin pouches, as well as *Ilex guayusa* leaves (Wassén 1972; Torres 1995). Wassén interpreted the artefacts to be the belongings of a medicine man or shaman. Another bundle of artefacts wrapped in a woven Tiwanaku style bag, including large, ornately decorated snuff trays, quartz crystals, mica flakes and miniature llama amulets (*illas*) was found at Amaguaya, department of La Paz, Bolivia. This has been interpreted as a “ritual” bundle, probably the belongings of a shaman (Wassén 1972; 1973).

There was also evidence, albeit less, to suggest hallucinogens were used by the Wari culture. Highland sites, such as those inhabited by the Wari are generally wetter than the hyper-arid coast. Moist soil conditions (combined with other environmental conditions such as available oxygen, soil pH, and presence of

microorganisms) are generally not conducive to the preservation of wooden artefacts (Blanchette 2000; Kim and Singh 2000) such as snuff trays.

However, Patricia Knobloch (2000) eloquently describes what she believes to be *Anadenanthera colubrina* iconography in Wari and Tiwanaku material culture. She describes a plant icon within Tiwanaku and Wari religious iconography that is very similar to *Anadenanthera colubrina*. This plant icon is depicted on snuff trays from Tiwanaku and San Pedro de Atacama, textiles from Pachacamac and Nasca, stone sculpture (Bennett Monolith and the Gate of the Sun at Tiwanaku) as well as ritually smashed giant ceramics from Conchopata, a major Wari centre located near Ayacucho, Peru.

Knobloch suggests that the ceramic offerings at Conchopata were the end result of a ritual in which the ceramics were ritually smashed and burned. Due to the *Anadenanthera* iconography and the fact that *chicha* is important in Andean ritual, she suggests that *Anadenanthera* may have been added to *chicha* to make a hallucinogenic beverage that was consumed during a ritual by a large number of participants, with someone overseeing the proceedings (Knobloch 2000). The addition of *Anadenanthera* snuff to *chicha* was recorded by Polo de Ondegardo (1916 [1571] 3 : 29-30) as well as ethnographers in the Andes (e.g. Isbell 1978: 151-158).

### **6.7. Late Intermediate Period (AD 1000 – AD 1476)**

The Late Intermediate Period (LIP) spans the time between the fall of Tiwanaku and Wari around 1000 cal AD and the Inca expansion c 1476 cal AD. The LIP was a time

of major transformations in the Central Andes as new polities arose, reorganised and gained power (Mendieta 2000: 37; Conlee 2003; Conlee *et al.* 2004: 209). Perhaps the most significant was Chimú culture, with its capital at Chan Chan, near the present day city of Trujillo on the northern Peruvian coast. The Lambayeque (Sican) and Casma cultures were also located on the northern coast. On the central coast there was Ichma culture, whose principal centre was at Pachacamac, as well as the Chancay culture, famous for its spectacular funerary textiles (Bákula 2000: 115-117). On the southern coast around the Ica and Nazca valleys, the Ica culture flourished. Further south the Chiribaya occupied the Osmore valley, whilst the Chachapoya lived in the cloud forests (*yungas*) between the Marañon and Huallaga rivers in the northern highlands.

By the Late Intermediate Period coca had been cultivated in the Andes for hundreds of years and was a major economic crop for coastal and Amazonian peoples, including the Chachapoya. These people depicted coca chewing in stone sculpture. Stylised stone heads appear to have the characteristic bulge in the cheek representative of a coca quid (see fig 6.7c). Well preserved textiles found as part of burial offerings at Laguna de los Cóndores suggests that the Chachapoya carried coca leaves in small bags (*chuspas*), a tradition that persists amongst Quechua and Aymara people today. Coca leaves are also common grave offerings amongst coastal groups during the LIP. An example is the Chiribaya, who occasionally buried their dead with bags stuffed with coca leaves. Coca quids have also been found *in situ* in Chiribaya mummies. In one unusual case, the body of an elderly male had been eviscerated and a jar containing coca leaves has been placed inside the chest cavity (Guillén 2004). Sonia Guillén (2004) suggests that the inclusion of bags of

coca in Chiribaya funerary bundles was a ritual offering and that “the amount of coca leaves, and offerings reflect the importance of the individual within their society” (Guillén 2004: 150). Whilst the importance of coca in Andean ritual (especially as funerary offerings), is not disputed, it is unclear whether or not the amount of coca included in a funerary offering is related to the social status of the deceased, although other indicators such as hairstyles, body adornments, cranial modification, textiles and ceramics may help support this idea (Arriaza *et al.* 1986; Torres Rouff 2002; 2008; 2012).

Coca appears to have been widely used during the LIP. A number of authors have identified an oral pathology that is suggestive of coca chewing, particularly amongst coastal populations (Turner 1993; Langsjoen 1996; Indriati and Buikstra 2001). The pathological changes associated with coca chewing consist of cervical root caries with root exposure on the buccal surfaces of posterior dentition (particularly 2<sup>nd</sup> and 3<sup>rd</sup> molars) as well as antemortem tooth loss (AMTL). A comparison of individuals from the Chiribaya Alta, Algodonal, and Yaral populations in the Osmore drainage designated as coca chewers on the basis of oral pathology with hair tests for a cocaine marker (BZE) showed that 85.7% of those with the buccal cervical-roots caries also had positive hair drug tests, suggesting that these individuals chewed coca in the months prior to death (Indriati and Buikstra 2001). Turner (1993) suggested that calcium oxide (lime) that is used to extract cocaine from coca leaves has a detrimental impact on the cellular integrity of the gingiva, resulting in the pathological changes evident in skeletal remains.



On the northern coast of Peru there is ample evidence for the continued use of the San Pedro cactus during the LIP from Lambayeque ceramics. The Lambayeque (800-1350 AD) people were culturally and genetically descended from the earlier Moche culture. There appears to be a continuation of the use of San Pedro cactus, as it is depicted in Lambayeque ceramics. An example in Glass-Coffin *et al.* (2004) shows a female holding the top of a San Pedro cactus in her hand. Sharon (2000: 69) illustrates other Lambayeque ceramics of a similar nature: hooded female figures holding the tops of San Pedro cacti (see fig 6.7a and b). Each of these females makes a different gesture with her hands. It has been suggested that these are ancient gestures of reverence that are still used by *curanderas* today (Glass-Coffin 1999). Cactus representations are also evident in artefacts from other LIP cultures, e.g. ceramics from the Chimú culture. Richard Evans Schultes *et al.* (2001: 168) provide an example of an owl-faced woman holding a San Pedro cactus. Stylised felines and San Pedro slices have been depicted in Chancay (1200-1450 AD) textiles (Cáceres-Macedo 2004: 498, see fig. 6.7).

Further afield there is yet more evidence of the use of psychoactive plants during the LIP. In 1990 two naturally desiccated bodies were found in a rock shelter near Cusi Cusi, a small village in Puna de Jujuy, northwest Argentina. The bodies (no information regarding age or sex of these individuals is reported) were placed in a flexed position and were covered with two mantles of fine camelid hair. Both individuals showed evidence of artificial cranial modification and were buried with snuffing equipment, including trays and bone tubes (Pochettino *et al.* 1999).

Pochettino *et al.* (1999) examined powdered archaeological material from these snuff tubes using conventional light microscopy and scanning electron microscopy. They identified the powdered substance as *Anadenanthera colubrina* var. *Cebil*, whilst ruling out other substances, such as tobacco leaves (*Nicotiana* sp.) and *coro* (possibly *Trichocline* sp., although a definitive botanical identification of *coro* has not been established).

The southernmost evidence for snuffing comes from the Iglesia Valley, San Juan province, Argentina. Two snuff trays were found in funerary contexts and from the Angualasto culture that flourished in this area during the LIP (Gambier 2001). Gambier states that the trays are imported to the area, suggestive of trade links between this area and the Bolivian *altiplano* in antiquity.

#### **6.8. Late Horizon (AD 1476 – AD 1532) & Colonial Period (AD 1532 – 1821<sup>8</sup>)**

The Late Horizon is characterised by the imperial expansion of the Inca to form the biggest empire in Andean history. The Inca called their empire Tawantinsuyu, Quechua for “four parts together”, as their empire was split into four quarters (D'Altroy 2002: xiii). At the height of the empire, Tawantinsuyu stretched some 5,500 km along the Andes – from southern Ecuador as far as northern Chile and north-western Argentina with a population of around 10 million (Moseley 2001: 9; D'Altroy 2002: 2).

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<sup>8</sup> Peru became independent from Spain in 1821

Coca was important to the Inca. It was often part of sacrificial offerings, either as an offering in itself or as part of a bigger assemblage. Bags of coca leaves have been found with children sacrificed during the state-sponsored *capacocha* ceremony. Of the three children found in 1999 at the summit of Lullailaco, one aged around 15



**Fig. 6.7** – a. Chimú bottle showing a curandera holding a piece of San Pedro cactus (Sharon 1978 fig. 4.11); b. Lambayeque bottle showing a curandera holding a piece of San Pedro cactus (Glass-Coffin *et al.* 2004); c. Stone coquero from La Penitenciaría de La Meseta, Amazon region. N. Peru (Muscutt 2007); d. Painted Chancay textile with felines and star-shaped San Pedro slices (Cáceres-Macedo 2005: 498).

(but may be younger, around 13 – Chiara Villa, pers. comm 2012) found with coca leaves in her mouth and on her lips. A younger boy, aged around 7, was buried with bags of coca amongst other offerings (Ceruti 2004). Coca bags have been found with other *capacocha*, most notably the “Ice Maiden” discovered by Johan Reinhard on mount Ampato (Reinhard 2005: 97) and the 8 year old boy known as the “Prince of El Plomo” found in 1954 (Horne and Kawasaki 1984). The use of coca during *capacocha* was mentioned by the Spanish chronicler Alonso Ramos Gavilán (1976 [1621]: 26) – “when the hour of sacrifice came, they placed in the mouth a fistful of coca leaves with which they smothered the child”.

Small animal-shaped receptacles known as *conopas* were used to burn coca in rites relating to both herd and crop fecundity. Specific *conopas* in the shape of camelids (llamas, alpacas, vicuñas, guanacos) known as *illas* (see fig. 6.8f) from northwest Argentina were found to have a layer of carbonised material adhering to the inside. Using scanning electron microscopy, the carbonised material was compared to both wild and cultivated varieties of coca. The sample from the *illa* was identified as *Erythroxylum coca*, ruling out other possible plants such as maize and aromatic herbs (Cortella *et al.* 2001). Coca chewing is evident in small silver and gold statues thought to depict Inca nobility. The male figurines, sometimes called “*Orejones*” on account of their elongated ears, a marker of high status (Allison *et al.* 1983; Julien 2000: 29), are sometimes clothed with textiles and feathers and the characteristic bulge in the cheek is evident in both male and female figures (see fig. 6.8e). An example (Netherly 1988: 268) shows a male figure (Netherly 1988: 268) with a coca quid in his cheek and the large, elongated ears of an Inca élite. It is interesting that high status figures such as these, made from precious metals, depict a coca quid. It

may be that the coca quid was something instantly recognisable as a visual marker of high status. This is pertinent, as it has long been held by various scholars that during the Inca period (c AD 1450 – 1532) access to coca was controlled by the state (Rowe 1946; Garcilaso de la Vega 1987 [1609]). It is thought that imperial drive to restrict coca began around AD 1230, in the reign of Inca Roca, but did not become a monopoly until the early 15<sup>th</sup> century (Rowe 1946: 292). Coca acquired a divine status and its association with ritual is clear (Cortella *et al.* 2001; Ceruti 2004; Bray *et al.* 2005). As the Inca Empire expanded, coca became more accessible to the wider population (Rowe 1946; Mortimer 1974: 292; Phillips and Wynne 1980; Cartmell *et al.* 1994).

Coca bags, (*chuspas*), have been argued to be an indicator of social status (Hughes 2010). As the Inca did not have a system of writing, textiles were used to communicate visual information, for example religious ideology using specific iconography. The coca bag, being associated with a semi divine plant, Hughes argues, communicated ideas about Inca ideology, social practice and embodied the physicality of Inca rule (Hughes 2010). The argument is based on the assertion the coca was a precious commodity. It was often accompanied by other precious goods such as fine cloth, *chicha*, gold, silver and llamas as part of sacrificial offering. This has been suggested by a number of the Spanish chroniclers (Garcilaso de la Vega 1987 [1609]; Cobo 1990 [1653]). Coca was also a significant part of gift giving and reciprocity (Betanzos 1996 [1551-1557]: 56). Ceremonial gift giving was a way of formalising allegiances and showing political power and its reciprocal submission.

Hughes argues that coca given in an important and visible context, such as the *Sapa Inca* bestowing it onto his Lords and *caciques*, demonstrates the legitimisation of their power. These individuals, in wearing *chuspas* as adornments are making a visual display of their authority (Hughes 2010).

Coca chewing artefacts from the Inca period are well documented (see fig. 6.8). There are numerous examples of *chuspas*, lime spoons and containers in museum collections in South America, the US and further abroad (Cáceres-Macedo 2004: 521; Rättsch 2005: 249).



**Fig. 6.8.** – Chuspas, *illa* and lime spoons and container a. Chuspa (Cáceres Macedo 2005: 522); b. Chuspa (Cáceres Macedo 2005: 521); c. Lime spoon (ancientartifax.com); d. Lime container (Rätsch 2005: 249); e. A figurine of a high status male with long ears (Orejones) and a coca quid in his cheek (Longhena and Alva 1999 138); f. Llama-shaped *illa* for burning sacrificial offerings (Cortella *et al.* 2001).

## 6.9. Summary

From the earliest times the people of the Andes have incorporated depictions of psychoactive plants into their sculpture, ceramics, architecture and ritual objects.

The earliest evidence for coca chewing comes from the Nanchoc Valley on the western slopes of the Andes. Coca leaves and compacted lime (some of which contained organic ashes, probably from plant material) were found in secure contexts dating to 8,000 BP (Dillehay *et al.* 2010). It has been suggested that coca must have been domesticated some time before. Coca chewing is part of Andean identity, and, based on archaeological evidence, has been a part of Andean life for at least 8,000 years. Coca leaves have been found in Nasca, Cabuza and Chiribaya funerary contexts and the unique bulge in the cheek of the coca chewer has been depicted in ceramics and sculpture from the Nasca, Inca, Moche and Chachapoya cultures. Coca chewers carry their coca leaves in a *chuspa*, a small bag woven from camelid wool. Archaeological examples of these bags, still used by Quechua and Aymara people today, have been found throughout the Andes.

Snuffing also appears to have a long history in the Andes. Snuffing seems to have been a key part of the Tiwanaku culture, with *Anadenanthera* iconography appearing in pottery, textiles and ornately carved wooden snuff trays. These items have been found far from the Tiwanaku core area around Lake Titicaca, leading some scholars to infer Tiwanaku influence as far as San Pedro de Atacama and the Azapa Valley in northern Chile. Despite the relative abundance of snuff trays with Tiwanaku iconography, the earliest snuff trays come from the Peruvian coast (from



contexts dating to around 1200 BC). Mortars possibly used to grind snuff have also been found at Chavín de Huántar in the northern highlands of Peru.

Chavín de Huántar is well known for the shamanic imagery present in the friezes that surround the sunken courtyard and the tenon heads, which are thought to represent transformation. A long, smooth cactus is depicted in the circular sunken courtyard at Chavín de Huántar, leading researchers such as Richard Burger (Burger 1992; 2011) to maintain that the San Pedro cactus was used as part of shamanic ceremonies.

The San Pedro cactus is also depicted in earlier Tembladera/Cupisnique ceramics – the swirling volutes, cacti and jaguars thought to represent a triad of icons associated with shamanic transformation. The same iconographic elements are present in some Moche ceramics. Bonnie Glass-Coffin has written extensively on these ceramics, found at Moche ceremonial sites in regard to female healers and San Pedro ceremonies in both Moche and contemporary Peru (Glass-Coffin 1991; 1998; Glass-Coffin *et al.* 2004).

Even though there is some archaeological evidence of the use of psychoactive plants such as coca, San Pedro and *Anadenanthera* snuff it is likely this is not representative of the wide variety of psychoactive plants available to Andean people. Some plants, such as *ulluchu* have yet to be conclusively identified by botanists. Some psychoactive preparations, such as *ayahuasca*, are not represented in the archaeological record as they are prepared as liquids according to varied recipes and no specific implements are needed for ingestion.

The next chapter discusses the sites and material culture specific to the use of psychoactive plants from which hair specimens for this project were obtained.

# Chapter 7: Site Information & Cultural Context

## 7.1. Introduction

This chapter aims to place the individuals analysed as part of this research project in their cultural context. This includes reviews of each site and a discussion of the cultural background of each group. Therefore this chapter discusses the Cabuza culture of the Azapa Valley in northern Chile and Tiwanaku/Cabuza on the Chilean coast around Iquique. Forty nine individuals from the Azapa Valley and three from Iquique were included in this research project. The Inca influence on the Peruvian coast is discussed in relation to the necropolis at Puruchuco-Huaquerones on the outskirts of Lima on the Peruvian coast, from which 13 individuals were analysed.

## 7.2. The Azapa Valley: An overview

### 7.2.1. Location and environment

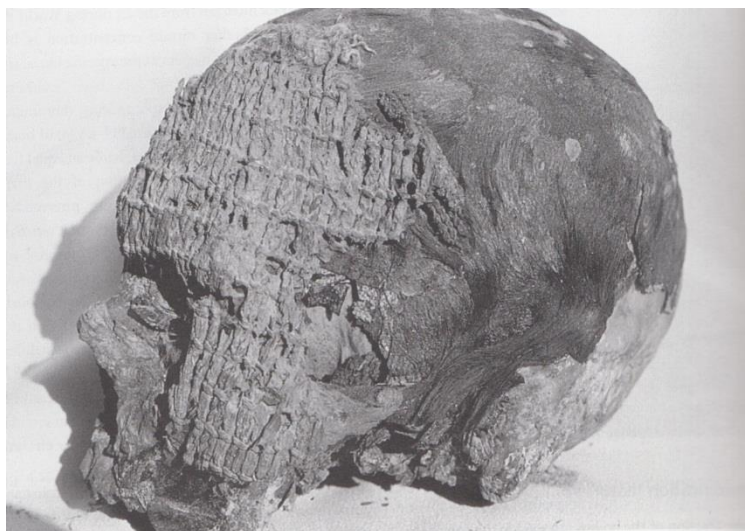
The narrow plain between the Pacific Ocean and Andes mountains is less than 100 km wide and extends from around 16° to 22° S. The east-west valleys were cut by glacial melt water and are dry *quebradas*. However, some also have seasonally flowing streams or rivers. In the case of the Azapa Valley, the Río San José flows through the summer months. This makes the fertile Azapa Valley ideal for irrigation and agriculture. The proximity to the ocean also makes it ideal for the exploitation of marine resources (Aufderheide 2003: 139). The climate of the Azapa Valley is

both temperate and hyperarid with very low annual precipitation, conditions ideal for the preservation of human remains and associated artefacts.

### **7.2.2. Early inhabitants: Chinchorro to Alto Ramírez**

The earliest inhabitants of the coastal area around Arica some 10,000 – 11,000 years ago were likely transhumant hunter-gatherers who were also heavily reliant on marine resources (although see Arriaza 1995a: 33-55 for an alternative argument). This tradition has become known as Chinchorro, after the beach on which hundreds of mummies dating to this period were found in the early 1900s by Max Uhle (Arriaza *et al.* 2008b: 45).

The Chinchorro practiced a complex form of anthropogenic mummification. The earliest evidence of such mummification from sites such as Camarones 14, Camarones 17 and Morro 1 indicate that the practice started around 5000 BC (Arriaza 1995b). Over 150 mummified bodies from this culture have been excavated from coastal sites from northern Chile (Aufderheide *et al.* 1993; Aufderheide 1996: 144). A naturally mummified adult male known as “Acha Man” (see fig. 7.1) was found at the Chinchorro residential site known as Acha-2, 5 km from the coast at Arica. Radiocarbon dating yielded an



**Fig. 7.1** – Acha Man (Aufderheide 2003: 142)

age of 7020 ± 255 BC. The body appears to have been naturally desiccated (Aufderheide *et al.* 1993).

Some mummies, such as “Acha Man”, were naturally mummified as a result of local climatic effects, whilst others have been mummified in an elaborate and intentional fashion, involving evisceration, defleshing, cleaning and wrapping the bones with fibres, then covering the body with clay and then painting the clay with a pigment, such as black manganese or red ochre (Allison *et al.* 1984; Allison 1985; Arriaza 1995b; Guillén 2004). The bodies were buried in an extended position and wrapped in twined mats, the distinguishing mortuary characteristics denoting the Chinchorro (Cassman *et al.* 2008). Grave goods consisted of tools associated with the exploitation of marine and terrestrial resources as well as clothing and personal adornments (Standen 2003). Snuff kits used for the inhalation of hallucinogens have also been found in late Chinchorro and Quiani burials, suggesting that this practice has a long history on the Pacific coast (Bird 1943: 243; Arriaza 1995b: 144).

The exploitation of maritime resources continued into what Chilean archaeologists call the Formative Period (1000 BC – AD 750, contemporaneous with the Peruvian Early Horizon and Early Intermediate Period). This era is associated with the first appearance of ceramics (Sutter and Mertz 2004; Sutter 2005b). Sand-tempered ceramics are found in middens and funerary contexts at the coastal sites of El Laucho and Faldas del Morro, both in modern day city of Arica (Daulsberg 1985; Focacci 1989). Inland sites associated with the Azapa (1000-800 BC) and Alto Ramírez (800 BC – AD 500) phases are associated with peoples who practiced a mixed agropastoral and maritime subsistence. The Alto Ramírez phase, according to

Mario Rivera (Rivera 1984; 1991; 2008: 968), seems to have been contemporaneous with Pukara and other *altiplano* societies of the Peruvian Early Intermediate Period, such as Qaluyu, Chiripa, Wankarani and early Tiwanaku (Rivera 2008: 964).

Some archaeologists contend that the appearance of textiles displaying Pukara motifs is some of the earliest evidence for highland colonists in the Azapa Valley (Santoro 1980; Kolata 1993a; Goldstein 1996), yet Richard Sutter states that the relatively low number and “ceremonial” nature of these artefacts suggests a locally-derived tradition with only very limited indirect exchange with the *altiplano* (Sutter 2000; Sutter and Mertz 2004). Stylistic similarities have also been noted with the Paracas and Nasca traditions on the Peruvian south coast (Goldstein 2000; Goldstein and Rivera 2004). The Alto Ramírez phase is associated with new developments in agricultural practices, such as the introduction of new crops such as quinoa and the growing and consumption of maize (Rivera *et al.* 1977; Goldstein and Rivera 2004: 171; Watson *et al.* 2010; Watson *et al.* 2011). This enabled population expansion by providing a food surplus. This in turn is thought to be partly responsible for the development of early settlements in the area (Rivera 2008: 964). During this phase local peoples prepared the dead as flexed bundles that were buried in cemetery mounds that grew to monumental proportions over time (Janusek 2008: 235). These mounds probably served as a focus for ancestor worship. Bodies tended to be buried in a semi-flexed position. Infants were sometimes buried in baskets. Typical grave goods included ceramics (plain and decorated), camelid fibre textiles decorated with geometric patterns, turbans, items associated with the use of hallucinogens (trays, tubes, spatulas), copper and silver

ornaments, coiled basketry and harpoons, nets, fishhooks and other items associated with the exploitation of marine resources, similar to that of the earlier Chinchorro tradition (Aufderheide 2003: 146; Rivera 2008: 966). Mario Rivera (1977: 41) also notes that trophy heads in net bags are also found in graves and the decapitated head appears as a motif on textiles.

### **7.2.3. Cabuza and Tiwanaku**

The Cabuza tradition is found along the fertile river valleys of Southern Peru (Sama, Caplina) and northern Chile (Azapa, Chaca, Camarones and Lluta). All are around 300 km west of the highland site of Tiwanaku, located in the Bolivian *altiplano*. Elevations range from 0-1000 metres above sea level for Cabuza sites (Goldstein 2002: 327). Cabuza habitation sites tend to overlook the open river floodplain and cluster around natural springs, suggesting a preference of zones suitable for irrigated cultivation (Goldstein 2002: 328). Cabuza dwellings were built of ephemeral material such as cane, leaving little remaining visible architecture. The Cabuza practiced an agricultural economy. Major crops included maize, beans, fruits and coca, probably supplemented by camelids, guinea pigs, shellfish, fish, seabirds and marine mammals (Goldstein 2002: 328; Sutter and Mertz 2004).

The relationship between the Cabuza tradition and the *altiplano* Tiwanaku polity has been debated by Andean scholars for a number of years. The Tiwanaku core region extends through much of the Bolivian and Peruvian highlands, with Tiwanaku influence evident in southern Peru, northern Chile (especially in the San Pedro de Atacama area) and eastern Bolivia (Goldstein 2002: 319). Key material culture

attributes associated with Tiwanaku include *kero* drinking vessels, zoomorphic/anthropomorphic and polychrome ceramics, spectacular textiles and snuffing equipment bearing “staff god” iconography and silver, bronze and gold metallurgy (Goldstein 2002; Goldstein and Rivera 2004: 319; Torres 2004b). The city of Tiwanaku, located near Lake Titicaca in Bolivia was the major ritual and administrative centre.

Tiwanaku influence in the Azapa Valley becomes apparent in the Cabuza phase, the dating of which has been disputed by various scholars. Most authors broadly place Cabuza around AD 300 – 1000 (Aufderheide *et al.* 2004; Goldstein and Rivera 2004; Rivera 2008: 966), whilst Sutter (2005b) places Cabuza in the latter part of this range, around AD 750 – 1100. Sutter recognised that Cabuza, Sobraya and Maitas-Chiribaya ceramic traditions in the Azapa Valley demonstrated “clear formal and stylistic similarities” to a number of Moquegua Valley ceramic traditions recovered from stratified post-Tiwanaku Late Intermediate period contexts (Sutter 2005b). These styles rarely have dates earlier than 900 AD, possibly indicating that Cabuza in the Azapa Valley must be later than originally thought. However other authors see these ceramics as heavily *influenced* by Tiwanaku precedents rather than being strictly contemporaneous with Tiwanaku (Bermann *et al.* 1989; Goldstein and Rivera 2004: 172). There is a lack of scientific dating of Cabuza material, probably stemming from the haphazard nature of excavations of Cabuza sites, usually undertaken as a response to agricultural or building projects.

Prior to Cabuza, the Alto Ramírez culture already had long-standing links to the *altiplano*, as evidenced by the presence luxury of highland goods in funerary



assemblages. These items, such as ceramics and textiles display iconography similar to that of Pukara, a precursor of Tiwanaku (Goldstein and Rivera 2004: 171). The height of Tiwanaku influence in the Azapa Valley appears to be during the Cabuza phase (c AD 500 – 1000). This phase is typified by the presence of Tiwanaku style textiles and fine redware pottery in funerary contexts (Goldstein and Rivera 2004: 171).

A number of suggestions have been put forward to explain the interaction between the Azapa Valley (and other peripheral areas such as San Pedro de Atacama and the Moquegua Valley) and *altiplano*. Models of indirect control of “ultra-peripheral regions” (regions furthest from the core region around Lake Titicaca) suggest an asymmetrical relationship between Tiwanaku and local élites. This theory assumes a greater degree of socio-political complexity for Tiwanaku compared to peripheral communities (Goldstein 1996). Goldstein suggests that these alliances may have been strengthened by grants of titles or rights, mutual political support, shared rituals, pilgrimages or periods of residence in Tiwanaku by élite families, intermarriage and other kinship ties, as well as the exchange of “labour-intensive prestige goods” such as fine ceramics and textiles, and possibly snuff trays/tubes . The material correlates of this relationship are the only physical evidence still visible to archaeologists (Goldstein 1996). As part of this indirect mode of control, both Browman (1980) and Berenguer and Daulsberg (1989) emphasised the importance of portable items with symbolically or iconographically charged exotic items that reinforce the association of the central polity (i.e. Tiwanaku) with implications of power or wealth (Goldstein 1996). Examples of these items include Tiwanaku style textiles and snuff kits bearing “religious” iconography, such as the “Staff God” seen

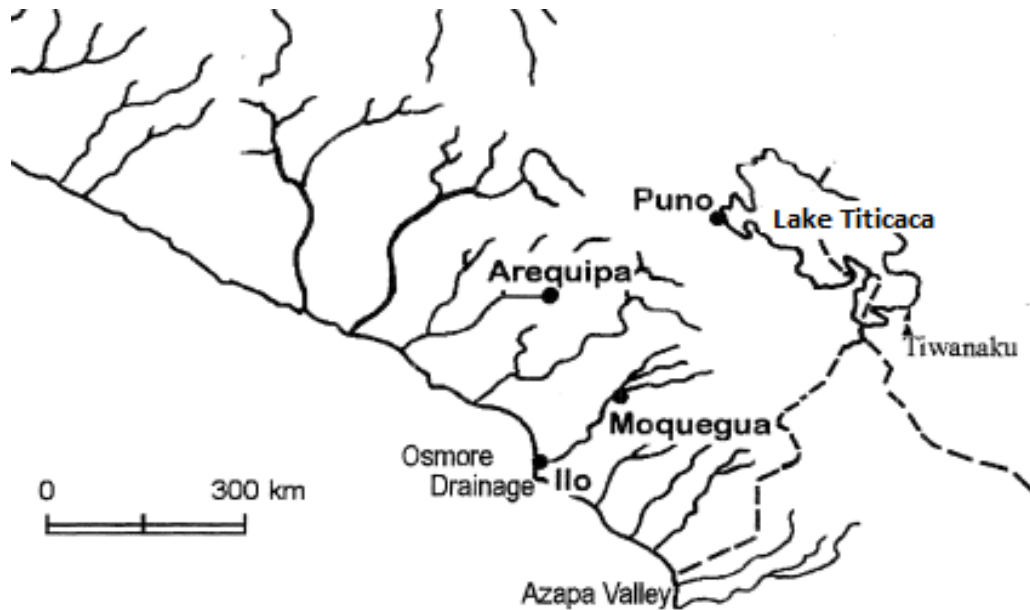
on Tiwanaku, Wari and earlier ceramics, textiles and architecture (see fig 7.2). These items have been found in élite mortuary contexts in San Pedro de Atacama and to a lesser extent in the Moquegua and Azapa Valleys (Llagostera *et al.* 1988; Focacci 1990; Chacama 2001).



**Fig. 7.2** – “Staff God” imagery on a. Tiwanaku snuff tray from Coyo Oriente (Torres 1986), Chile b. Tiwanaku/Wari textile fragments bearing “Staff God” imagery, c. AD 750 – 1000 (Brown University 2012), c. Ceramic with “Staff-God” figure from the north coast of Peru, c. AD 900-1000 (Viau-Courville 2012).

Certainly the end of Tiwanaku tradition in the Azapa Valley was concurrent with the local Cabuza style. Cabuza ceramics are typified by black on red slip ware, which Daulsberg believed to be derived from an earlier highland tradition (Goldstein 1996). The persistence of this style alongside the Tiwanaku style ceramics has led some researchers to suggest the co-existence of small enclaves of Tiwanaku colonists imposed on a local Cabuza population. In the Moquegua Valley (Osmore drainage) to the north of the Azapa Valley, Tiwanaku presence is much more obvious. There are around 30 sites with Tiwanaku features, including a Tiwanaku style sunken court temple and spatially distinct Tiwanaku cemeteries. There is also

bioarchaeological evidence for mass migration of highland people to Moquegua



**Fig. 7.3** – Moquegua in relation to the Azapa Valley (after Blom *et al.* 1998).

during the Tiwanaku V phase (10<sup>th</sup> century) (Goldstein 1993; Hoshower *et al.* 1995; Blom *et al.* 1998). Goldstein suggests that the presence of large Tiwanaku style specialised ceremonial architecture in Moquegua is indicative of state control (Goldstein 1993).

This differs from the situation in Azapa. The presence of small enclaves of Tiwanaku people has been interpreted in different ways – from a symbiotic relationship between the two groups to a system of marked stratification, with the Tiwanaku style ceramic tradition representing the “highest status” (Berenguer and Daulsberg 1989). This assumption is based on the higher quality of textiles, ceramics and the presence of snuff trays, spoons and *altiplano* style four-pointed hats, thought to be a marker of élite status during the Middle Horizon (Goldstein 1996).

#### **7.2.4. Tiwanaku presence in the Azapa Valley: Archaeological evidence**

Azapa sites with Tiwanaku ceramics are much rarer than sites with Cabuza ceramics. Polychrome *keros*, *tazones*, and pitchers as well as *ollas* and *tinajas* have been found at habitation sites. Only three habitation sites in the Azapa Valley have been found that are believed to be unambiguously affiliated with Tiwanaku. One of these is AZ-83, located near to three small Tiwanaku cemeteries – AZ-9, AZ-14 and AZ-19. Azapa-83 was bulldozed in 1974, but prior to demolition of this site, archaeological excavations recovered both Tiwanaku and Cabuza ceramics and textiles. Two uncalibrated radiocarbon dates of AD 560 ± 110 and AD 760 ± 70 were obtained from this material (Rivera Diaz 1987).

Generally burials have received more attention than habitation sites in the Azapa Valley, as cemeteries are more numerous than habitation sites. Tiwanaku cemeteries in the Azapa Valley are rare and small in size. Some have exclusively Tiwanaku grave goods, whilst others have a mixture of both Cabuza and Tiwanaku style artefacts. The Tiwanaku cemetery AZ-143 in the Quebrada del Diablo is a cluster of some 30 stone-lined cists. The site was heavily looted, but excavations revealed Tiwanaku style domestic ceramics, warp-face and tapestry textiles as well as Tiwanaku style quartzite tipped arrows. Another much larger cemetery is located only 10m away. This cemetery, AZ-144, contains around 200 looted burials and is spatially separate from the smaller Tiwanaku cemetery. An examination of pottery scatter over the cemetery suggests a Cabuza affiliation. AZ-143 and AZ-144 are thought to be contemporaneous. Goldstein suggests that the juxtaposition of the “high status” Tiwanaku cemetery and the lower status, more populous cemetery

supports Berenguer and Daulsberg's (1989) theory of elite but small enclaves of Tiwanaku groups present in the Azapa Valley.

Tiwanaku burials have been found in relation to earlier sites. Around 30 Tiwanaku stone lined cist tombs containing high status grave goods such as four-pointed polychrome hats were found at AZ-14, a large tumulo site dating to the Alto Ramírez phase. The burials were scattered along the south-eastern edge (Goldstein 1996). Goldstein argues that the deliberate placement of high status individuals and offerings in earlier impressive landscapes served to help the Tiwanaku to "glean local prestige by reference to previously sanctified places" (Goldstein 1996: 65).

#### **7.2.5. Tiwanaku presence in the Azapa Valley: Skeletal evidence**

Bioarchaeological analyses of Azapa Valley populations have drawn differing conclusions on the matter of Tiwanaku or *altiplano* colonisation.

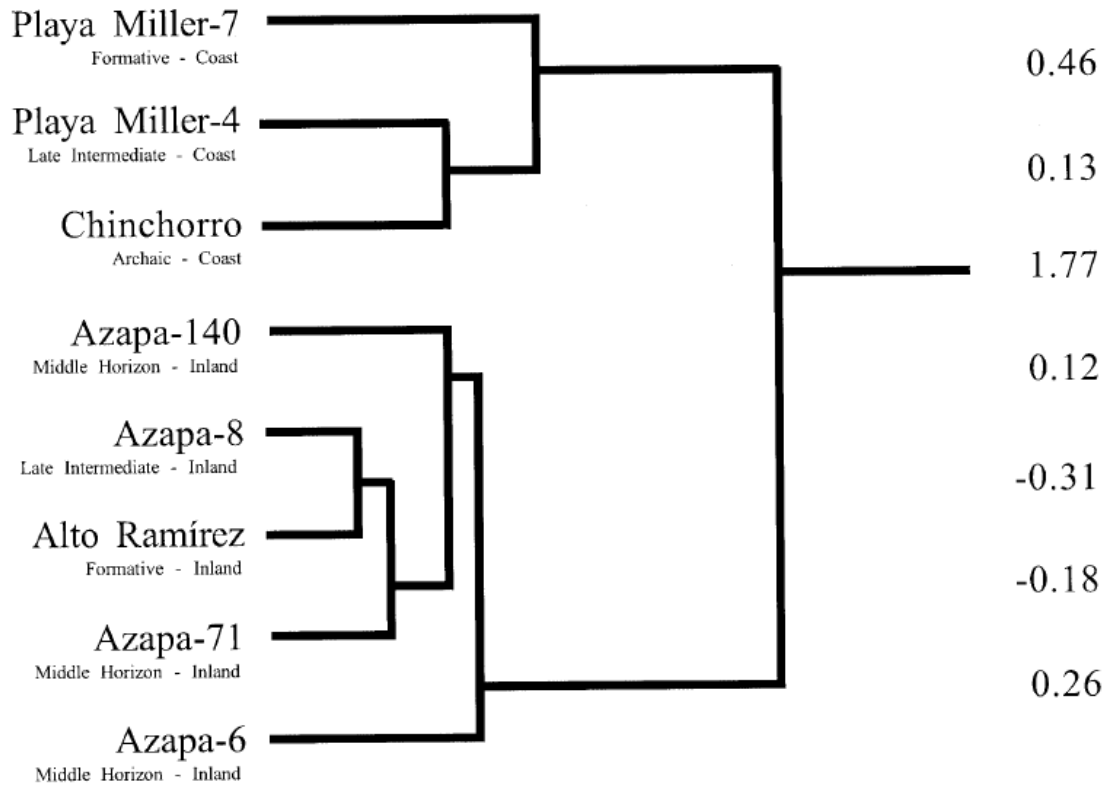
Francisco Rothhammer and Calogero Santoro (2001) used craniometric measurements to determine biological distance between 11 populations (223 individuals) from the Azapa Valley and Arica littoral. The populations dated from the Formative Period to the Late Intermediate Period. They concluded that the earliest inhabitants arrived from the highlands some 9,000 years ago. A decrease in biological distance between *altiplano* and coastal groups was noted during the Formative Period, which the authors suggest was caused by gene flow (i.e. population movement). During the Alto Ramírez phase, the biodistance measurements (using cranio-facial measurements) become statistically non-significant (Rothhammer *et al.* 2002), suggesting that the Tiwanaku people and

coastal people were genetically similar. Rothhammer *et al.* (2002) note that during this period archaeological data suggests that *altiplano* influence started in the coastal valleys. They state that “this result agrees with the hypothesis that population displacements from the highlands may have been responsible for cultural development in the valley during and after the Alto Ramírez phase” (Rothhammer *et al.* 2002). Their data suggests that the peak of gene flow was during the Middle Horizon and Late Intermediate Period (Rothhammer and Santoro 2001) as a result of significant population movement from the highlands to valleys as a result of the collapse of Tiwanaku. Sutter and Mertz (2004) were critical of their methods and interpretation stating that “the investigators did not control for sex, cranial modification, or observation error in the craniometric measures they used”. Cranial modification was a common practice throughout the Andes and was highlighted as a particular issue. Rothhammer *et al.* have suggested that intentional cranial modification did not significantly affect facial measurements. This assumption was based on a series of papers by José Cocilovo (Cocilovo 1973; 1975; 1978). Sutter and Mertz cite a number of studies (Anton 1989; Cheverud *et al.* 1992; Rhode and Arriaza 2006) that suggest cranial modification does significantly affect measurements, casting some uncertainty on the results of earlier studies.

In contrast, the results from a series of biodistance studies utilising cranial and dental non-metric traits (as opposed to cranial measurements) from Azapa populations suggests that there was non-significant gene flow from the *altiplano* (Sutter 2000; Sutter and Mertz 2004; Sutter 2005a; Sutter 2005b). For example, Sutter and Mertz examined 37 non-metric cranial traits in eight Azapa mortuary populations. Cranial traits were eliminated from subsequent multivariate

biodistance analyses if they did not exhibit any variation. In addition, contingency  $\chi^2$  tests were used to exclude traits that were not significantly different. Cranial traits found to be significantly correlated with sex of the individual were also removed from subsequent statistical analyses. None of the biodistances calculated were significant, which suggests that the Azapa Valley mortuary samples represent a relatively coherent breeding population through time, with ancestral-descendant relationships from the Archaic/Preceramic period through the Late Intermediate period (Sutter and Mertz 2004). Cluster analysis (see fig. 7.4) revealed two clusters. The first cluster contained all three coastal groups, whilst the second cluster contained all five inland samples. None of the biodistances were significant. These results suggest that a degree of “breeding isolation” (i.e. limited gene flow) existed between prehistoric coastal and inland populations.

Sutter’s analysis of heritable dental traits in 12 populations from the south central Andes also supports a theory of demic diffusion over time as opposed to complete population displacement in the Andean valleys (Sutter 2005a). Other research using both dental and cranial non-metric traits has suggested that the gene flow between the *altiplano* and coast was non-significant (Guillén 1992; Sutter and Mertz 2004; Sutter 2005b).



**Fig. 7.4** - Cluster analysis of non-metric cranial traits from Azapa populations (Sutter and Mertz 2004).

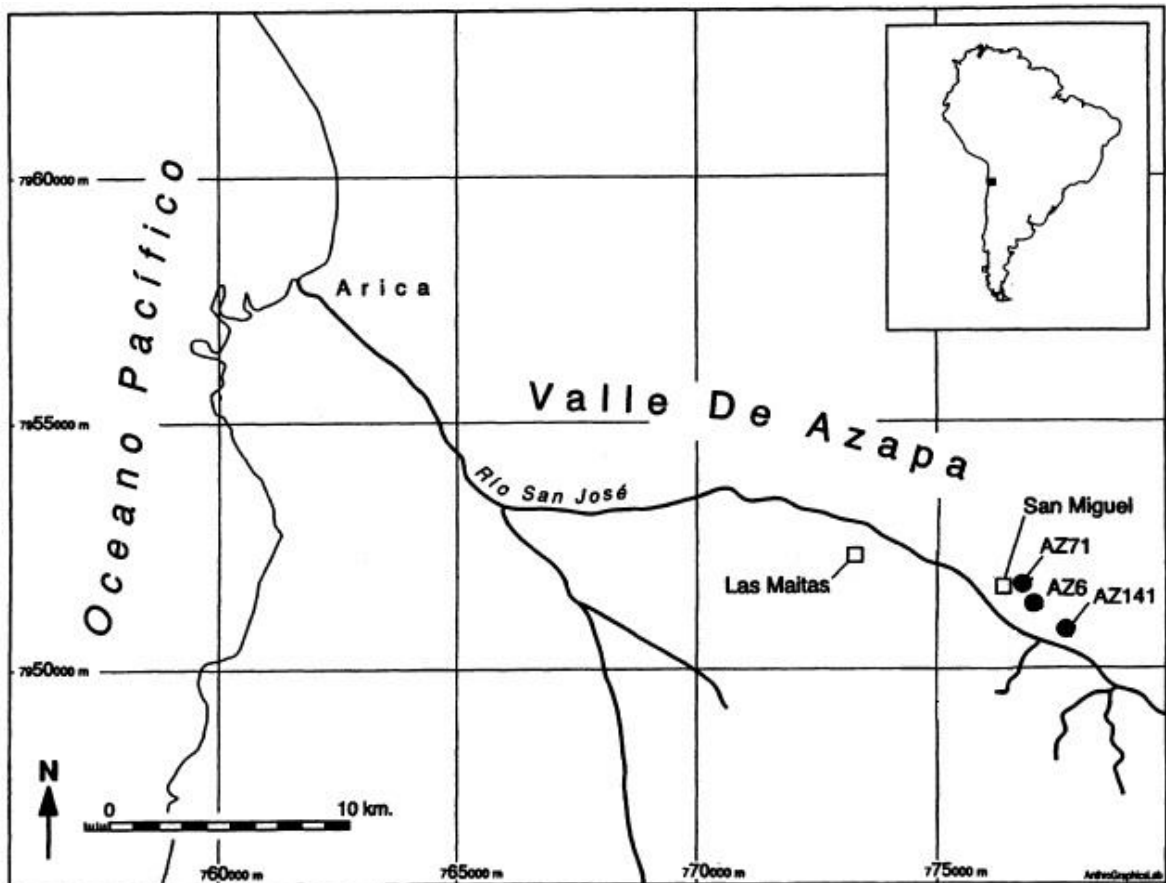
### **7.2.6. Cabuza cemeteries in the Azapa Valley**

Hair samples from Cabuza individuals for this project were collected by Arthur Aufderheide in the early 1980s from three cemetery sites: Azapa-6, Azapa-71 and Azapa-141 (Aufderheide 2003: 115). Aufderheide was part of the excavation team led by Guillermo Focacci, Calogero Santoro and Iván Muñoz. This team excavated cemeteries in the Azapa Valley to make way for agricultural expansion. As Paola Ponce notes in her doctoral thesis, no maps were made during initial excavation, so valuable spatial and temporal information on Cabuza mortuary practices has been lost (Ponce 2010).



Azapa-6 (AZ-6) is a Late Intermediate Period cemetery located 13 km from the coast of the port town of Arica along the northern slope of the Azapa Valley. This cemetery is located adjacent to the modern cemetery of the town of San Miguel and the contemporaneous Late Intermediate Period cemetery Azapa-71 (Santoro 1980; Santoro 1981; Sutter 2005b). The cemetery was uncovered by workmen using bulldozers to build a poultry yard and subsequently excavated by Guillermo Focacci in 1973 as a salvage project (Rivera 1977:58). The cemetery contained 206 burials, 104 of which were Cabuza. Fifty of these remaining burials belonged to Maitas Chiribaya (Tiwanaku period) and San Miguel (Desarollo Regional period) cultures (Focacci 1990).

Azapa-141 is a Cabuza cemetery located approximately 22 km from the coast on the north side of high terrace along the San Jose River. The cemetery was excavated by Guillermo Focacci in 1984 and the analysis of the skeletal remains was carried out by Dr Marvin Allison the following year. No written report resulted from this excavation, therefore all the available information came from the fieldwork notes left by Focacci (Ponce 2010).



**Fig. 7.5.** – The Azapa Valley showing the locations of AZ-6, AZ-71 and AZ-141 (after Rothhammer and Santoro 2001: 60).

A total of 55 bodies were found. Individuals of all ages, sexes and in various degrees of preservation were represented. Eighteen were males, ten were females, 14 were subadults and the remainder ( $n=13$ ) were incomplete, represented only by crania (Ponce 2010). Radiocarbon dates suggest the cemetery was in use between AD 900 and 1300, according to the records held at the Laboratorio de Antropología Física, Museo Arqueológico San Miguel de Azapa in Arica (Ponce 2010).

Typical Cabuza burials, like those at AZ-6, AZ-71 and AZ-141 consist of a body in a flexed or semi-flexed position wrapped in camelid wool textiles. The textiles are sometimes held in place with vegetable fibre ropes. The body is usually accompanied by grave goods. Common findings in Cabuza funerary assemblages

include wooden spoons, coiled basketry, plain and decorated ceramics, coca leaves, gourds, food items (e.g. maize, potato, beans, camelid bone), textiles and other clothing items, hunting and fishing equipment. Weapons and snuff kits are also found, but less frequently (Focacci 1990).

### **7.3. Mummies from the Museo Regional de Iquique, Chile**

#### ***7.3.1. Los Verdes and Bajo Molle-1: An overview***

As the result of a salvage project in 1995, seven spontaneously mummified bodies were found at sites near to the coastal city of Iquique on the Chilean coast (Aufderheide 2003: 156). The mummies from which hair specimens were collected originated from either Bajo Molle or Los Verdes and were curated at the Museo Regional de Iquique.

Bajo Molle is located 8 km from Iquique. The initial discovery in the late 1950s revealed a tomb containing the bodies of three adults and two subadults (Moragas 1995). The site was disturbed in 1969 during the construction of a coastal road. More mummies and associated objects were found in 1992, whilst further mummies were discovered during a later salvage operation (Moragas 1995; Aufderheide 2003: 156). Burials were generally semi-flexed, covered with totora mats, sea kelp and the feathers of sea birds. Red pigment was found on the lower limbs of some individuals. Other grave goods included brown ceramics (*pucos*, bottles), maize cobs, vegetable fibre bundles, anthropomorphic and ornithomorphic vessels and polychrome jars. Generally the ceramics were rare and somewhat crude in appearance. Wooden artefacts included simple paddles, snuff trays and tubes,

boxes of various shapes and spindle whorls. Textiles found at the site are typical Tiwanaku influenced Cabuza items, including high status items such as four-pointed polychrome hats.

Most mummies from Bajo Molle dated to the Desarrollo Regional<sup>9</sup> period that post-dates the Cabuza phase (c. AD 1000 - 1400, Rivera 2008: 970). Aufderheide suggests a range from AD 1115 to AD 1310. However at least two individuals from Bajo Molle, an adult female (IQU-95 T3) and an infant male (IQU-95 T7) were thought to belong to the Cabuza/Tiwanaku period. One individual, IQU-95 T2, an adult female from the Los Verdes site was also believed to be Cabuza or Tiwanaku related.

The Los Verdes site is a sheltered cove some 24 km to the south of Iquique. The cemetery site (Los Verdes-1) is associated with a nearby habitation site (Los Verdes-2). The site is thought to be a late Tiwanaku colony, based on the presence of Tiwanaku style material culture, such as snuffing equipment with Tiwanaku iconography and polychrome textiles (Moragas 1995).

### **7.3.2. Bioarchaeological studies**

These mummies were examined and recorded in great detail by Arthur Aufderheide in 1995 as part of a bigger project to obtain biological information on mummies with no or very limited provenance to “....test the hypothesis that useful anthropological/archaeological and biomedical information could be derived from bodies even when no reliable provenience for recovered mummies is known”

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<sup>9</sup> This period correlates roughly with the end of the Middle Horizon and Late Intermediate Period using the Rowe-Lanning system described in Chapter 1.

(Arriaza *et al.* 2008a: 55). A number of scientific tests were carried out, including aDNA analysis for tuberculosis and Chagas disease, hair tests for BZE using RIA, radiocarbon dating, and osteological and palaeopathological analysis (see table 7.1). These individuals were curated at the Museo Regional de Iquique until 1995. As part of the study previously mentioned, these individuals were subject to destructive postmortem examinations. Fuller descriptions of these individuals based on Aufderheide's original field notes are in Appendix 3.

The following analyses were carried out on the Iquique mummies in addition to traditional osteological analyses: radiocarbon dating, stable light isotope analysis of bone apatite (carbon and nitrogen), aDNA testing for *Mycobacterium tuberculosis* and *Trypanosoma cruzi*, and RIA assay for benzoylecgonine (BZE). The table below summarises the scientific testing of the three Iquique individuals included in this current research project: IQU-95 T2 (Los Verdes), IQU-95 T3 and IQU-95 T7 (Bajo Molle-1)

**Table 7.1** – Summary of scientific analyses of the Iquique individuals

<i>Mummy</i>	<b>Dating</b>	<b>Stable light isotopes</b>		<b>aDNA analyses</b>		<b>RIA assay</b>
	<i>C14 date</i> (BP)	$\delta^{13}C$	$\delta^{15}N$	<i>Chagas</i>	<i>TB</i>	<i>BZE</i>
<b>IQU-95 T2</b>	1310 (125)	-8.80	22.46	-	+	-
<b>IQU-95 T3</b>	935 (75)	-8.16	22.46	+	-	+
<b>IQU-95 T7</b>	760 (85)	-18.39	25.25	+	-	NT

The stable light isotope values obtained from bone apatite suggest the diets of these coastal peoples had a significant marine component (high  $\delta^{15}\text{N}$  values). Arriaza *et al.* (2008a) suggest that a portion of the C4 components of the diet came from marine sources, leading them to conclude that the diets of these individuals composed a “generous quantity of terrestrial plants of which maize constituted only a minor fraction, supplemented by abundant marine resources” (Arriaza *et al.* 2008a: 63).

A DNA probe directed at a kinetoplast DNA segment of the protozoan *Trypanosoma cruzi* was used to detect the presence of the causative agent of Chagas disease in the Iquique mummies. Two individuals, IQU-95 T3 and IQU-95 T7 both tested positive for Chagas disease. Both of these individuals were included in this current research project into psychoactive compounds in ancient hair.

Chagas disease, discovered in 1909, is also known as American trypanosomiasis. This chronic, incurable parasitic disease is endemic to central and south America where some 10 million people are thought to be carriers (Aufderheide *et al.* 2004). The disease reservoir is in around 150 species of mammal, including cats, dogs, guinea pigs, rodents, marsupials and armadillos (Rassi *et al.* 2010). The disease itself is spread via large blood sucking insects from the Triatominae family (Aufderheide *et al.* 2004; WHO 2009; Rassi *et al.* 2010). When these insects bite a human, the ingestion of the blood meal causes the insect (vector) to defecate. When the itching bite area is rubbed, the disease-laden faeces are pushed into the bite wound. By these methods, the trypanosome gains access to the victim’s blood stream, initiating the acute stage of the disease. The parasite penetrates tissue cells (in

particular the myocardium and meninges). The death rate for persons in the acute stage is 10% due to myocarditis or meningoencephalitis (Aufderheide *et al.* 2004). Those who survive the acute stage either pass into an asymptomatic stage or the chronic stage of the disease, which can last decades. The parasite eventually causes massive damage to the myocardium, resulting in extensive inflammation and fibrosis. Eventually the heart becomes enlarged and cannot pump blood effectively (dilated cardiomyopathy). The ganglion cells in the gut may also be destroyed, leading to paralysis of parts of the digestive system, resulting in intestinal obstruction (Aufderheide *et al.* 2004).

Macroscopic examination of mummies from South America has yielded individuals with megacolon, megaesophagus and other changes associated with Chagas disease. Histological, immunological and DNA testing has confirmed the presence of Chagas in Peruvian, Chilean and Brazilian mummies (Rothhammer *et al.* 1985; Fornaciari *et al.* 1992; Gühl *et al.* 1999; Ferreira *et al.* 2000; Madden *et al.* 2001; Aufderheide *et al.* 2004; Fernandes *et al.* 2008; Araújo *et al.* 2009). Megacolon has been identified in ancient human remains (c 1200 years old) as far north as the Lower Pecos region of south Texas (Barth and Kundrotas 2011).

One individual, also included in this current study on psychoactive compounds in ancient hair, an adult female aged 40-50 (IQU-95 T2), was noted to have lung adhesions and destruction of vertebral bodies as well as a lytic lesion with periosteal bone formation on the scapula. These lesions were compatible with a diagnosis of tuberculosis. Bone samples were tested for *Mycobacterium tuberculosis* aDNA. A sample from a rib lesion yielded no positive amplification of

mycobacterial aDNA, a sample from an affected vertebral body indicated the presence of *M. tuberculosis* DNA (Arriaza *et al.* 2008a).

Tuberculosis is an infectious disease caused by the genus *Mycobacterium*, which affects humans and non-human mammals. Tuberculosis is spread via infected droplets in the air from human to human (*M. tuberculosis*) or by ingesting infected animal products leading to a disease not involving the lungs (*M. bovis*). Contact with wild and domesticated animals with the infection may lead to pulmonary or gastrointestinal routes of transmission. Tuberculosis is a disease of poverty. Risk factors for tuberculosis in ancient (and modern) populations include proximity to animals (in particular cattle), overcrowding and poor living conditions, poor diet, poor hygiene, occupation, ethnicity, disasters and migration (Roberts and Buikstra 2003: 12).

Advances in aDNA methodologies, in particular polymerase chain reaction (PCR) techniques have allowed for the investigation of lesions suspected to be caused by tuberculosis. In addition to IQU-95 T2, a number of other Andean mummies have tested positive for *M. tuberculosis*, including an adult female from Chiribaya Alta in the Lower Osmore drainage (c 1000 AD) (Salo *et al.* 1994) and in a 11-13 year old girl with Pott's disease. Radiocarbon dating placed her in the late Maitas Chiribaya or early San Miguel phase (AD 1040 ± 70) (Arriaza *et al.* 1995). The girl was buried with a sash and belt. It was suggested that this was designed to provide mechanical support and pain relief. In addition, a quid of coca leaves was found in her mouth. The authors suggest this may have been chewed to relieve pain (cocaine has analgesic action), but RIA testing of her hair for BZE yielded a negative result



(Arriaza *et al.* 1995). The negative hair test may be a result of significant postmortem degradation rather than this individual not having chewed coca leaves.

#### **7.4. The Late Horizon cemetery at Puruchuco-Huaquerones**

##### ***7.4.1. Archaeology of the Central Peruvian Coast***

The central Peruvian coast has been continuously occupied for at least 10,000 years. Over 650 sites have been identified in the Chillón and Lurín valleys dating from the Preceramic period to the Late Horizon (Patterson and Lanning 1964; Feltham 1984). The Chavín culture was one of the earliest presences in the Rimac Valley, present from around 1700 BC. The Lima, Wari and Ichma cultures also are evident in the archaeology of this valley. During the Late Horizon the inhabitants of the valley were part of the *Ate/Lati curacasgo*, part of the larger Ichma *señorio*, under the religious rule of Pachacamac in the neighbouring Lurín valley (Williams 2005: 79).

Occupation in this area tended to be restricted to hillsides and the edges of valleys. However, during the Late Horizon habitation sites moved to valley floors. Some researchers suggest that this may be due to an increase in reliance on agriculture after this area was brought under Inca imperial control around AD 1471 (Rowe 1946).

## **7.4.2. Puruchuco-Huaquerones**

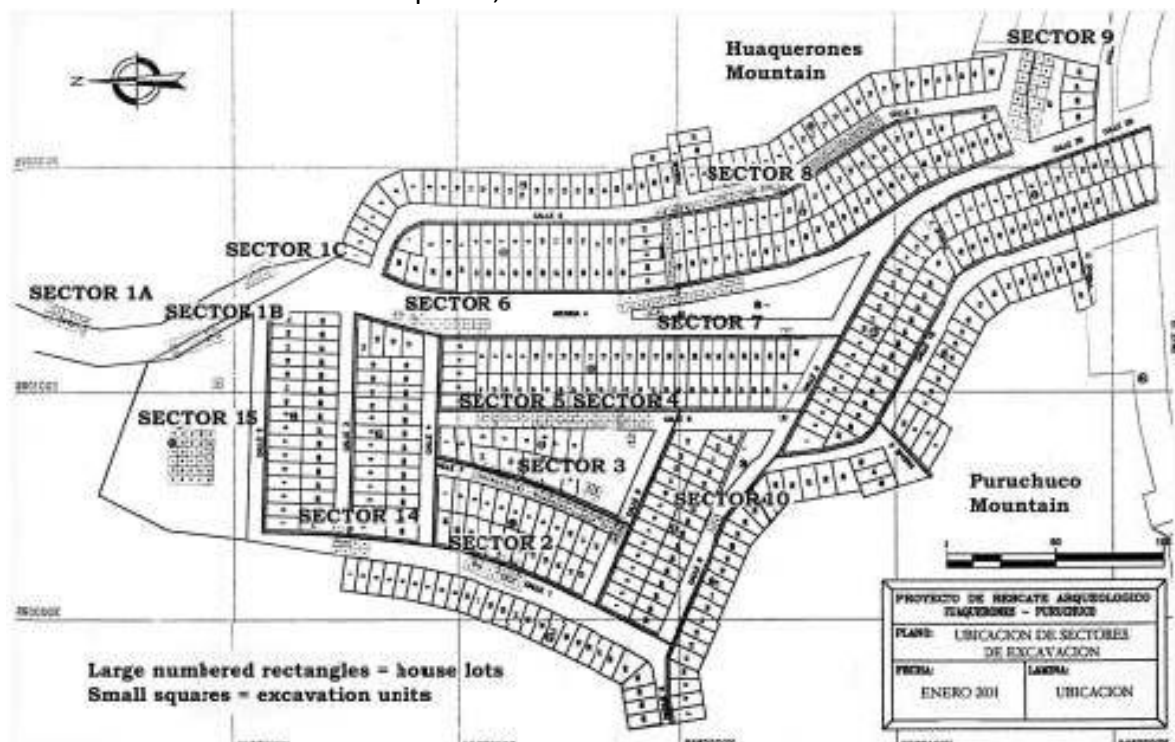
### *7.4.2.1. Site location and history*

Puruchuco-Huaquerones is primarily a Late Horizon (AD 1476-1532) cemetery located in the Rimac Valley in the Department of Lima, on the central Peruvian coast. The site lies on the eastern border of metropolitan Lima (Haun and Cock Carrasco 2010: 201). Puruchuco-Huaquerones is south of the Rimac River, which is 150 km long with origins at 4800 metres above sea level in the Western Andes (Williams 2005: 78) . The river runs year-round. The surrounding land is very suitable for agriculture, and at an altitude of 300 metres above sea level, the area is at the transition between the coast and *yungas* (warm forests). This makes the Rimac valley ideal for coca cultivation, similar to the neighbouring Lurín valley (Williams 2005: 78). The site encompasses two hills (*cerros*), Puruchuco and Huaquerones as well as the intervening *quebrada* of Huaquerones. The site occupies an area that was once part of the Lati (or Ate) minor *Señorio* during the Late Intermediate Period (Haun and Cock Carrasco 2010: 201) and contains a number of cemeteries and buildings, including the palace of Puruchuco, which was restored in the 1950s by Arturo Jiménez Borja (1988). The palace was probably built for a local *curaca* during the Late Intermediate Period, but remodelled during the Late Horizon.

Much of the site is now underneath the shanty town of Túpac Amaru. The town was founded in 1989 by some 340 families fleeing guerrilla activity in the highlands. The mummies are less than 2 metres below the surface. As a result of the influx of water and sewage from the shanty town and the use of bulldozers in construction, the

mummies are degrading rapidly. In some cases they have been dug up and burned by the town inhabitants hoping to avoid scientific excavation that would delay town development, despite the site being designated a national monument (Cock 2002). Preservation at the site varies from very well preserved mummies (including textiles and other organic materials) to poorly preserved bone (Williams 2005: 89; Murphy *et al.* 2010). The site has also been heavily looted over the years (Haun and Cock Carrasco 2010: 201).

In 1999 the Instituto Nacional de Cultura del Perú (INC) initiated an archaeological assessment of the site prior to the installation of sewage pipes and electricity. The excavations, carried out by Guillermo Cock and a team of Peruvian archaeologists, uncovered hundreds of well-preserved burials. Large scale excavations funded by the people of Túpac Amaru and the National Geographic Society were undertaken on 2001-2002. The site was divided into 16 sectors, usually 8x20 m. Each sector was divided into 3x3 or 5x5m squares, which in turn were subdivided into 1x1m units.



**Fig. 7.6** – Sectors and excavation units at Puruchuco-Huaquerones (Williams 2005, after Cock 2001).  
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A variety of burials have been identified at Puruchuco-Huaquerones, including mummy bundles (*fardos*), *camillas* (reed stretchers, see fig. 7.7) and secondary burials. Six types of *fardo* were identified: these include simple cotton textile shrouds, woven mats made from plant fibres, layered cotton textiles with alternate layers of grasses/leaves, layered cotton textiles with alternate layers of unprocessed cotton, layered cotton textiles with alternate layers of cotton seeds and a cotton textiles with alternate layers of unprocessed cotton with cottonseeds. Around 6% of individuals had false heads (*falsa cabeza*, see fig. 7.7). These false heads are made



**Fig. 7.7** – Left: A *falsa cabeza* being excavated at Puruchuco-Huaquerones. Right: An infant buried on a reed *camilla* (Ira Block, [www.irablock.com](http://www.irablock.com)).

from textile and sometimes have facial features and wigs. *Falsa cabezas* were only associated with *fardos* stuffed with cotton or cotton seeds (Haun and Cock Carrasco 2010: 203). Typically these *fardos* contain more than one individual and have a

greater variety and generally higher quality grave goods including woven vegetable fibre mats, ceramic vases, string bags containing vegetable offerings, gourds with maize and beans, weaving kits and weaving tools made of wood and rope wrapping (Cock 2001 cited in Williams 2005: 87). Reed stretchers “*camillas*” were primarily used to bury children. Secondary burials were unusual at Puruchuco-Huaquerones. A total of 21 identified, where the individual was exhumed after the decomposition of soft tissue and reburied as a mummy bundle by inserting a dried reed stick through the vertebral foramen and weaving the ribs together using string (Williams 2005: 87).

### **7.2.3. Bioarchaeological investigations at Puruchuco-Huaquerones**

#### *7.2.3.1. Health and diet*

The people buried at Puruchuco-Huaquerones witnessed the expansion, domination and collapse of the Inca Empire (c 1476-1532) (Murphy 2004). Melissa Scott Murphy hypothesised that power shifts from local to imperial Inca administrators may have affected the health of communities buried at Puruchuco-Huaquerones. A total of 207 individuals from Late Horizon contexts were examined for evidence of nonspecific indicators of stress, fractures, dental caries and degenerative joint disease. Evidence of social differentiation with the mortuary sample was inferred from the presence of (1) false heads, (2) shell offerings, specifically *Spondylus princeps*, (3) metal offerings, and (4) ceramic offerings (Murphy 2004). Murphy found no association between social status and health. She noted that “health differences between different social status groups are

significantly less hierarchical than anticipated". The skeletal remains at Puruchuco-Huaquerones also provide evidence of violence around the time of the Spanish conquest of the Inca Empire, including some of the earliest evidence of trauma inflicted with European weapons, such as steel edged weapons and firearms (Murphy *et al.* 2010).

Jocelyn Williams analysed mummified soft tissues and bone from 45 individuals for carbon and nitrogen isotopes as a means of palaeodietary reconstruction (Williams 2005). These individuals were from of Late Intermediate Period contexts at Puruchuco-Huaquerones. Hair, nail, skin and muscle record short term dietary information, whilst bone records longer dietary trends, somewhere in the region of 10-25 years (Williams 2005). Williams' analyses revealed a strong C4 dietary component, indicating a diet based on maize, with some C3 resources (probably tubers) and low trophic level animal protein, most likely camelids and *cuy* (guinea pig). Segmental analysis of hair showed a cyclical intake of C3 resources. Tubers are a wet season (winter/dry season) crop, therefore probably only consumed in winter (there is no archaeological evidence that food in any significant quantity was stored). Of the 45 individuals, 47% had depleted  $^{13}\text{C}$  levels at the time of death (most proximal hair segment), suggestive of death during winter or spring. 53% had enriched  $^{13}\text{C}$  levels, suggestive of death during summer or autumn (wet season). These differences were not significant. However, the slightly higher death rates during the wet summer months in Peru are characterised by increased morbidity from bacterial diarrhoea, cholera, parasitic infection and malaria (Yeager *et al.* 1991; Lanata *et al.* 1992; Marín *et al.* 1996; Franco *et al.* 1997; Guthmann *et al.*

2001; Martinson *et al.* 2003; Ortega and Bonavia 2003; Lama *et al.* 2004; Williams and Katzenberg 2012).

In addition to the stable light isotope analyses, Williams also carried out osteological analyses of the 63 of the 72 individuals she used for her isotopic work, Sex was estimated using the criteria in Buikstra and Ubelaker (1994). Age was estimated using sternal rib end methods (İşcan *et al.* 1985) and diaphysal bone length for subadults (Ubelaker 1989). The remains were also examined for the presence of cribra orbitalia, porotic hyperostosis, tuberculosis, trauma and osteoarthritis.

Williams concludes that health was relatively good at Puruchuco-Huaquerones. Her findings indicate that stature was comparable with other Andean sites and sexual dimorphism is evident. However, when she compared the average statures at Puruchuco-Huaquerones with modern growth charts, she found that males were below the 5<sup>th</sup> percentile and females at the 10<sup>th</sup> percentile. She suggests that both males and females were under chronic stress which compromised growth and development. In addition, rates of chronic stress markers such as cribra orbitalia (33% adults, 65% subadults), porotic hyperostosis (36% adults, 53% subadults) and periostitis (17% all individuals) were relatively high, indicating a persistent stress, such as parasitism, dietary insufficiency, or diarrhoeal disease, which is still a chronic stressor for modern Andean populations (Yeager *et al.* 1991; Checkley *et al.* 2000).

7.2.3.2. *Puruchuco-Huaquerones* – a *mitmaqkuna* community of weavers?

The majority of individuals buried at Puruchuco-Huaquerones had weaving tools in their funerary offerings (see fig 7.8). Agricultural, metallurgical and tools for the production of ceramics are overwhelmingly absent (Haun and Cock Carrasco 2010: 204). Ethnohistorical evidence suggests that the Inca relocated whole communities with specific craft specialisations as a means



**Fig. 7.8**– Weaving tools found in the grave assemblage of an individual at Puruchuco-Huaquerones (Ira Block, [www.irablock.com](http://www.irablock.com)).

of optimising land use and production (Haun and Cock Carrasco 2010: 194). These people kept all their traditions, including dress styles and maintained ties of reciprocity and kinship, although Rostworowski de Diez Canseco (Rostworowski de Diez Canseco 1999: 172) suggests that at the height of Inca expansionism, ties between the communities and their places of origin would have been weakened or lost due to the distance between them. These relocated communities are known as *mitmaqkuna* in Quechua (from *mitma* – “meaning to spread, distribute,” and *kuna* meaning “people”). *Mitmaqkuna* were kin based groups that were relocated to other areas and set up with lands and herds to provide a stable, skilled and permanent community of craft specialists in the employment of the state (Costin 1996: 216).



Relocation appears to have been a widespread phenomenon (D'Altroy 2002: 248) but problems arise when trying to identify these state colonies in the archaeological records (Haun and Cock Carrasco 2010: 196).

The overwhelming presence of weaving tools in burials at this site may be an indicator that these inhumations represent a community of weaving specialists, possibly relocated from elsewhere. However, bioarchaeological evidence suggests that the people buried at Puruchuco-Huaquerones were local people. Williams (2005: 323) compared the average carbon and nitrogen isotopic composition of the soft tissues (recording short term diet) with the average isotopic composition of bone collagen (recording long term diet). She found these data were very similar. Although diet varied in the months before death (due to seasonal intake of C<sub>3</sub> foods), the foods that were being consumed were similar in their isotopic composition to the foods consumed for the last 10-25 years of life. This suggests that the Puruchuco-Huaquerones individuals lived on the coast for at least the last 10-25 years of their life and were probably not relocated from the highlands during the Inca expansion. It does not discount relocation from another part of the coast. Haun and Cock Carrasco's (2010) preliminary biodistance (skeletal and dental metric and non-metric traits) and grave goods analysis also suggests the population of the Huaquerones cemetery were largely local people rather than *mitmaqkuna* or migrants from the highlands.

## 7.5. Summary

The majority of hair specimens come from three contemporaneous cemeteries located in the Azapa Valley in northern Chile. The cemeteries date from the Late Intermediate Period, with all individuals thought to be affiliated with the Cabuza culture. The Cabuza herded animals and cultivated a wide variety of crops, including coca, which is occasionally found in Cabuza grave assemblages.

The exact nature of the relationship between the coastal Cabuza people and *altiplano* Tiwanaku polity is debated. However, it is clear from various archaeological discoveries that there was some form of Tiwanaku presence in the Azapa Valley during the Cabuza period. *Anadenanthera* iconography is well documented in Tiwanaku ceramics and snuff trays. The “snuffing complex” – whilst not unique to Tiwanaku - is most associated with this culture. Snuff trays are rare in Azapa; however, they are not unheard of. Snuff trays have been found at the three Azapa cemeteries included in this study, but not with any of the individuals being tested for this project.

Therefore, based on archaeological evidence, it would be expected that some individuals will test positive for coca metabolites (cocaine, BZE). *Anadenanthera* snuff was probably only used by “high status” males. Snuff trays are overwhelmingly associated with this group, leading to the assumption that it was these males who used hallucinogenic snuff. Fewer individuals may test positive for bufotenine, the active compound in *Anadenanthera* snuffs.

The samples from Puruchuco-Huaquerones represent a coastal community of weavers who probably identified as Ichma, but would have been under Inca

imperial control during the Late Horizon. It has been postulated that coca was controlled during the Inca period. Only 13 hair samples were analysed from an “Inca” population, but 9 of these tested positive (Cartmell *et al.* 1994). The same may be expected for the individuals from Puruchuco-Huaquerones. Snuffing equipment is not commonly found in contexts dating to the Inca period, although the Spanish chroniclers recorded their use by Andean people. The destruction of snuffing equipment was ordered during the “extirpation of idolatries” by the Spanish in the 17<sup>th</sup> century (Arriaga 1968 [1621]). Rowe (1946: 291) also suggests coca was the main psychoactive plant available to the Inca “Narcotics were unimportant in Inca culture. No narcotic was taken expressly to obtain visions...strongest drug-containing substance used by the Inca was coca, next tobacco, and finally, perhaps wil’ka”. Based on what is known about the distribution and importance of coca, it is likely that a significant proportion of the Puruchuco-Huaquerones will test positive for coca metabolites.

## Chapter 8: Materials & Methods

### 8.1. Introduction

This chapter details the analytical standards chosen for the method development for this project. The analytes were chosen as they come from the most well-known psychoactive plant species available to Andean people as well as compounds already detected in ancient hair and depicted in Andean material culture (see chapters 2, 3, 5 and 6). A summary of the 65 hair specimens used for this project is also presented. Detailed cultural and contextual information for the Cabuza, Tiwanaku and Inca/Ichma individuals is presented in Chapter 7 and Appendices 1-3.

### 8.2. Analytical standards

#### 8.2.1. *Choice of analytes*

A number of drug compounds with similar chemistry were chosen as target analytes for hair analysis following a detailed examination of the literature on the archaeological evidence for the use of psychoactive plants in the Andes, coupled with an awareness of commercially available analytes,. Table 8.1 summarises these plant species.

**Table 8.1** – Plant species containing psychoactive compounds (Schultes et al. 2001; Rättsch 2005).

<b>Species</b>	<b>Active compounds</b>
<i>Brugmansia</i> and <i>Datura</i> sp.	atropine, scopolamine
<i>Viola</i> sp.	<i>N,N</i> -dimethyltryptamine, 5-methoxy- <i>N,N</i> -dimethyltryptamine
<i>Psychotria viridis</i>	<i>N,N</i> -dimethyltryptamine
<i>Banisteropsis caapi</i>	harmine, harmaline, tetrahydroharmine, <i>N,N</i> -dimethyltryptamine
<i>Echinopsis</i> sp.	3,4,5-trimethoxy- $\beta$ -phenethylamine (Mescaline)
<i>Anadenanthera</i> sp.	5-hydroxy- <i>N,N</i> -dimethyltryptamine (Bufotenine)
<i>Erythroxylum</i> sp.	cocaine (+ ethanol = cocaethylene), BZE

### **8.2.2 Certified standards for analysis and other reagents**

Table 8.2 below summarises the analytical standards purchased for the development of a multi-component assay for psychoactive compounds in ancient hair.

Corresponding deuterated internal standards were chosen on the basis of availability and price. Deuterated scopolamine was prohibitively expensive ( $\approx$ \$2000 USD) and therefore not purchased.

LC-MS grade acetonitrile, ammonium formate, and formic acid were purchased from Sigma-Aldrich (Gillingham, UK). Analytical grade (18 M $\Omega$ ) deionised water was used to make up the aqueous buffers.

**Table 8.2** – Analytical standards for method development. With the exception of cocaine HCl (Sigma, Gillingham, UK) all standards were purchased from LGC Standards (Teddington, UK).

Compound	Class/Schedule	Batch/Lot number	Expiry date
Atropine (1 ml in acetonitrile)	Not controlled	31346-011	09/2010
Bufotenine (1 ml in acetonitrile)	A/1	FE042709-02	06/2012
Benzoylecgonine (BZE) (crystals)	A/2	n/a	n/a
Cocaine (as cocaine HCl crystals)	A/2	n/a	n/a
<i>N,N</i> -DMT (crystals)	A/1	n/a	09/2010
Harmaline (crystals)	Not controlled	08040-491	07/2013
Harmine (crystals)	Not controlled		01/2011
Mescaline HCl (1 ml in methanol)	A/1	35326-78C	06/2011
Scopolamine (as scopolamine hydrobromide crystals)	Not controlled	K0G033	n/a
Cocaethylene (1ml in acetonitrile)	A/1	n/a	08/2014
Cocaine-D3 (1ml in acetonitrile)	Not controlled	FE080607-03 FE112008-02	08/2012 11/2013
Cocaethylene-D8 (1 ml in acetonitrile)	Not controlled	FE081707-01	08/2012
Mescaline-D9 (1 ml in methanol)	Not controlled	FE010610-01	01/2014

### **8.2.3. Compliance with Misuse of Drugs Act 1971 and The Misuse of Drugs Regulations 2001**

All standards were securely stored and were weighed before and after aliquots were taken from them, fulfilling the requirement for security and record keeping as required by the Home Office license to possess Schedule 1 controlled drugs held by the University of Bradford. The log book for the standards in table 8.2 is retained the University of Bradford Analytical Centre and will be kept for a minimum of two

years after the last date of entry is the log book, as required by the Schedule 1 License to Possess issued by the Home Office.

### **8.3. Hair samples**

A total of 65 samples were acquired for this project. The table below summarises the key features of each set of samples. Chapter 7 and appendices 1, 2 and 3 provides the cultural and contextual information for these individuals. The amounts of hair in each sample bag donated by Arthur Aufderheide varied greatly, from over 60g of hair, including intact braids in one case (IQU-95 T3 from Bajo Molle) to a few strands. In one case, (AZ-71 T46) there was only some desiccated scalp and a maize kernel.

The Cabuza hair samples were chosen from Arthur Aufderheide's archive of hair specimens collected over a number of years as part of a long-term research programme at the Universidad de Tarapaca. The Cabuza specimens represent the largest number of individuals from the same cultural group (see table 8.3). There is also snuffing equipment in contexts from the Azapa Valley this time period (Focacci 1990; Ogalde *et al.* 2009). The Tiwanaku samples from the sites around Iquique were included, as they had previously been tested for BZE and could act as controls for reproducibility, as well as being roughly contemporaneous with the Cabuza specimens. The Inca/Ichma samples provided by Jocelyn Williams as part of a wider programme of stable light isotope analysis (Williams *et al.* 2011a) were included as it is thought that coca chewing was widespread during the Inca period.

**Table 8.3** – Hair samples from Azapa, Iquique and Puruchuco-Huaquerones

Culture	Site	Date	Number of samples	Donated by
Cabuza	Azapa-6	“Late Horizon”	8	Arthur Aufderheide
Cabuza	Azapa-71	“Late Horizon”	28	Arthur Aufderheide
Cabuza	Azapa-141	“Late Horizon”	13	Arthur Aufderheide
Tiwanaku?	Los Verdes	1310 ± 125 ybp	1	Arthur Aufderheide
Tiwanaku?	Bajo Molle	935 ± 75 ybp 1115 ± 120 ybp	2	Arthur Aufderheide
Inca/Ichma	Puruchuco-Huaquerones	15 <sup>th</sup> /16 <sup>th</sup> century	13	Jocelyn Williams
<b>Total</b>			<b>65</b>	

#### 8.4. LC-MS/MS instrumentation and conditions

Chromatographic separation was achieved using a Waters 2695 separations module (Waters, Manchester, UK) interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters, Manchester, UK), equipped with MassLynx 4.0 software. Ionisation was achieved using electrospray ionisation in positive ion mode (ES+).

The separation module was equipped with an Allure™ PFP Propyl column (Thames Restek, Buckinghamshire, UK). Column dimensions: 30 x 2.1 mm, (Particle size: 5µm, pore size 60Å) with a 10 x 2.1 mm Allure™ PFP Propyl guard cartridge (Thames Restek, Buckinghamshire, UK) fitted into a Trident™ 1 cm holder with in-line filters (Thames Restek, Buckinghamshire, UK).



Mobile phase A was 100% acetonitrile, whilst mobile phase B was 5mM ammonium formate in water (pH 2.65), at a ratio of 80:20. The flow rate was 0.2 ml/min. Table 8.4 summarises the parent (Q1) and daughter ion (Q3) and retention time (RT) for each analytical compound.

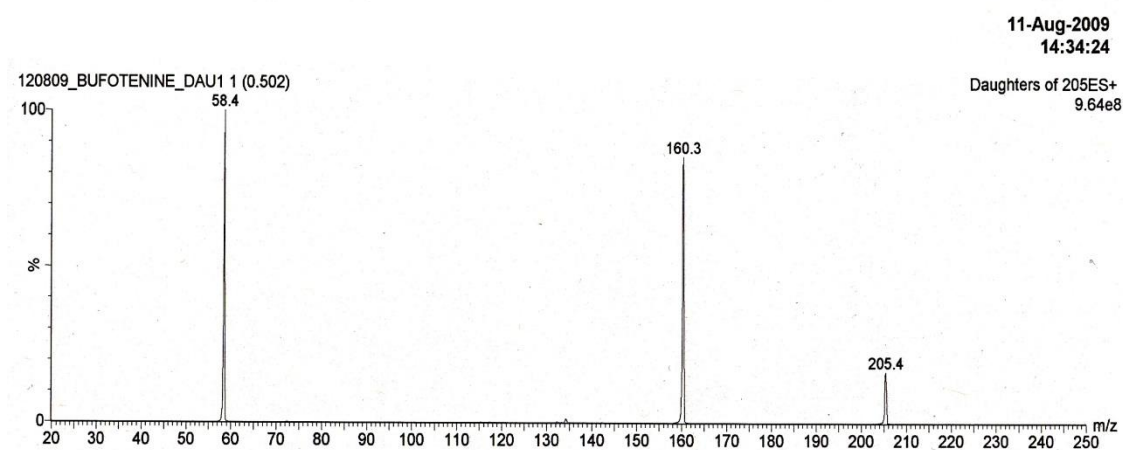
**Table 8.4**– Mass spectrometer settings for each compound. RT = retention time, CE = collision energy.

Compound	Q1	Q3	RT	Dwell	Cone (V)	CE (eV)
	Quantifier					
Atropine	290	124	4.59	0.2	35	28
Bufotenine	205	160	3.86	0.2	35	15
BZE	290	168	0.79	0.2	35	n/a
Cocaine	304	182	8.20	0.2	35	25
<i>N,N</i> -DMT	189	144	5/90	0.2	35	12
Harmaline	215	174	n/a	0.2	35	n/a
Harmine	213	198	n/a	0.2	35	n/a
Mescaline	212	195	4.23	0.2	35	12
Scopolamine	304	138	n/a	0.2	35	n/a
Cocaethylene	318	196	9.30	0.2	35	35
Cocaine-D3	307	185	8.26	0.2	35	25
Cocaethylene-D8	326	204	9.30	0.2	35	35
Mescaline-D9	262	204	4.23	0.2	35	12

Blank samples (100% acetonitrile) were run alternately between sample extracts from ancient hair to ensure that there was no carry-over from previous samples. The samples were also run out of numerical sequence. Samples were only matched to contextual information after analysis.

The parent and daughter ions were established by injecting a standard into the MS/MS system and fragmenting the compound (see fig. 8.1). The Q1 (table 8.4) is the mass of the compound + 1 due to the addition of a proton during ionisation. The quantifier ion in this case is the most abundant ion produced during fragmentation (Zumwalt and Moore 2006).

### 8.5. Method development: Linearity, LOD and LOQ



**Fig. 8.1** – An example of compound fragmentation in a triple quadrupole system. The parent compound is the peak with the highest mass. For bufotenine this is 205. This pseudomolecular ion [M+1] breaks down into two major fragments with masses of 58 and 160. The mass fragment at 160 was used as the daughter ion in this method. (Peters *et al.* 2007).

Linearity is a measure of the relationship between the concentration of analyte in the sample and the corresponding detector response. Calibration lines were generated for each compound at various concentrations (see Appendix 4). Each concentration also had a fixed amount of deuterated internal standard added. The ratio of the response of the deuterated internal standard and compound were plotted to create the calibration lines (see table 8.5 and appendix 4). The same fixed amount of deuterated internal standard is added to the unknown sample. The ratio of the response of the internal standard to the unknown analyte can then be used to quantify the unknown in the sample.

During method development phase, harmine and harmaline were found to be insoluble in acetonitrile, and were therefore removed from the assay as they caused blockages in the guard column, resulting in high pressures in the LC-MS/MS system that would otherwise result in damage to the column and instrumentation. Scopolamine was found to have significant carry-over, requiring a number of blank (100% acetonitrile) samples to be run overnight to clean the column.

**Table 8.5** – Linearity for psychoactive compounds

Compound	Linear range (pg on column)	Equation of line	R <sup>2</sup>
Atropine	25000 – 50	$y = 0.0059x + 1.519$	0.9924
Bufotenine	25000 – 50	$y = 0.0033x + 1.8328$	0.9919
Cocaine	50,000 – 50	$y = 0.0014x + 0.233$	1
Cocaethylene	50,000 – 250	$y = 0.0002x + 0.0035$	0.9971
Mescaline	25000 – 50	$0.0044x - 2.1739$	0.9972
<i>N,N</i> -DMT	50,000 – 5	$y = 0.1317x + 20.952$	0.9956

NB: BZE was added later in the project. There is no linearity data available.

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using Masslynx 4.0 software employing the RMS method. The LOD is defined as a signal to noise ratio (S/N) of 3 or greater, whilst the LOQ is defined as having a signal to noise ratio of 10 or greater (Peters *et al.* 2007). These data are summarised in table 8.6 for the selected compounds for this method.

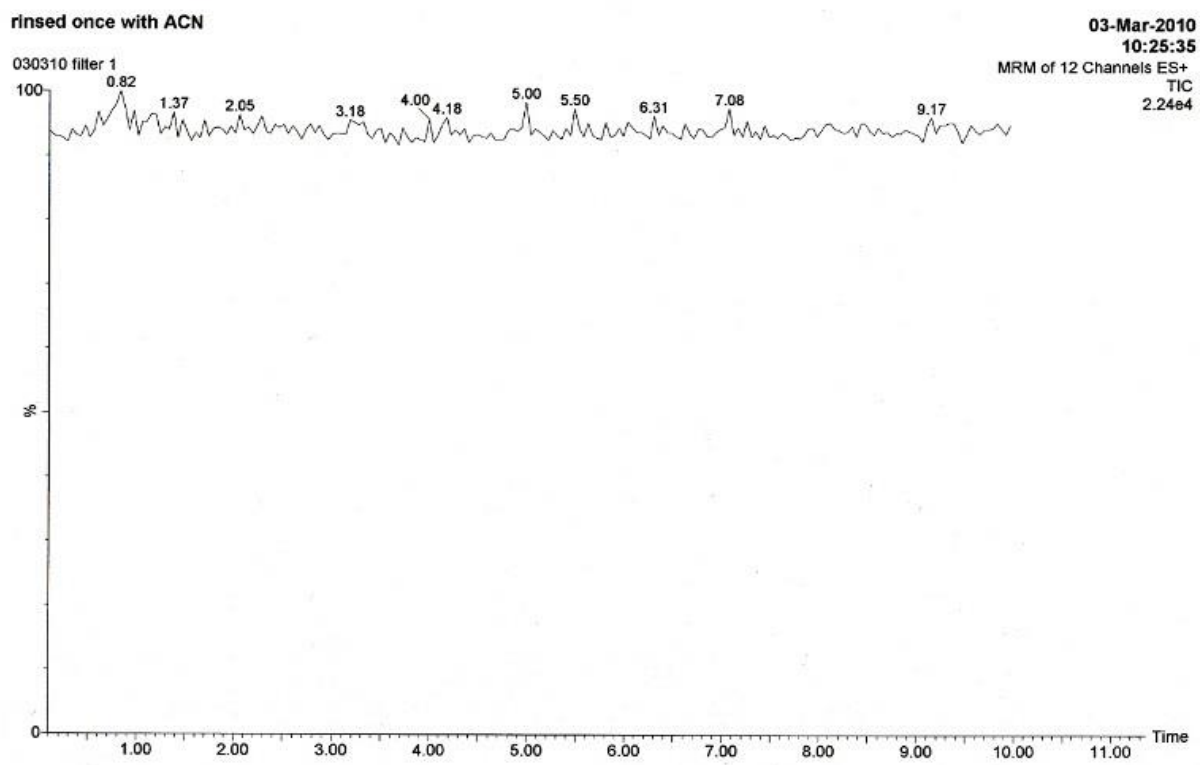
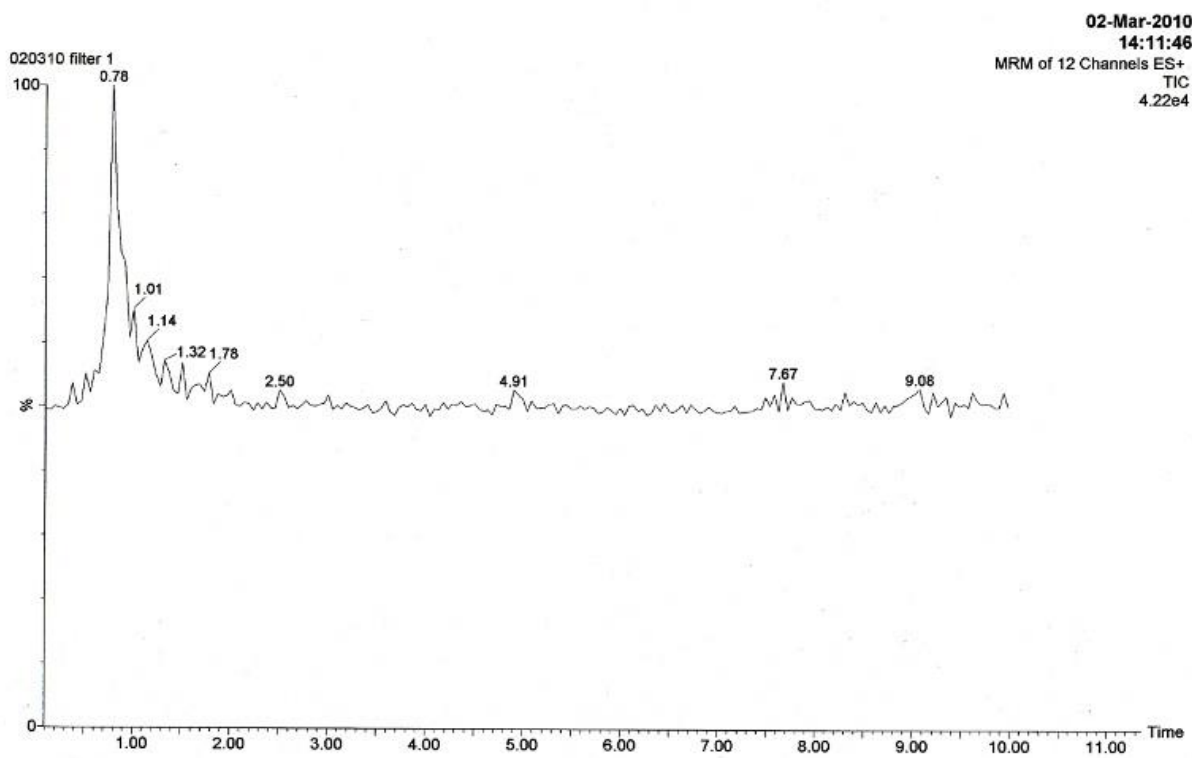
**Table 8.6** – Upper limit of quantification (ULOQ), limit of detection (LOD) and limit of quantification (LOQ) for psychoactive compounds determined in this study.

	ULOQ (pg)	LOD (pg)	LOQ (pg)
Atropine	25000	50	50

Bufotenine	25000	50	50
Cocaine	50000	50	500
Cocaethylene	50000	250	2500
Mescaline	25000	50	50
<i>N,N</i> -DMT	50000	< 50	250

### 8.6. Sample preparation for LC-MS/MS

For each individual where there was a sufficient amount of hair, 2.5 mg and 50 mg hair was prepared for analysis with LC-MS/MS. Each hair sample was weighed and recorded. The sample was then digested with 200  $\mu$ l (for 2.5 mg samples) or 1 ml (for 50 mg samples) 0.1M hydrochloric acid at 45°C for 20 hours according to Valente *et al.* (1981). The temperature was maintained using an electric heating block. The samples were then filtered using a 3ml Luer-lok syringe with a Minispine Acrodisc syringe filter (13mm diameter; with GHP membrane; pore size 0.2 $\mu$ m, VWR International, Lutterworth, UK) that had previously been conditioned with LC-MS grade acetonitrile as experiments indicated omitting this step introduced contaminants into the sample (see fig. 8.2). The samples were blown down under nitrogen at 45°C and re-constituted with 200  $\mu$ l LC-MS grade acetonitrile.



**Figure 8.2** – Top image shows a peak at retention time 0.78 min, indicating a contaminant that would be passed into a sample if not rinsed first. The bottom image shows that this can be eliminated by rinsing the filter prior to use with the sample.

**8.7. Summary**

A multi-component assay for LC-MS/MS was developed for rapid analysis of extracts from ancient hair samples. The analytes were chosen based on archaeological and

ethnographical and botanical evidence of their use in antiquity and modern cultures. Scopolamine, harmine and harmaline were removed from the assay, as scopolamine adhered to the column and carried over into subsequent samples. Harmine and harmaline were not completely soluble in the mobile phase and precipitated out, causing blockages in the guard columns resulting in high pressures within the LC-MS/MS system. BZE was added as a qualitative compound later in the project.

## **Chapter 9: Results from LC-/MS/MS analyses of hair from Pre-Columbian Andean individuals**

### **9.1. Introduction**

This chapter reports the results from the LC-MS/MS multi-component assay of hair from desiccated human remains from cemetery sites in northern Chile and the central coast of Peru.

Positive results were based on a peak at the retention time  $\pm 0.05$  min and ion transition of a specific compound, for example a positive BZE result must have had a peak at  $0.79 \text{ min} \pm 0.05 \text{ min}$  with an ion transition of  $290 > 168$ . The peak must also have had a signal to noise ratio of 3 or more.

### **9.2. Iquique, Chile (Bajo Molle and Los Verdes)**

Two of the three individuals from sites around Iquique (IQU-95 T2 and IQU-95 T3) had previously been tested for BZE (Arriaza *et al.* 2008a). In that study, IQU-95 T3, a female aged around 50 tested positive for BZE. IQU-95 T2, another adult female aged around 50 did not test positive for BZE. The same was found when these individuals were tested during this study. IQU-95 T7, an infant male aged around 1 was not tested for BZE by Arriaza *et al.* (2008a). Other tests were performed, including an aDNA test for Chagas Disease, which was positive. This individual

tested positive for BZE during this study confirming coca consumption. Table 9.1 compares the results from Arriaza *et al.* (2008a) and this study. These specimens acted as negative and positive controls for BZE. The results for the Iquique individuals were the same in both the RIA and LC-MS/MS tests.

**Table 9.1** – Comparison of results from the current project and Arriaza *et al.* (2008a)

Individual	Site	BZE test (RIA) Arriaza <i>et al.</i> (2008)	BZE test (LC- MS/MS)	Notes
IQU-95 T2	Los Verdes	-	-	+ TB + lung adhesions
IQU-95 T3	Bajo Molle	+	+	+ Chagas + lung adhesions
IQU-95 T7	Bajo Molle	NT	+	+ Chagas

### 9.3. Azapa Valley, Chile (AZ-6, AZ-71 and AZ-141)

A total of 49 individuals from three cemetery sites in the Azapa Valley (AZ-6, AZ-71, AZ-141) were tested for psychoactive compounds. Fifty milligram samples and 2.5 mg samples were tested. Typically 30 to 200 mg samples, although large, have been used in the past for RIA and GC-MS analyses of ancient hair (Cartmell *et al.* 1991a; Springfield *et al.* 1993; Cartmell *et al.* 1994; Ogalde *et al.* 2009). Previous work carried out by the author (Brown *et al.* 2008) on hair samples from frozen contexts used ≈2 mg samples. Given that hot arid conditions are known to be less conducive to biomolecular preservation, two masses were prepared to see if individuals that tested positive with 50 mg hair samples also tested positive with 2.5 mg hair samples.



In some cases there was not enough hair to prepare 50 mg samples. Those individuals with insufficient hair samples (IS) are indicated in the tables below.

**Table 9.2.** Results from hair tests for Azapa-6 individuals

Individual	MS File name				Result	Age/Sex	Notes
	090311 2.5 mg	100511 2.5 mg	110511 50 mg	120511 50 mg			
16	BZE	BZE	-	-	+ BZE	I/I	
19	-	BZE	BZE	-	+ BZE	16/F	Buried with coca leaves
22	-	-	-	-	Neg	I/I	
29-1	BZE	BZE	BZE	BZE	+ BZE	I/I	
36	-	-	-	BZE	Neg	25/M	
71	-	-	Buf	Buf	+ Buf	30/M	Orejones/ Braids
108	BZE	BZE	-	BZE	+ BZE	I/I	
TMCA3	BZE	BZE	BZE	BZE	+ BZE	40/F	

Buf = Bufotenine, Neg = negative, I=indeterminate

The MS file names relate to the date of analysis. Each sample, a 2.5 mg and 50 mg were run. Underneath the MS file name the sample size tested is indicated. If BZE (or bufotenine, Buf) were present in the sample this is indicated in the table. The results indicate that generally if BZE is detected in a 2.5 mg sample it was detected in the 50 mg sample (if there was enough hair to prepare a 50 mg sample). In some cases BZE was detected in one sample, but not the other. There may be a number of reasons for this. Although care was taken to ensure hair samples were homogenous by using entire lengths of hair, excluding grey hairs, there may be differential preservation of the drug in hair, or different rates of incorporation, depending on what stage of the hair cycle the hair was in, as there may be a lag in

incorporation. Williams *et al.* (2011b) noticed this phenomenon in stable isotope analysis of hair. They termed it “growth cycle error”. This is more likely to be a problem for the 2.5 mg samples, as it is a small sample where the presence of one or two hairs with an incorporation lag would significantly affect the amount of drug compound in the sample.

From the eight individuals from Azapa-6 (table 9.2), five tested positive for BZE, a metabolite of cocaine, although no cocaine was detected. One individual, AZ-6 T71, an adult male with “*orejones*” tested positive for bufotenine, a psychoactive compound found in *Anadenanthera* snuff.

Two individuals from Azapa-71 (46 and E1) did not have large enough hair samples to carry out any hair tests (either 2.5 mg or 50 mg). A further eight individuals (48, 76A, 94B, 200, 285, 322, 331 and AA) did not have large enough samples to prepare 50 mg samples. Of 26 individuals, 15 tested positive for BZE (see table 9.3). Figure 9.1 compares a BZE standard with examples of positive BZE tests.

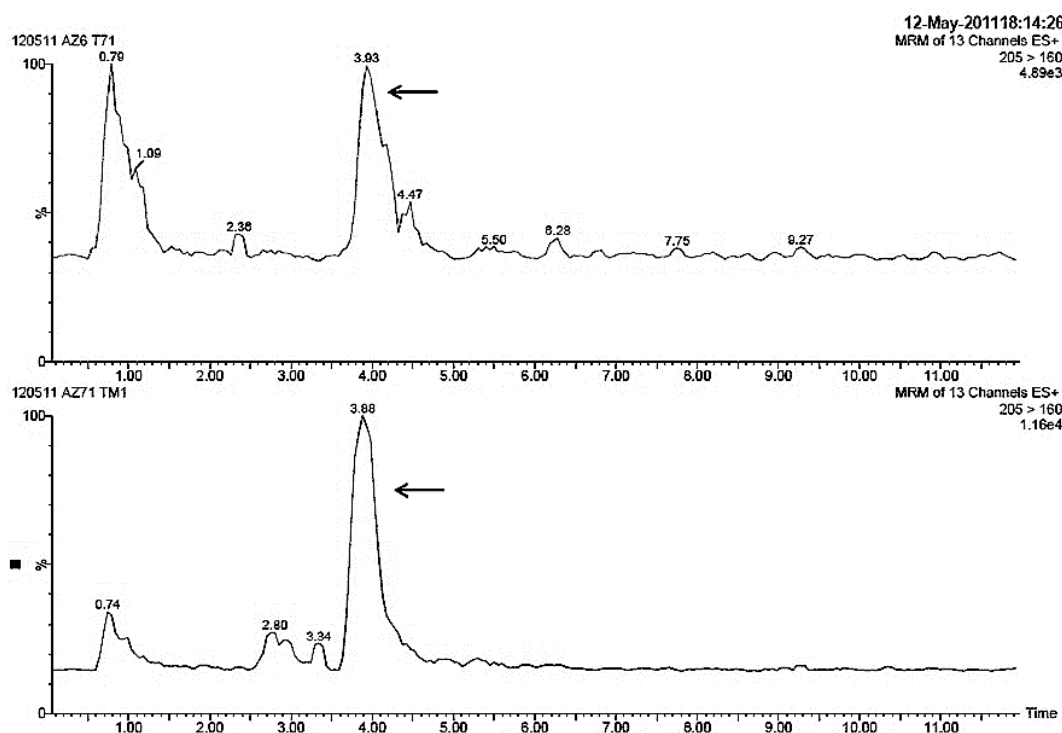
One individual, AZ-71 TM1, a young adult female, tested positive for both BZE and bufotenine, an alkaloid in *Anadenanthera* snuff.

**Table 9.3.** Results for hair tests for Azapa-71 individuals

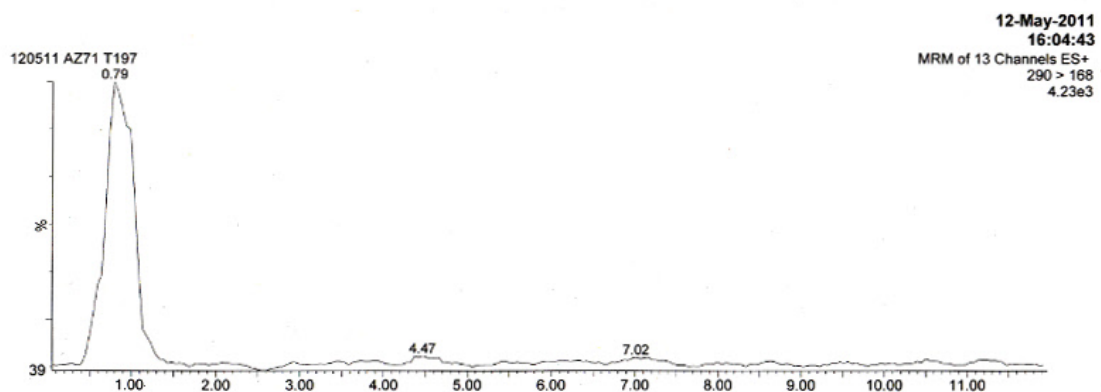
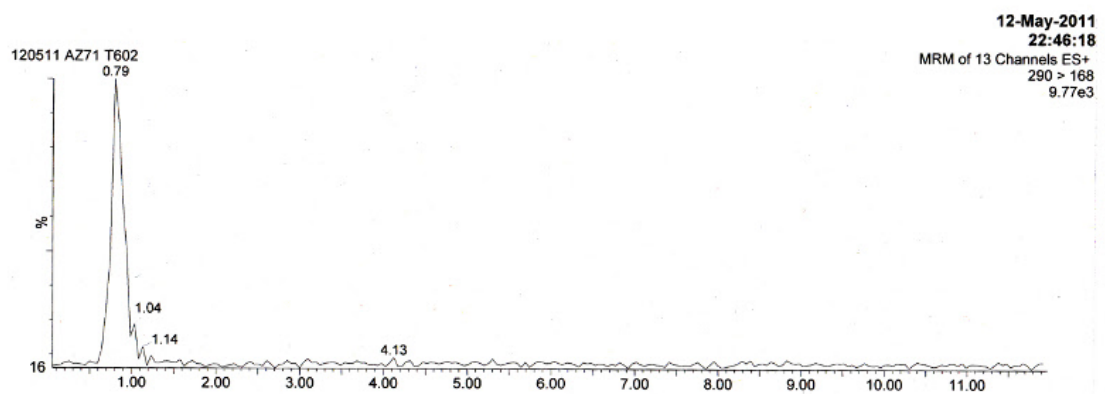
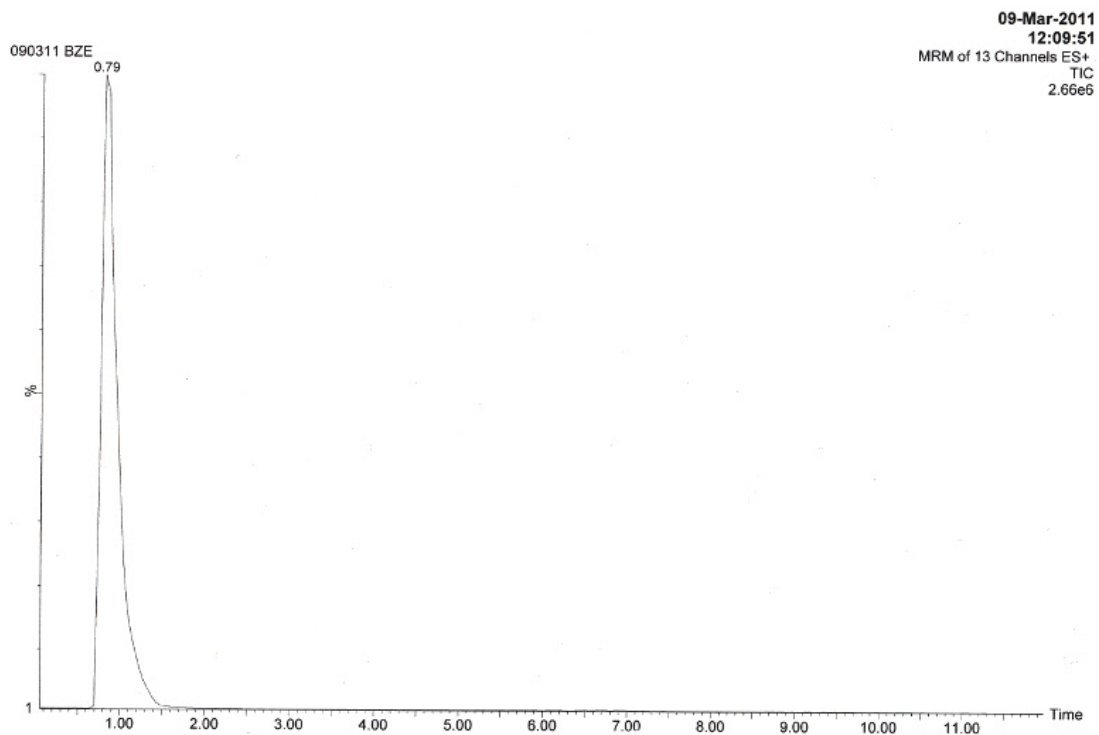
Individual	MS file name				Result	Age/Sex	Notes
	090311 2.5 mg	100511 2.5 mg	110511 50 mg	120511 50 mg			
36	-	BZE	BZE	-	<b>+BZE</b>	< 1/M	
46	IS	IS	IS	IS	<b>ND</b>	I/I	
48	BZE	-	IS	IS	<b>Neg</b>	<1/I	
76 A	-	-	IS	IS	<b>Neg</b>	I/I	
94 B	-	BZE	IS	IS	<b>Neg</b>	I/I	
105	-	BZE	BZE	BZE	<b>+BZE</b>	15/M	
127	BZE	BZE	BZE	-	<b>+BZE</b>	45/F	
197	-	-	BZE	BZE	<b>+BZE</b>	1/F	Possibly Tiwanaku, GI problem
200	-	BZE	IS	IS	<b>Neg</b>	I/I	
201B	-	BZE	BZE	BZE	<b>+BZE</b>	35/F	
210	-	BZE	BZE	-	<b>+BZE</b>	1/M	
230	-	BZE	BZE	-	<b>+BZE</b>	12/M	Chagas disease (megacolon)
240	-	BZE	-	-	<b>Neg</b>	<1/F	GI problem
263	-	BZE	-	-	<b>Neg</b>	1/F	GI problem
285	-	-	IS	IS	<b>Neg</b>	1/M	
322	-	BZE	IS	IS	<b>Neg</b>	5/I	
331	BZE	BZE	IS	IS	<b>+BZE</b>	20/M	Cranial modification
470	-	BZE	BZE	BZE	<b>+BZE</b>	2/I	
504	-	BZE	BZE	-	<b>+BZE</b>	3/M	
601	-	BZE	-	-	<b>Neg</b>	55/M	
602	-	BZE	BZE	BZE	<b>+BZE</b>	55/M	
AA	-	-	IS	IS	<b>Neg</b>	12/F	
A3	-	BZE	BZE	-	<b>+BZE</b>	I/M	
B1	-	BZE	-	-	<b>Neg</b>	20/F	Buried with

							coca leaves
DD	BZE	BZE	BZE	-	<b>+BZE</b>	40/F	
E1	IS	IS	IS	IS	<b>ND</b>	20/F	Cranial modification
M1	BZE	BZE	BZE/Buf	BZE/Buf	<b>+BZE/Buf</b>	22/F	Cranial modification
N1	BZE	BZE	BZE	-	<b>+BZE</b>	3/M	Cranial modification

Buf = Bufotenine, ND = no data, GI = gastrointestinal, Neg = negative, I – indeterminate, IS = insufficient sample



**Fig 9.1** – Arrows indicate peaks for bufotenine. In both samples bufotenine presents a weak signal. However, the peaks appear at the right retention time and right ion transition for bufotenine (205 > 160).



**Fig. 9.2** – Comparison of a BZE standard (top) and two examples of positive tests from Cabuza individuals with peaks at 0.79 min.

**Table 9.4.** Results for hair tests for Azapa-141 individuals

Individual	MS file number				Result	Age/Sex	Notes
	090311 2.5 mg	100511 2.5 mg	110511 50 mg	120511 50 mg			
12	-	-	BZE	BZE	<b>+BZE</b>	2/I	
13	-	-	-	-	<b>Neg</b>	7/I	
18	-	-	-	-	<b>Neg</b>	2/F	
26	IS	IS	IS	IS	<b>ND</b>	2/I	
28	-	BZE	BZE	BZE	<b>+ BZE</b>	1/I	
34	-	BZE	BZE	BZE	<b>+ BZE</b>	7/M	
35	BZE	-	IS	IS	<b>Neg</b>	19/M	Buried with ceramic <i>kero</i>
47	-	-	IS	IS	<b>Neg</b>	2/M	
53	-	-	BZE	BZE	<b>+BZE</b>	20/M	
54	BZE	BZE	BZE	-	<b>+BZE</b>	1/M	
DESC 2	BZE	BZE	BZE	BZE	<b>+ BZE</b>	35/F	
DESC 8	-	-	-	-	<b>Neg</b>	2/I	
T-TOL	-	-	-	BZE	<b>Neg</b>	I/I	

ND = no data, Neg = negative, I – indeterminate, IS = insufficient sample

One individual from Azapa-141 (AZ-141 T26) did not have sufficient hair for analysis.

A total of six individuals from this site tested positive for BZE. No individuals from this site tested positive for any compound other than BZE (see table 9.4).

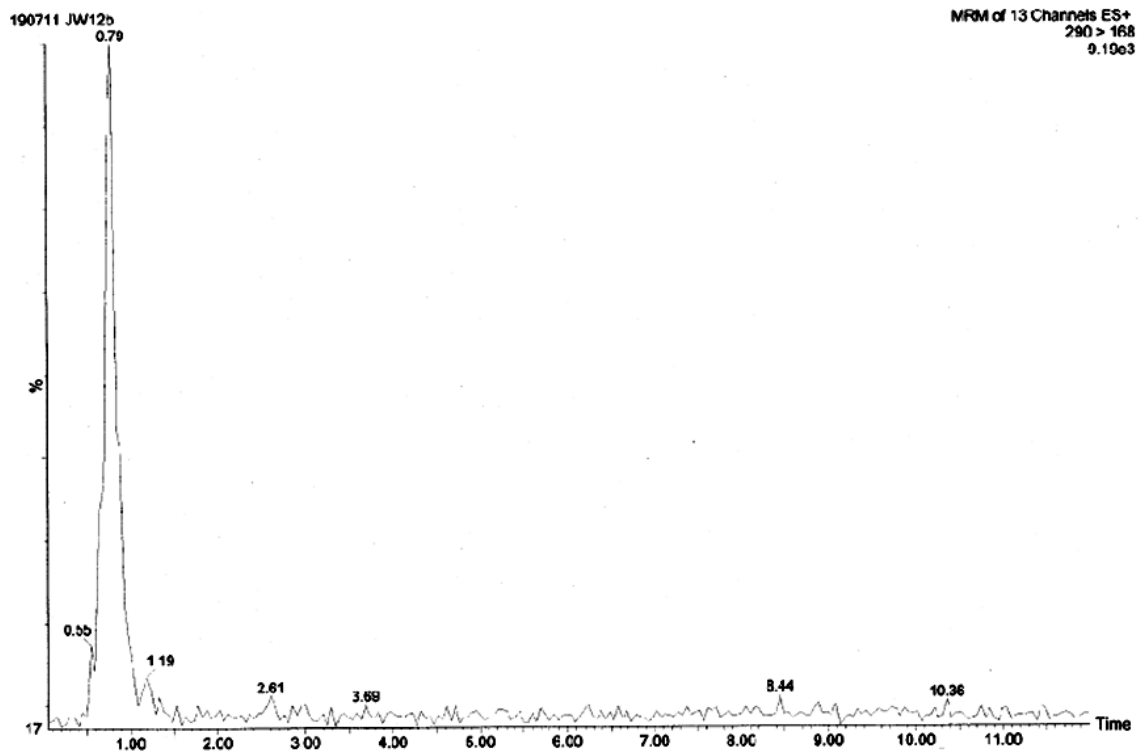
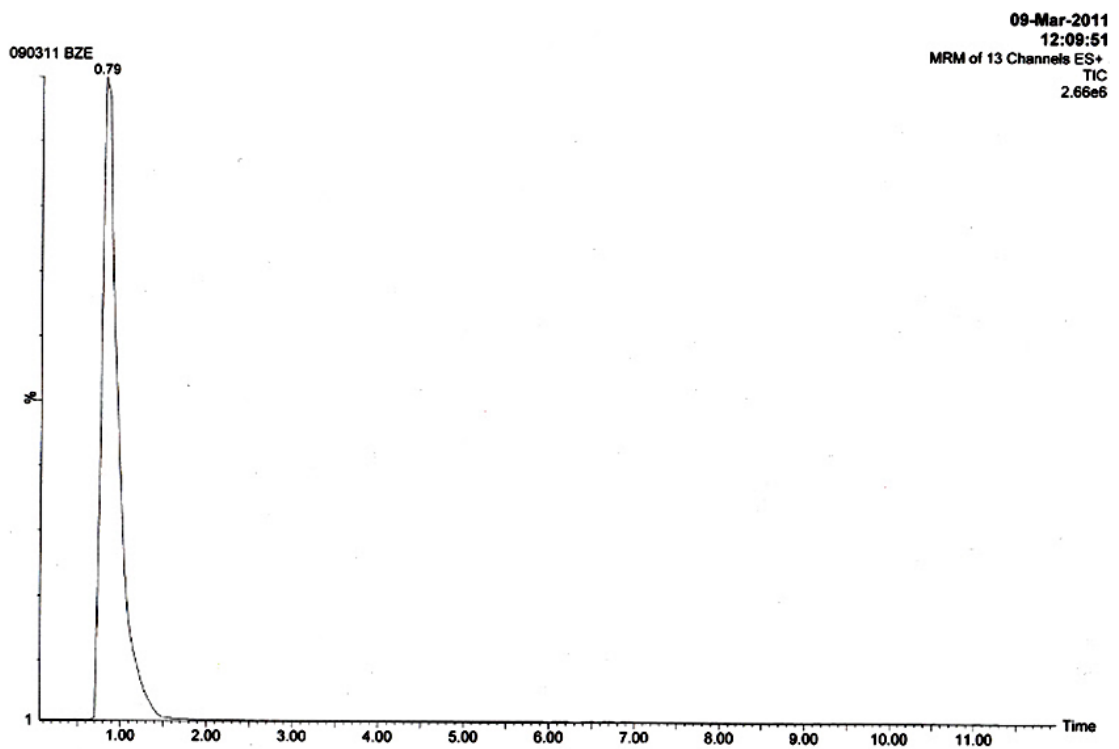
### 9.3. Rímac Valley, Peru

Of the thirteen hair samples available, two did not have sufficient amounts of hair to prepare a 50 mg sample for analysis. Three individuals of the thirteen did not test positive for any psychoactive compounds. Eight individuals tested positive for BZE (see Fig 9.2 for an example of a positive BZE test in one of the Puruchuco-Huaquerones individuals). In seven of these eight individuals BZE was detected in successive runs (see table 9.5).

**Table 9.5.** Results for hair tests for Puruchuco-Huaquerones individuals

Sample ID	Burial No	MS file name		Result	Age	Sex
		190711	190711			
12	163	BZE	BZE	<b>+BZE</b>	20-35	F
16	171	-	-	<b>Neg</b>	35-50	M
18	-	BZE	BZE	<b>+BZE</b>	4-8	I
20	2	-	-	<b>Neg</b>	20-25	M
22	179	BZE	BZE	<b>+BZE</b>	20-35	M
25	38	-	-	<b>Neg</b>	35-50	F
28	58	BZE	BZE	<b>+BZE</b>	50+	F
33	129	BZE	BZE	<b>+BZE</b>	20-35	F
41	56-1	-	BZE	<b>+BZE</b>	35-50	F?
53	-	IS	IS	<b>ND</b>	4-8	SA
54	-	BZE	BZE	<b>+BZE</b>	35-50	M
59	143	IS	IS	<b>ND</b>	35-50	M
68	-	BZE	BZE	<b>+BZE</b>	8.5-13.5	SA

ND = no data, Neg = negative, SA = sub adult



**Fig. 9.3** – An example of a positive BZE test (bottom) from an individual from Puruchuco-Huaquerones compared to a BZE standard (top).





#### 9.4. Summary

Hair samples from 60 individuals from the Azapa Valley, Iquique and Puruchuco-Huaquerones were tested for psychoactive compounds. Two compounds were detected in these ancient hair samples: benzoylecgonine (BZE) and 5-hydroxy-*N-N*-dimethyltryptamine (bufotenine). BZE is the major metabolite of cocaine, the main alkaloid in coca leaves, whilst bufotenine is an alkaloid in *Anadenanthera* snuff. The table below summarises the findings from this research project.

**Table 9.6** – Summary of results from LC-MS/MS analyses of Cabuza and Ichma hair samples (50 mg samples).

Site	Cultural group	Number tested	Positive for BZE (%)	Positive for bufotenine
Iquique (Los Verdes & Bajo Molle)	Tiwanaku/Cabuza	3	2 (66%)	0
Azapa-6	Cabuza	8	5 (63%)	1
Azapa-71	Cabuza	26	15 (58%)	0
Azapa-141	Cabuza	12	6 (50%)	1
Puruchuco Huaquerones	Ichma/"Inca"	11	8 (72%)	0

## Chapter 10: Discussion

### 10.1. Review of aims and objectives

#### 10.1.1. Hair specimens

The aim of this project was the development and implementation of an LC-MS/MS method suitable for detecting coca metabolites and other psychoactive compounds such as mescaline, *N,N*-dimethyltryptamine and bufotenine in small ( $\approx 2\text{mg}$ ) hair samples. Previous research into the ingestion of coca and alcohol in the final months of a young woman destined to be ritually killed by the Inca showed that BZE, cocaine, ecgonine methyl ester (EME) and cocaethylene were detectable in 2 mg (and smaller) samples (Brown 2007). In this case the hair samples came from a high altitude mountain shrine (Llullaillaco, 6739 metres above sea level), where the environmental conditions (cold, dry) have resulted in exceptionally well preserved organic remains. The individuals from Llullaillaco dated to the Inca period (C14 date  $400 \pm 25^{10}$ ). In this case, LC-MS/MS proved to be an excellent tool for detecting low amounts of drug compounds in hair. This instrumental method was chosen again for the current research project due to the success of the Llullaillaco project (Brown 2007; Brown *et al.* 2008).

The hair specimens obtained for this project were obtained from Dr. Arthur Aufderheide's hair collection from the University of Minnesota at Duluth Paleobiology laboratory. The Cabuza hair specimens were chosen as they

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<sup>10</sup> calibrated using Oxcal version 3.10 and the INTCAL04 data set at 95.4% probability 1430 A.D. (82.3%) 1520 A.D. and 1590 A.D. (13.1%)1620 A.D.

represented the largest amount of specimens allocated to a single cultural group. There are also known material correlates of snuffing (snuff tubes, trays) and bioarchaeological evidence of coca chewing from the area which this cultural group lived – the Azapa Valley of northern Chile, making the Cabuza an ideal group to test the LC-MS/MS method on. Dr Aufderheide also included three specimens from three individuals from sites around the Chilean city of Iquique thought to belong to Tiwanaku individuals, roughly contemporaneous with the Cabuza individuals (there is a paucity of radiocarbon dates for Cabuza, however the Iquique individuals have dates that range from the 6<sup>th</sup> to 15<sup>th</sup> centuries). Two of these individuals had tested positive for BZE in a previous study (Arriaza *et al.* 2008a). These specimens acted as control samples for BZE.

The hair specimens from Puruchuco-Huaquerones date to the Inca period (15<sup>th</sup>/16<sup>th</sup> century), but were probably from a group of people known as the Ichma, a weaving specialist group under Inca imperial control, who lived just inland from the coast along the Rímac valley from what is now the modern city of Lima. Previous studies have shown that coca chewing was widespread during this time period (Cartmell *et al.* 1991a; Cartmell *et al.* 1991b; Springfield *et al.* 1993; Cartmell *et al.* 1994). Other psychoactive compounds, although most likely used, have not been detected in hair.

During the early stages of this project, a paper was published by Ogalde *et al.* (2009) who analysed 32 individuals from Azapa cemeteries for  $\beta$  carboline alkaloids and *N,N*-dimethyltryptamine. Although this research group used a different analytical method with higher limits of detection, their study provides some useful

comparative data, as some of the same individuals were tested by Ogalde *et al.* (2009) (discussed in further detail in s10.2).

#### **10.1.2. Sample sizes and control specimens**

Experiments with 2 mg hair samples from the Cabuza individuals indicated that in some cases BZE was detectable even in these small samples, but bufotenine was not detectable at all. Generally the signals for BZE were low, even with 50 mg samples (although well above the detection limits (i.e. with signal to noise ratios of 100 or more) as ascertained by calculating signal to noise ratios using the MassLynx software. The amount of hair required for a bulk sample indicated that these particular hair specimens were unsuitable for segmental analysis for compounds other than BZE.

Typically 20-100 mg samples have been used in previous analyses of ancient hair for coca metabolites (Cartmell *et al.* 1991a; Cartmell *et al.* 1991b; Springfield *et al.* 1993; Cartmell *et al.* 2001; Cartmell and Weems 2001; Cartmell *et al.* 2002; Rivera *et al.* 2005; Arriaza *et al.* 2008a). Ogalde *et al.* (2009) used 200 mg hair to investigate the use of hallucinogens and harmine/harmaline in ancient hair. Given that compounds like mescaline and scopolamine are only incorporated into hair at the low to mid pg range (Kintz *et al.* 2006; Gambelunghe *et al.* 2012), a larger hair sample would be required for analysis. Ogalde *et al.* (2009) used a 200 mg sample for their investigation into hallucinogen use in a similar population, using GC-MS. LC-MS/MS has lower detection limits, therefore requiring a smaller sample. In many cases the specimens donated for this project were not large enough to prepare 200

mg samples, so a lower amount of hair, 50 mg, was chosen, as this is about the average for previous studies on coca metabolites in hair.

### **10.1.3. Taphonomy and drug preservation in ancient hair**

Cocaine and its metabolites bind readily to dark pigments in hair (Gygi *et al.* 1996; Joseph *et al.* 1996; Joseph *et al.* 1997; Hubbard *et al.* 2000). Given that pigments (melanin granules) are the most resistant component of the cortex of human scalp hair to degradation by microbial/chemical attack (Wilson *et al.* 2007a) it is not surprising drug molecules are detectable for very long periods after death, although eumelanin, a dark pigment, is less stable than other pigments (Wilson *et al.* 2001). However, based on preliminary experiments done at Bradford (Conlon 2006; Brown 2007) and forensic case reports (Mari and Bertol 1997; Arado *et al.* 2001; Tsatsakis *et al.* 2001; De Giorgio *et al.* 2007; Borriello *et al.* 2008; Kintz *et al.* 2008; Gaillard *et al.* 2011) it is likely that the amount of drug left in hair after an extended postmortem period in a hyperarid environment is a fraction of what it was during life (Conlon 2006; Brown 2007). The environmental and chemical conditions of the depositional environment are key factors in the preservation of drug compounds in hair. In the case of the young woman found on Lulllaillaco, the levels of cocaine, BZE and EME were comparable to modern coca chewers. The ratio of cocaine to BZE was also similar to what would be expected in modern coca chewers, with cocaine being about five times the amount of BZE detected in hair (Henderson *et al.* 1992)

In living cocaine users and coca chewers (and the young woman from Lulllaillaco) cocaine is the major alkaloid incorporated to hair (Nakahara *et al.* 1992). Cocaine

was not detected in any of the individuals from Iquique, Azapa or Rímac valleys. This seems to suggest that any cocaine that was originally in the hair has degraded into BZE and other smaller compounds within the depositional environment. Although BZE is a metabolite of cocaine produced via *in vivo* metabolic processes it can also be produced by hydrolysis. Several authors report that cocaine is not particularly stable *in vitro* and is prone to breakdown into BZE and EME over relatively short time periods if buffers and preservatives are not added (Isenschmid *et al.* 1989; Giorgi and Meeker 1995; Warner and Norman 2000; Skopp *et al.* 2001). The rate of hydrolysis of cocaine into BZE and EME is increased by temperature and high pH (Isenschmid *et al.* 1989), both of which are significant taphonomic variables in the process of decomposition within the burial environment, as even well-preserved mummies will have undergone significant putrefactive change (Janaway *et al.* 2009).

The degradation of various psychoactive compounds in hair is important to consider in terms of other more unusual drug compounds in hair that are only incorporated in trace amounts to begin with. For example, Kintz *et al.* (2006) tested the hair of a patient who ingested *Datura innoxia* flowers and was hospitalised for one week. The patient recovered. A 200 mg sample of hair was taken and tested for atropine and scopolamine. Atropine was not detected, but scopolamine was – in three segments of hair. The range was 14-48 pg/mg. These levels are very low. Another case study of mescaline in hair reported similarly low levels (1.3mg/L) (Gambelunghe *et al.* 2012). Given that these substances are incorporated at a low level it seems unlikely that these substances would be detected in archaeological hair.

Hair can be contaminated with drug compounds in putrefactive fluids during decomposition. Pascal Kintz *et al.* (2008) calls this “external postmortem artefact”. Kintz’s group were unable to remove the contamination with repeated solvent washes. Whilst this is an obvious issue for drug testing hair in forensic cases, this is also a consideration for ancient hair samples, particularly for bodies buried with large amounts of coca leaves, as has been observed in coastal burials in Peru and Chile (coca leaves became common burial inclusions after c AD 400). The combination of a decaying body in the presence of coca leaves would likely result in the release of cocaine from the leaves which could contaminate hair, resulting in a possible false positive. This would depend on the containment of coca leaves (i.e. in a *chuspa* or wrapped in an *inkuñā* – a large woven cloth) and the placement on them in relationship to the body. Although, it should be noted that individuals found buried with coca leaves do not always test positive for BZE (Cartmell *et al.* 1991a; Aufderheide 2003: 158).

#### **10.1.4. Method development and implementation**

From what was learned in the Lulluillaco project, a column was picked that was specifically suitable for drug compounds, in particular cocaine and its metabolites BZE and EME. Other more unusual drug compounds, such as mescaline, *N,N*-DMT, bufotenine, harmine, harmaline and scopolamine were trialled on the column to find their ion transitions and retention times, as well as their linear ranges.

Harmine and harmaline were removed from the assay as they did not dissolve fully in acetonitrile. These compounds also precipitated out of the solvent and blocked



the guard columns attached to the main column. This resulted in a build-up of pressure in the system, which could potentially cause damage to the instrument and column. The guard columns were replaced with new ones and the column flushed with solvent. Typically LC-MS/HPLC methods for the detection of harmine and harmaline in biological samples tend to have a lower organic solvent component of the mobile phases (e.g. Yu *et al.* 2003).

Scopolamine was also removed, as it adhered to the column and was hard to remove once there. It caused significant carry-over from one sample to the next, resulting in scopolamine being detected in blank samples.

The linearity and limits of detection and quantification were established for all compounds, except BZE, which was added to the assay late in the project. The calibration lines were constructed using the ratio of the peak areas of a deuterated internal standard to a standard at decreasing concentrations to establish linearity and also allow for quantification of unknown compounds in the ancient hair samples. Due to a number of significant instrument problems and a lack of facilities to correctly and securely store the deuterated compounds, they degraded, resulting in the original compound being present in the internal standard. This made them unsuitable to use as internal standards, as it would be impossible to tell if they were present in the hair or the internal standard. As it had become clear at this point that relatively large hair samples would be needed to be able to detect compounds such as bufotenine, it was decided to present qualitative results. These results have identified two individuals that tested positive for bufotenine, which has not been

detected in ancient hair previously. These two individuals could be analysed in more detail in the future.

## **10.2. *Anadenanthera* snuff in the Azapa Valley**

Snuffing has been well documented in the Andes and Amazon by archaeologists and ethnographers, and there is a specific material culture associated with snuffing. Examples include snuff trays, mortars, spoons, and spatulas (Burger 1992: 271; Torres 1995; Quilter 1998; Chacama 2001; Llagostera 2001). The focus of studies on snuff use in the Andes has focussed on the material correlates of snuffing. Snuff trays have received the most attention, particularly those from San Pedro de Atacama (Torres 1986; 1987a; Llagostera *et al.* 1988; Torres *et al.* 1991; Torres 1995; Torres and Conklin 1996; Llagostera 2001; Torres 2004a; Llagostera 2006; Carod-Artal and Vázquez-Cabrera 2007b). In funerary contexts snuff kits are generally found with relatively high status adult males (Goldstein 1996; Torres Rouff 2002). Material correlates of high status in the Azapa Valley during the Middle and Late Horizons include four-pointed woollen hats, panpipes, snuff trays and the presence of “*Orejones*” – elongated ears for wearing ear decorations (Allison *et al.* 1983). Two individuals from Azapa cemeteries tested positive for bufotenine, an alkaloid present in *Anadenanthera* snuff. One of these, AZ-6 T71 was an adult male identified as an *Orejon*.

*Anadenanthera*, a species of tree native to the Andes has been identified as a source of hallucinogenic snuff used by ancient populations in northern Chile. The seeds and bark are ground to make snuff, known as *vilca*, *cebil* (when made from *A.*

*colubrina*) or *yopo*, *cohoba*, *parica* (when made from *A. peregrina*) that produces visual hallucinations and sensations of bodily heaviness when inhaled (Schultes *et al.* 2001: 122, see s3.2.1). *Anadenanthera* contains a number of alkaloids, but the key alkaloid is 5-hydroxy-*N,N*-dimethyltyptamine (bufotenine). This compound is used to differentiate *Anadenanthera* from other plants, such as *Virola* sp, which are also used to make snuffs in the Amazon region (Torres *et al.* 1991). Bufotenine is also found in a species of toad (*Bufo alvarius*), and at least two other less well known plant species: *takini* (*Brosimum acutifolium*), a hallucinogenic preparation made from the latex of a large canopy tree in the Amazon used by indigenous groups in Suriname for Shamanic and medicinal purposes (Moretti *et al.* 2006) and *Mucuna pruriens*, a tropical legume with a wide distribution with known ritual uses in the Caribbean (Davis 1983).

Iconographic representations of *Anadenanthera* have also been identified in Chavín, Wari and Tiwanaku sculpture and ceramics (Knobloch 2000; Isbell and Knobloch 2006; Burger 2011), suggesting that *Anadenanthera* has been culturally and ritually significant in the Andes for over 2,000 years (Burger 2011). Snuff trays and tubes have been found at Azapa sites, including all of the cemeteries from which the individuals in this research project originated (Focacci 1990; Torres 1995; Ogalde *et al.* 2009).

One adult male (T33) from Azapa-141 was found with a snuff tray, panpipes, and a four-pointed hat, trappings of “high status” (Goldstein 1996). A sample of hair from this individual was tested for a number of compounds using GC-MS (Ogalde *et al.* 2009). This individual tested positive for harmine The authors of this study stated

that this indicated the use of *Banisteriopsis*, probably as part of a preparation similar to *ayahuasca*. The authors also state the following regarding their results:

“This information is extremely useful, because it shows the snuffing kits used in Azapa Valley were not related to *Anadenanthera* consumption. Our findings are controversial regarding the classical archaeological interpretations of snuffing kits and chemical evidence of snuff powder found in San Pedro de Atacama (Ogalde *et al.* 2009: 469”).

The findings from the LC-MS/MS analyses directly contradict these findings, as two individuals from contemporaneous sites have tested positive for bufotenine, one of the alkaloids in *Anadenanthera* snuff. This does not rule out the possibility that something similar to *ayahuasca* was used. However, there currently is a debate about the antiquity of *ayahuasca*, with a number of authors providing compelling arguments for *ayahuasca* being a relatively recent development (Gow 1994; Brabec de Mori 2011). A more likely scenario is that the discovery of harmine in hair does indicate the use of *Anadenanthera* snuff. Harmine has been detected in *Anadenanthera* snuff samples prepared by the Piaroa of the Orinoco basin (Holmstedt and Lindgren 1967; De Smet and Rivier 1985). Although Torres and Repke (2006: 73) state that there is no ethnographic evidence of *Banisteriopsis* as a snuff admixture, Rodd (2002) observed the Piaroa adding fresh *Banisteriopsis* roots to *Anadenanthera* snuff. The Guahibo of eastern Colombia have also been documented as chewing *Banisteriopsis* alongside inhaling snuff (Torres and Repke 2006: 73). Given there is some ethnographic evidence of other plants being added to snuff powders (albeit in modern groups further north than the Azapa Valley), it is

possible that the ancient inhabitants of the Azapa Valley had a similar practice, although there is no archaeological evidence to suggest this was the case. Torres *et al.* (1991) did not detect harmine or any other  $\beta$  carboline alkaloids in the ancient snuff samples they analysed.

AZ-6 T71 is an adult male, aged around 30-35 when he died. His hair tested positive for bufotenine. There is no information in the original field notes regarding his grave goods. However, it was noted that this individual is an “*Orejon*”, a male with elongated ears for wearing ear spools. In northern Chile these ear spools were made from corn cobs or wrapped camelid fur capped with silver and gold plates (Allison *et al.* 1983). These individuals tend to be taller and have less pathology than males without elongated ears, giving rise to the suggestion that they were a privileged group (Allison *et al.* 1983). Christina Torres-Rouff discussed body modifications in northern Chile and suggested “alterations and ornamentations of the body transform the physical self into a vehicle for displaying identity and conveying social information” (Torres Rouff 2012: 153) – these modifications include permanent alterations such as cranial modification, tattoos and the wearing of ear spools and labrets. The conspicuous display of elongated ears reinforced his special status within his community.

There is no osteological or dietary information from stable light isotope analysis to support the idea that this particular individual had a different diet from the rest of the population. However, it is recorded in the original field notes that this individual had a number of cranial injuries on the left temporal and occipital bones. There is less contextual information for the other individual, AZ-71 TM1, a young adult

female with type 5 cranial modification, flattening of the occipital bone using boards (Gerszten 1993). This type of cranial modification is found in around 14% of individuals from the Azapa Valley, and is associated with the Tiwanaku culture. Cabuza cranial modification was of a different style, achieved using binding rather than boards (Gerszten 1993). No grave goods, other than a poorly preserved mantle is recorded. No pathological information is available for this individual. Whilst only circumstantial, the association between a cranial modification style associated with Tiwanaku and a positive bufotenine test is interesting, as snuffing is almost synonymous with Tiwanaku influence, even though snuffing seems to have been practiced on the coast long before the rise of Tiwanaku in the highlands during the Middle Horizon. It is also interesting as it does suggest snuffing was not limited to males. Archaeological evidence also suggests females may have also practiced snuffing. Snuffing artefacts have been found with females, for instance, a snuff kit (bag and hollow tube) was found with an adult female at Solcor 3, San Pedro de Atacama (see s6.6) whilst snuff spoons and tubes were found with high status female burials dating to the Middle Horizon at the Wari site of Qoripata (Llagostera *et al.* 1988; Torres *et al.* 1991; Glowacki 2002: 282; Torres Rouff 2002; Janusek 2008: 268). However, snuffing artefacts are overwhelmingly found with males.

The findings from this research project also partially confirm the results of Ogalde *et al.* (2009). This research group tested 32 individuals for *N,N*-dimethyltryptamine and harmine using GC-MS. Six of these individuals (AZ-6 T19, AZ-6 TMCA3, AZ-71 T127, AZ-71 T602, AZ-141 T34, AZ-141 T53) were also tested in this research project. In both studies, using different analytical methods, none of them tested positive for *N,N*-dimethyltryptamine.

### 10.3. Coca chewing

Coca chewing has been practiced in the Andes for thousands of years. The earliest evidence of coca leaves and lime production (for *illipta*, an alkaline substance that facilitates the extraction of alkaloids from coca leaves) comes from secure contexts dating to 8,000 cal BP in the Nanchoc valley in northern Peru (Dillehay *et al.* 2010). The tradition of coca chewing continues to the present day and is an integral component of Quechua and Aymara cultural identity (Allen 1981; 2002), despite international efforts to eradicate coca as it is the botanical source of cocaine (Weil 1995; Metaal *et al.* 2006; Grisaffi 2010).

Coca has, over the centuries, been used for medicinal, ritual and social purposes (Martin 1970; Weil 1978; Allen 2002). The Inca ascribed a divine status to coca and it had been suggested that during the Inca period coca was controlled and restricted to the upper echelons of society (Rowe 1946: 291). However John Murra (1986) suggests there is no evidence to support this idea whatsoever.

The results from hair tests for BZE support the idea that most people had access to coca, although only a small number of “Inca” individuals have been tested. Cartmell *et al.* tested 13 “Inca” individuals from sites on the northern coast of Chile (Cartmell *et al.* 1991a; Cartmell *et al.* 1994). In the current study 11 individuals from Puruchuco-Huaquerones, a site in the Rímac valley near the modern city of Lima, Peru were tested. Cartmell *et al.* had 9/13 individuals test positive for BZE using RIA/GC-MS. Of the 11 individuals from Puruchuco-Huaquerones, 8/11 tested positive for BZE. In both cases there is evidence that the majority, around three

quarters of individuals, imbibed coca in some form – either by chewing or possibly drunk as an infusion.

Around half of the individuals from Azapa-6 (5/8), Azapa-71 (15/26) and Azapa-141 (6/12) also tested positive for BZE. This is a similar proportion of Cabuza (10/16) and Maitas Chiribaya (54/97) individuals from the Arica/Azapa Valley tested by Cartmell *et al.* (1991a). Again, this suggests that the inhabitants of the Azapa Valley used coca during the Late Intermediate Period and Late Horizon. This is not surprising, given that coca was a crop grown by Azapa Valley populations from at least the Middle Horizon (Molina *et al.* 1989). There is evidence of widespread coca production at the same time in the Ica valley in southern Peru (Beresford-Jones *et al.* 2011), as well as ethnohistorical accounts of coca production in coastal valleys (Rostworowski de Díez Canseco 1988; Rostworowski de Díez Canseco 1989).

The Iquique individuals, of which there were only 3 in the study, are thought to be contemporaneous with the Cabuza populations in the Azapa Valley. Two of the three individuals tested positive for BZE. IQU-95 T7 had not been previously tested, but IQU-95 T2 and IQU-95 T3 had. The results from this research project concur with those of Arriaza *et al.* (2008a). T3 tested positive in both studies, whilst T2 did not.

The sample sizes are too small to make generalisations about coca chewing in both populations. However, it is worth noting that males and females of all ages test positive for BZE, including infants under the age of one. Cocaine can pass from the mother to an unborn child *in utero* (Woods 1998) and can be detected in the hair of neonates (Klein *et al.* 1994; Garcia-Bournissen *et al.* 2007). It can also be excreted



in breast milk (Chasnoff *et al.* 1987; Wiggins *et al.* 1989) which may be why very young infants test positive for BZE.

Coca use in both the Cabuza and Ichma people seems to have been widespread, and there may be many reasons for this. For some individuals there is pathological information available – it is notable that those with gastrointestinal problems, including Chagas disease, tested positive for BZE – for example IQU-95 T3, IQU-95-T7, AZ-71 T197 and AZ-71 T230 had positive BZE tests and Chagas disease or “gastrointestinal problems”. Coca has been used extensively to treat disorders of the gastrointestinal tract, including dysentery, diahorrea, stomach aches, indigestion, cramps and other painful conditions (Martin 1970; Carter *et al.* 1980; Grinspoon and Bakalar 1981; Weil 1981; Plowman 1986a: 8). This interpretation is hampered by the fact that there is not pathological information for all individuals.

There are a number of other reasons Andean people may have chewed coca other than the treatment of illnesses. Ethnohistorical and ethnographic accounts suggest Andean people chewed coca to suppress hunger and increase their ability for work (Martin 1970; Hanna 1974; Hanna and Hornick 1977). Coca is also known to help alleviate the effects of altitude sickness (Hanna 1974; Fuchs 1978). It is unlikely that coastal people would chew coca for this reason. However, if these people practiced a vertical economy, as proposed by Murra (1956) they may have chewed coca when visiting different ecozones at higher altitude. As yet there is no archaeological evidence that this was the case, but it is clear there were links between the Azapa Valley and the *altiplano* during the Formative Period (Romero *et al.* 2004) onwards, although the extent of the relationship between the Cabuza and Tiwanaku polity is

still debated (Rothhammer and Santoro 2001; Rothhammer *et al.* 2002; Sutter 2005b; Sutter 2006).

Coca plays an important social function in contemporary groups in the Andes. Amongst Quechua groups the sharing of coca is called *hallpay*. This involves an elaborate ritual and exchanging of coca leaves (Allen 1981, see s.2.6.3). To decline offered coca is to decline hospitality. Coca may have had a similar role in past Andean societies, in that chewing coca was a group activity that helped reinforce social ties.

## Chapter 11: Conclusions & Further Work

### 11.1. Conclusions

This project achieved a number of things, but also raised more questions that could be the foundation for future research.

A multi-component assay using LC-MS/MS was developed to test ancient hair for psychoactive compounds suspected of having a long history of use in the Andes. This method was applied and a total of 60 individuals from three locations (Azapa Valley and Iquique in Chile and Puruchuco-Huaquerones, near Lima, Peru) were tested for a number of psychoactive compounds.

The results from the Cabuza population from the Azapa Valley indicated the widespread use of coca, as evidenced by the detection of BZE in hair. Two individuals also tested positive for bufotenine, the key alkaloid in *Anadenanthera* snuff. Snuff tubes and trays have been found in funerary contexts in all three cemeteries these individuals came from, yet the two individuals that tested positive for bufotenine were not buried with snuffing equipment. Only basic contextual information was available for these two individuals. The male (AZ-6 T71) who tested positive for bufotenine had elongated ears. Male with elongated ears are known as Orejones. Orejones were thought to be a marker of high social status. Snuffing, rather the discovery of snuff trays/tubes in funerary contexts has also been suggested to be a marker of high status. Even though this male did not have these items in his burial goods, the combination of a snuff marker in his hair and

elongated ears may suggest he had a special status within his community. A young adult female also tested positive for bufotenine, suggesting females also practiced snuffing. Archaeological evidence from other sites suggests females (to a much lesser extent) practiced snuffing. This female also had a type of cranial modification associated with Tiwanaku, rather than the local Cabuza style. Both snuffing and cranial modification produced by boards have been suggested to be markers of altiplano influence. There is no direct evidence to suggest this individual was a migrant from the *altiplano*.

In all populations BZE was detected in around half-to three quarters of individuals. The results from this study are comparable to previous studies of ancient hair, with similar proportions of each group testing positive for BZE.

Cocaine and BZE bind very easily to hair. Drug compounds bind to various components of hair, but melanin is particularly important (Nakahara *et al.* 1995; Gygi *et al.* 1996; Joseph *et al.* 1996; Joseph *et al.* 1997; Pötsch *et al.* 1997b; Bourges *et al.* 2003; Mieczkowski and Kruger 2007). Melanin is the most robust component of the hair, which may explain why drug compounds are detectable in ancient hair samples, although the amounts detected in ancient hair samples from certain depositional environments may be a fraction of what they were when the individual died. This may also explain why other drug compounds, such as scopolamine and mescaline that are incorporated into hair at much lower levels are not detected in ancient hair.

## 11.2. Further work

This research project has identified a number of areas for further work. First, further research into the preservation and degradation of drug molecules in hair in laboratory based simulated burial environments, particularly arid environments, would aid interpretation of data from archaeological hair from South America. The results may give us some idea of how fast drug molecules in hair degrade under these conditions. Another avenue of research is the preservation of drug compounds in hair in association with a decaying body (potentially using a human body analogue) in a simulated desert environment. To investigate what Kintz *et al.* (2008) call the “external postmortem artefact”, drug free hair could be buried with a decaying body and coca leaves in a simulated desert environment to see to what extent coca metabolites contaminate the hair.

The use of LC-MS/MS for archaeological specimens is a very new development. LC-MS/MS is prone to matrix effects that can result in either ion suppression or enhancement of the target analyte. As archaeological specimens are potentially degraded could be a source of co-eluting compounds, a very valid area of further research could be the investigation of potential sources of these effects and developing a method to recognise and minimise these effects.

The data obtained from this research project is qualitative, but has indicated individuals for further analysis. Quantifying the levels of bufotenine in the hair of the two Azapa individuals who tested positive for this drug and carrying out segmental analysis of some of the individuals with large enough hair samples, for

example the Iquique individuals, who tested positive for BZE, may also shed some light into coca chewing behaviour in these individuals.

Relatively little is known about the Cabuza culture, as research on archaeological populations around Arica has focussed largely on earlier groups (i.e. Chinchorro). The following suggestions may help understanding of contentious ideas about Cabuza populations:

- a. Radiocarbon dating of Cabuza material. There are few dates for the Cabuza period. A series of radiocarbon dates based on a sensible sampling strategy should help place the Cabuza within a more defined temporal framework.
- b. Stable light isotope analysis of hair samples could shed light on seasonal changes in diet and possible short term locational changes (Wilson *et al.* 2007b; Williams and Katzenberg 2012).
- c. Comparison of strontium isotopes in tooth and bone could indicate whether the Cabuza were highland migrants or local people with long-standing connections to the coast and river valleys, as has been debated by various researchers (Sutter 2000; Rothhammer and Santoro 2001; Rothhammer *et al.* 2002; Sutter 2005b; Sutter 2006). Similar work on other Andean populations has been able to differentiate between highland and coastal people from Tiwanaku and Chen Chen in the Moquegua valley (Knudson *et al.* 2004).
- d. The analysis of hair for cortisol, a marker of systemic stress has been detected in hair from various sites in northern Chile (Webb *et al.* 2010). This type of analysis combined with stable light isotope analysis and pathological

data may provide a detailed life history of disease and dietary/physiological stress.

- e. Investigation of very short term dietary changes at an individual level is possible with the use of liquid-chromatography isotope ratio mass spectrometry (LC-IRMS). Although a relatively new technique, it has provided interesting data regarding specific dietary inputs (i.e. lipids and carbohydrates) into Chinchorro diet (Smith *et al.* 2011). This technique may be a useful tool for investigating resource use (and the possibility of a vertical economy) by Cabuza people.
- f. The data from stable light isotopes, radiocarbon dating and hair tests for cortisol and psychoactive compounds integrated with archaeological data such as burial type and grave goods could aid understanding of the Cabuza culture. This is particularly relevant, as so much contextual information was lost during excavation, as these were a response to building work and land development during the 1970s/1980s.

The analytical method developed and used for this project was only capable of detecting a small number of compounds. The method was not suitable for some compounds, for example scopolamine, harmine and harmaline. The following suggestions would aid further method development for other compounds:

- a. Development of a GC-MS or LC-MS/MS method using solvents other than acetonitrile for the detection of harmine, harmaline and scopolamine in ancient hair samples, as the method developed for this project was unsuitable for these compounds.

- b. Nicotine is present in a number of plant species, including the tobacco plant (*Nicotiana* sp.) and is known to have been used in the Andes. Analysis of hair samples for nicotine and its metabolite cotinine may give a fuller picture of the use of plants containing psychoactive compounds by ancient populations. The post-excavation histories of any of the individuals tested should be taken into account, particularly if they have been housed in European museum collections for long periods of time, as nicotine solutions were used as insecticides and may be present as a contaminant (Buckland and Panagiotakopulu 2001).



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## Appendix 1: Mummies from the Azapa Valley, Chile

### A2.1. Introduction

The samples originating from Chile (Azapa Cabuza and Museo Regional de Iquique samples) were donated by Dr. Arthur Aufderheide. These samples were collected by Dr. Aufderheide in the 1980s whilst he was on fieldwork in Chile. The samples were stored in plastic sample bags for almost 30 years. The majority of the samples had not been opened since they were collected. The contextual information was gathered and translated from Spanish by the author from the field note books of Guillermo Focacci and others who undertook emergency excavation of the Azapa sites in the 1980s and the 1990 site report of AZ-6 (Focacci 1990).

**Table A2.1:** Cabuza individuals from Azapa-6

<b>Tomb</b>	<b>Age</b>	<b>Sex</b>	<b>Grave goods</b>	<b>Pathology</b>
16	I	I	None recorded	None recorded
19	16	F	Ceramic frags., wooden spoon, basketry, bag, poncho, cactus needle, maize cob (4), shirt, coca leaves, potato, bottle, copper needle, animal skins (on feet), belt, lower garment	None recorded
22	I	I	None recorded	None recorded
29-1	I	I	Kero, wooden spoon	None recorded
36	25	M	None recorded	None recorded
71	30	M	<i>Orejones</i> , trenzas	Trepanned?
108	I	I	None recorded	None recorded
TMCA3	40	F	None recorded	None recorded

**Table A2.2:** Cabuza individuals from Azapa-71

<b>Tomb</b>	<b>Age</b>	<b>Sex</b>	<b>Grave goods</b>	<b>Pathology</b>
36	0.1	M	Ceramic jar decorated in Cabuza style (fragment), courseware <i>kero</i> , undecorated bottle gourd	None recorded
46	I	I	Undecorated ceramic jar, woollen hat (blue & brown), wooden spoon, spindle & spindle whorl	None recorded
48	0.1	I	Piece of gourd	None recorded
76A	I	I	Wooden spoon, comb, cactus needles, camelid bone, woollen bag, arrows (broken)	None recorded
94B	I	I	Vegetable fibres, undecorated plant fibre bowl, sewing kit, undecorated gourd,	None recorded
105	15	M	Olla (2 handles), ceramic jar, gourd, basket fragment, 5 "stained" stones, 2 wooden spoons, one with camelid figure, one broken, four arrows with fox fur ends (?)	None recorded
127	45	F	Reed ropes, mantle	None recorded
197	1	F	Wooden spoon, ceramic fragment, undecorated ceramic jar	GI problem
200	I	I	Ceramic frag, two bags, one undec., two poles/sticks, sewing kit with cactus needles and wool, camelid bone, wooden spoon, pyroengraved gourd (2?), ceramic jar	None recorded
201B	35	F	Poorly preserved blankets	None recorded
210	1	M	Head band - white, blue & red; reed ropes, brown cords	None recorded
230	12	M	None recorded	Pneumonia, megacolon
240A	0.5	F	None recorded	GI problem, megacolon
263	1	F	None recorded	Pneumonia, GI problem
285	1	M	None recorded	Pneumonia, septicaemia, cranial def. Type 12
322	5	I	None recorded (head only)	None recorded
331	20	M	None recorded	None recorded
470	2	I	white feather (simple headdress)	None recorded
504	3	M	Brown/beige wool "cape", red wool string around head, brown shirt with coloured stripes, maize cob, 1 camelid foot, 1 potato, leather sandals, beige/brown striped shirt, <i>tari</i> , coca leaves, <i>illipta</i>	None recorded

601	55	M	2 shirts, maize cobs, potato, 2 camelid legs, <i>kero</i> , bowl, basket	None recorded
602	55	M	Poncho, loincloth, head band	None recorded
AA	12	F	5 maize cobs, 3 potatoes, sandals, 2 ponchos	None recorded
A3	I	M	None recorded	None recorded
B1	20	F	Thread, sash/belt, leather sandals, <i>tari</i> , coca leaves	Subcranial haemorrhage
DD	41	F	Reed ropes, sandals, poncho, sewing kit	None recorded
E1	25	F	reed ropes, red bag, sash/belt, wool, 2 shirts, sewing implements	OA (minimal), occupational change, cranial mod (type 4 or 5)
M1	22	F	mummy in poor condition, skin loose, buried with a blanket	cranial mod (type 5)
N1	3	M	shirt, poncho (both poor cond.), sandals	cranial mod (type 3)

**Table A2.3** – Cabuza individuals from Azapa-141

<b>Tomb</b>	<b>Age</b>	<b>Sex</b>	<b>Grave goods</b>	<b>Pathology</b>
12	2	I	Kero (veg, fiber), wooden spoon with camelid figure, gourd, wooden spoon, ceramic jar, sandals	None recorded
13	7	I	rope, hair, wooden stake that held false head in place, 2 wool shirts (brown)	None recorded
18	2	F	Bags (5), cactus needles, braid of human hair, pair leather sandals, box	None recorded
26 1-2	2	I	Fragment of blue textile, sandals (pair), veg. Fiber rope, corn, striped shirt	Enthesopathies
28-1	1	I	Wool shirt, textile frags, mummy bundle ropes, wool bag with strap	None recorded
34	7	M	Brown shirt, red thread, piece of wood, textile, tie strings, sandals, gourd	None recorded
35	19	M	Kero (ceramic)	None recorded
47	2	M	Ceramic jar, basketry, wooden spoon	None recorded
53	20	M	Kero (veg. Fiber), ropes for mummy bundle, textile frags (3), 2 wool shirts, red headband, sandals	Enthesopathies, OA
54	1	M	Striped shirt, basketry, textile frag, mummy bundle ropes, maize grains	None recorded
DESC 2	35	F	None recorded	Enthesopathies, OA
DESC 8	2	I	None recorded	None recorded
T-TOL	I	I	None recorded	None recorded

## Appendix 2: Mummies from Museo Regional de Iquique, Chile

### A3.1. Introduction

The descriptions in this Appendix are based on the original field notes taken by Arthur Aufderheide in July and August 1995. The notes were taken on University of Minnesota “Mummy Autopsy Protocol” proformas. The proformas record site information, age, sex, pathologies, grave goods, preservation status, autopsy details, metrics, skeletal and dental inventory, hairstyle and cranial modification types. Also included in this appendix are photographs of the mummies that were taken by Arthur Aufderheide. Only images of IQU-95 T2 and IQU-95 T3 were available.

The mummies IQU-95 T2, IQU-95 T3 and IQU-95 T7 were included in a study by Bernardo Arriaza *et al.* (2008a) on obtaining biological information from mummies without contextual information using scientific dating and biomedical testing alongside traditional anthropological means of investigating human remains.

## **A3.2. IQU-95 T2 (adult female, Los Verdes)**

### *A3.2.1. Basic information*

IQU-95 T2 is an adult female from the Los Verdes site near Iquique on the Chilean coast. This individual was excavated by J. Checura and analysed by A. Aufderheide on 31 July 1995. Originally the individual was thought to date from the Regional Development phase, but based on radiocarbon dating this was re-evaluated. This individual probably belongs to the end of the Cabuza/Tiwanaku period.

### *A3.2.2. Age, sex and stature*

The determination of sex was based on the morphology of the Ossa coxae (wide sciatic notch) and cranium (mastoid process, supraorbital ridges and occipital protuberance consistent with typical female appearance) and the presence of breasts and vagina.

Age was estimated to be between 40 and 50 based on wear on the pubic symphysis (specific method not mentioned) and the presence of “moderate osteophytes”.

### *A3.2.3. Burial information*

The body was buried in a flexed position. The arms and legs were painted with a red pigment and buried dressed in three woven shirts of a brown colour. The head protrudes from the bundle, suggesting that there may have been another covering at some point, but no longer present. A piece of grey ceramic had been sewn to the

**Figure A3.1**– IQU-95 T2, Adult female from Los Verdes. Image: ACA



outer part of the outermost garment. The whole bundle was held in place with a 2 cm thick wool rope. Heavy hide sandals were present on the feet.

#### *A3.2.4. Preservation of soft tissues*

##### *A3.2.4.1. Skin*

The body of this individual was spontaneously mummified. The preservation of the skin on the torso was scored as “intermediate” on a three step scale of good to poor. The skeleton was scored as good on the same scale.

The presence of tissue was scored on a scale of 0-5. This was then used to calculate a soft tissue index using the following calculations:

$$\text{Bone: (head + chest + abdomen + arms + legs)*4 = B}$$

$$\text{Soft tissue: (head + chest + abdomen + arms + legs)*4 = ST}$$

$$\text{Soft tissue index} = (\text{ST}/\text{B}) * 100$$

**Table A3.2** – Soft tissue and bone scores for IQU-95 T2

	Head	Chest	Abdomen	Arms	Legs	%Preservation
<b>Bone</b>	5	5	5	5	5	100
<b>Soft tissues</b>	4	3	3	5	5	80

$$\text{Soft tissue index for IQU-95 T2} = (100/80) * 100 = 80\%$$

No tattoos or skin lesions were noted. Aufderheide noted the presence of breasts, although only the right one was identifiable.

#### *A3.2.4.3. Hair and nails*

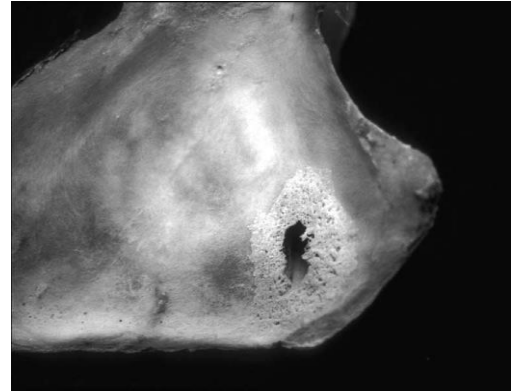
Observation of scalp hair revealed the presence of head lice. The hair itself was black in colour and covers about 80% of the cranium. The hair was braided into multiple small braids which were then gathered into a single braid on either side of the head (hairstyle type 2.16). No body hair was present, but fingernails were present.

#### *A3.2.4.4. Internal organs*

During autopsy Aufderheide noted the presence of the following organs: heart, lungs (both), small intestine, large intestine, bladder, brain and diaphragm. None the organs present showed any sign of pathology. The following organs/structures were not present: oesophagus, liver, gallbladder, spleen, stomach, kidneys, ureter, aorta, trachea, and uterus. No coprolites were present in any part of the digestive system. Aufderheide assigned an organ preservation score of 40% for this individual.

#### *A3.2.5. Skeletal preservation and pathology*

This individual had a number of dental pathologies, including moderate dental caries and antemortem tooth loss (AMTL). In particular Aufderheide notes the left maxillary second premolar had been lost antemortem. A large periapical abscess was present with a fistula, but there was no sign of remodelling, suggesting the lesion was active when this individual died.



**Figure A3.2** – Lytic lesion on the left scapula of IQU-95 T2 (Arriaza *et al.* 2008).

In the postcranial skeleton lytic lesions were noted on ribs and defects were evident in the parietal pleura. Lesions were also noted on the second thoracic vertebral body. The lesion was

5 mm wide and exposed trabecular bone. A 1 cm lesion was also noted on the left scapula. The defect was surrounded by a ring of periosteal bone formation. Aufderheide's original diagnosis was septicaemia as a complication of the periapical abscess. However, aDNA analysis of the lesions revealed the presence of *Mycobacterium tuberculosis*, the causative agent of tuberculosis.

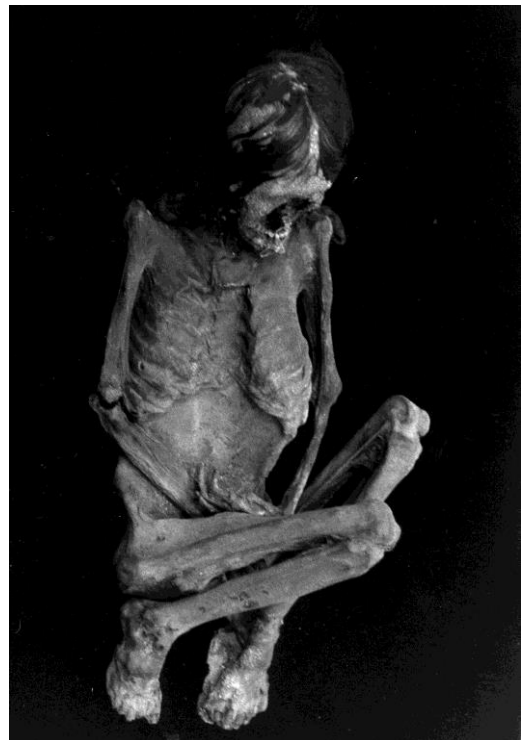
### **A3.3. IQU-95 T3 (adult female, Bajo Molle)**

#### *A3.2.1. Basic information*

IQU-95 T3 is an adult female from Bajo Molle-1. This individual was analysed by Arthur Aufderheide on 1 August 1995. The culture assigned to this individual is coastal regional development with Tiwanaku influence.

#### *A3.2.2. Age, sex and stature*

IQU-95 T3 is an adult female aged between 40 and 50 based on age-related changes in the pubic symphysis, dental wear and the presence of osteophytes. Sex determination was based on the morphology of the Ossa coxae and the presence of external genitalia. Using the formulae from Trotter (1970) stature was estimated to be 153.4 cm using the formula for Mongoloid males (even though this individual is female) for combined length of femur and tibia.



**Fig A3.3** – IQU-95 T3, adult female from Bajo Molle-1. Extensive postmortem damage is evident in the facial area.

#### *A3.2.3. Burial information*

The body was buried in a flexed position and partially wrapped in multiple layers of finely woven textile. Thick soled sandals were present on the feet. Extensive postmortem damage is evident in the facial area. Aside from the textiles this individual was buried in, no other grave goods were found.

#### *A3.2.4. Preservation of soft tissues*

##### *A3.2.4.1. Skin*

There was no evidence of any artificial means of preservation on this individual. Skin preservation of the abdomen was rated as “intermediate”. No tattoos or other body modification was present.

**Table A3.2** – Bone and soft tissue scores for IQU-95 T3

	Head	Chest	Abdomen	Arms	Legs	%Preservation
<b>Bone</b>	4	5	5	5	5	96%
<b>Soft tissues</b>	4	5	3	5	5	88%

Soft tissue index for IQU-95 T3 = 92%

A red pigment was observed painted on the arms, legs and lower chest of this individual.

##### *A3.2.4.3. Hair and nails*

Abundant scalp hair was present on this individual. The hair was dark brown in colour and braided into multiple braids that were gathered on each side of the head. Head lice were found on the scalp hair. Finger and toenails were present. Body hair was absent.

#### *A3.2.4.1. Internal organs*

The following organs were present: heart, lungs, liver, small and large intestine, bladder, brain and diaphragm. The lungs were noted as showing some signs of pathology. The following organs/structures were not present: oesophagus, gallbladder, spleen, stomach, kidneys, ureters, aorta, trachea, and uterus. No coprolites were found. Aufderheide assigned an organ preservation score of 45% for this individual.

#### *A3.2.5. Skeletal preservation and pathology*

All skeletal elements with the exception of some facial bones were present. Bone preservation was described as “good”.

Aufderheide noted flattened areas on the femoral condyles and suggested this may be an effect of squatting. He also noted that the bones were “unusually light” and suggested osteopenia as the cause. Osteopenia is a general descriptive term for a loss of bone mass. It is caused by a number of conditions including osteoporosis, osteomalacia, hyperparathyroidism, cancer and severe malnutrition (Ortner 2003: 410). No vertebral fractures were noted.

In addition, a number of teeth were lost before death. Only the mandible was present. All three molars on both sides were lost, as was the left central incisor. No carious lesions were noted in surviving teeth. However, abrasion between the right mandibular second incisor and canine was noted. This was thought to be occupational, as the teeth were used as a tool.

### **A3.3. IQU-95 T7 (infant male, Bajo Molle 1)**

#### *A3.2.1. Basic information*

IQU-95 T7 is an infant male, aged 9-12 months from the Bajo Molle 1 site near Iquique on the Chilean coast. This individual was excavated by Cora Moragas and analysed by Larry Cartmell on 2 August 1995. The cultural affiliation of this individual is stated as “Pica-Tarapaca Regional Development with Tiwanaku influence”. No photographs of this individual are available.

#### *A3.2.2. Age, sex and stature*

Determining biological sex of juvenile remains is often difficult or impossible. In this case it was possible to determine sex as the individual was mummified. The scrotum was identifiable. Age was determined by the sequence of tooth eruption.

#### *A3.2.3. Burial information*

The body was buried in an extended position dressed in an undecorated brown tunic. Sandals were present on the feet. This child was buried with a pink and white bivalve shell, a corn cob and a seagull (bird bones and feathers were found in the grave). The body is headless, but some cranial fragments and hair was found.

#### *A3.2.4. Preservation of soft tissues*

##### *A3.2.4.1. Skin*

Skin preservation was scored as “intermediate”. The skin over the right chest cavity was absent, revealing ribs and no thoracic contents. The majority of the skin of the posterior thoracic and abdominal cavities was absent.

**Table A3.3** – Bone and soft tissue scores for IQU-95 T7

	Head	Chest	Abdomen	Arms	Legs	%Preservation
<b>Bone</b>	0	5	5	5	5	80%
<b>Soft tissues</b>	0	2	2	5	5	56%

Soft tissue index for IQU-95 T7 = 70%

##### *A3.2.4.3. Hair and nails*

Loose hair was found associated with the body. It was not attached to the scalp. Finger and toenails were present.

##### *A3.2.4.4. Internal organs*



The only internal organ identified was the small intestine, which was poorly preserved. The other organs/structures on Aufderheide's inventory were not present.

#### *A3.2.5. Skeletal preservation and pathology*

Skeletal preservation was scored as "good". No pathology was noted in the skeleton or dentition

## **Appendix 3: Mummies from Puruchuco-Huaquerones, Peru**

### **A3.1. Introduction**

The table below summarises the contextual information of 13 “Inca” individuals that were tested for cocaine and hallucinogens. These 13 individuals were from the Inca necropolis at Puruchuco Huaquerones, on the outskirts of Lima, Peru. These 13 were some of the individuals included in Jocelyn Williams’ Doctoral Thesis entitled *“Investigating diet and dietary change using the stable isotopes of carbon and nitrogen in mummified tissues from Puruchuco-Huaquerones, Peru.”*

Thirteen hair samples were sent to Bradford for stable light isotope analysis at the University of Bradford. The hair samples were prepared for segmental analysis by ELB in September 2008 (this work was later presented at the 7<sup>th</sup> World Congress on Mummy Studies in San Diego in 2011). Dr. Williams allowed for the remaining hair to be analysed as part of ELB’s doctoral research.

The information presented in this appendix was collated from Dr. Williams’ Doctoral Thesis.

**Table A4.1** – Contextual and pathology information for 13 individuals from Puruchuco Huaquerones, Lima, Peru

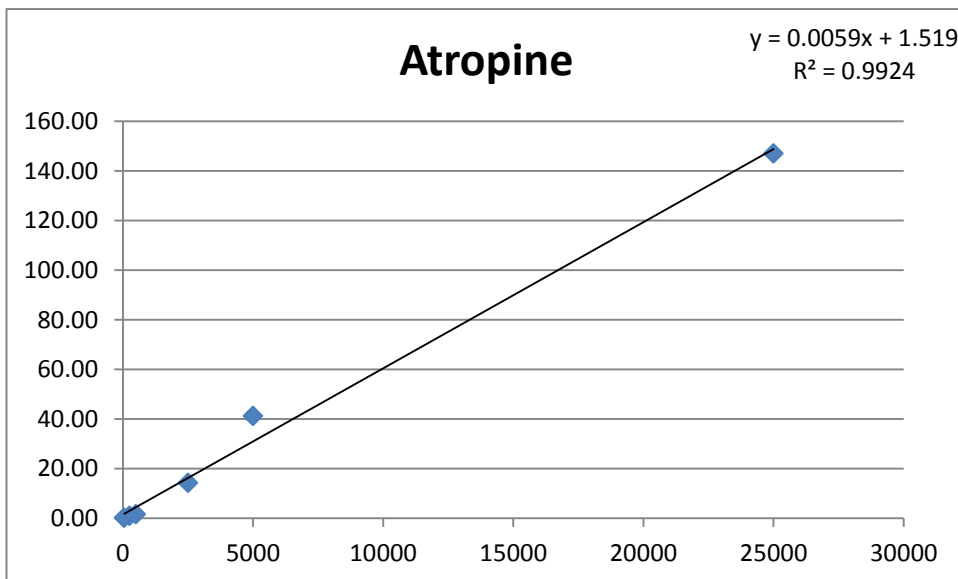
ID	Age	Sex	Burial reference*			Grave goods	Pathology	
			S	C	U			
12	20-35	F	7	J9	2	163	Disturbed context – no objects recorded	Healed skull fracture
16	35-50	M	7	K1	4	171	IPB, rope encased cotton filled fardo, gourds	OA ( Ulna, tibia, radius, femora, metacarpals, vertebrae)
18	4-8	I	8	28			Disturbed context – no objects recorded	None recorded
20	20-25	M	9	F	2	2	IPB, rope encased cotton filled fardo, weaving tools, decorated ceramics, gourds	OA (vertebrae), healed rib #
22	20-35	M	8	X'	35	179	Textile wrapped partially disturbed primary burial, metal objects, weaving tools, textile bag/belt	OA (Vertebrae, calcaneus, talus, navicular)
25	35-50	F ?	16	F3	1	38	Partially disturbed cotton filled fardo, primary burial, weaving tools, textile bag/belt, decorated ceramics, gourds	PH, OA ( tibia, femur, humerus, ulna, vertebrae)
28	50+	F	15	I2	2	58	IPB, rope encased <i>fardo</i> filled with cotton seeds, metal, weaving tools, Spondylus shells	None recorded
33	20-35	F	8	S'	68	129	Disturbed burial, no grave goods recorded	CO, PH, periostitis (femur)
41	35-50	F	16	P3	4	56-1	Partially disturbed burial, <i>fardo</i> filled with cotton seeds, metal, weaving tools, plain ceramics	CO, PH
53	4-8	S	3	S'	4		Disturbed burial, no	No pathology

		A					grave recorded	goods recorded	recorded
<b>54</b>	35-50	M	3	I	2		Disturbed burial, no grave recorded	goods	Cribra orbitalia
<b>59</b>	35-50	M	7	M	4	143	IPB, Cotton fardo, encased. Decorated ceramics, abalone shells	filled rope gourds,	Cribra orbitalia, porotic hyperostosis, healed fracture of radius
<b>68</b>	8.5- 13.5	S A	3	V	1		Disturbed burial, no grave recorded	goods	None recorded

## Appendix 4: Linearity and calibration data

### A4.1. Atropine

<b>Atropine</b>						
	ATR	IS	ATR/IS	Mean	St dev	%Precision
10 ug	7216735	30643	235.510			
	7631447	32301	236.260			
	7867889	35528	221.456			
	8164398	36546	223.401			
	7347983	30917	237.668	230.86	7.77	3.36
5 ug	3980282	27589	144.271			
	4783335	33016	144.879			
	3898891	24596	158.517			
	3854366	29186	132.062			
	4747348	30573	155.279	147.00	10.44	7.10
1 ug	219389	4748	46.207			
	149507	3399	43.986			
	113950	3075	37.057			
	75476	2610				
	70744	1888	37.470	41.18	4.62	11.21
500 ng	21127	1357	15.569			
	17201	1762				
	18012	1258	14.318			
	19888	1389	14.318			
	23503	1824	12.885	14.27	1.10	7.68
100 ng	962	436	2.206			
	913	576	1.585			
	937	499	1.878			
	932	542	1.720			
	847	620	1.366	1.64	0.22	13.24
50 ng	350	353	0.992			
	311	297	1.047			
	232	225	1.031			
	303	284	1.067			
	259	314	0.825	0.99	0.11	11.36
10 ng	49	347	0.141			
	58	365	0.159			
	45	296	0.152			
	92	292				
	39	190	0.205	0.16	0.03	17.18

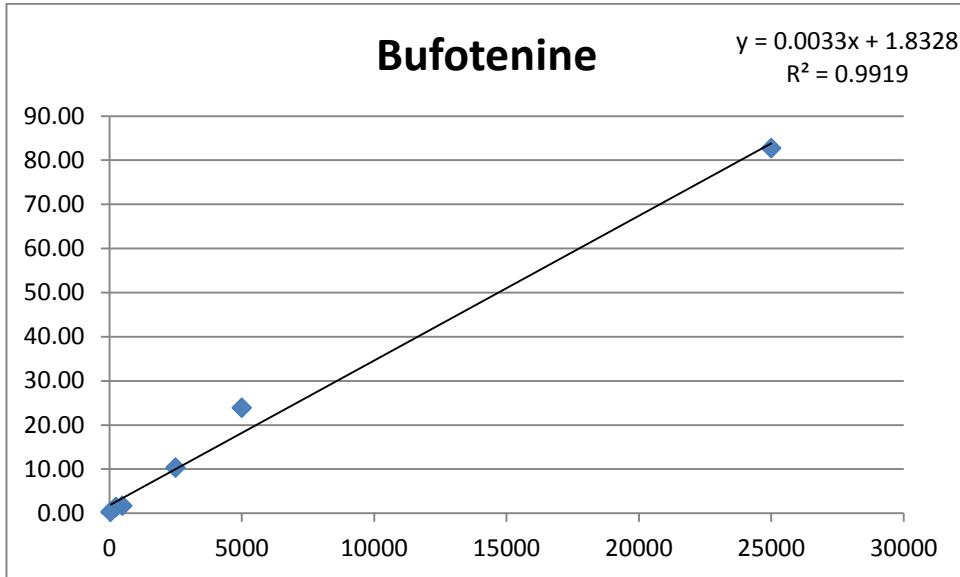


Data used to plot chart. Values in the pg column represent on-column concentrations.

<b>pg</b>	<b>ratio</b>
25000	147.00
5000	41.18
2500	14.27
500	1.64
250	0.99
50	0.16

#### A4.2. Bufotenine

Bufotenine						
	BUF	IS	BUF/IS	Mean	St dev	%Precision
10 ug	4227989	30643	137.976			
	4531123	32301	140.278			
	4445576	35528	125.129			
	4689575	36546	128.320			
	4163212	30917	134.658	133.27	6.40	4.80
5 ug	2245022	27589	81.374			
	2705568	33016	81.947			
	2006571	24596	81.581			
	2529112	29186	86.655			
	2500352	30573	81.783	82.67	2.24	2.71
1 ug	121055	4748	25.496			
	74110	3399	21.803			
	71674	3075	23.309			
	63408	2610	24.294			
	46285	1888	24.515	23.88	1.40	5.86
500 ng	17703	1357	13.046			
	15542	1762	8.821			
	13689	1258	10.882			
	15644	1389	11.263			
	13786	1824	7.558	10.31	2.15	20.86
100 ng	1082	436	2.482			
	925	576	1.606			
	844	499	1.691			
	965	542	1.780			
	977	620	1.576	1.66	0.09	5.54
50 ng	252	353	0.714			
	315	297	1.061			
	385	225	1.711			
	407	284	1.433			
	415	314	1.322	1.38	0.27	19.50
10 ng	71	347	0.205			
	69	365	0.189			
	75	296	0.253			
	75	292	0.257			
	52	190	0.274	0.24	0.04	15.52



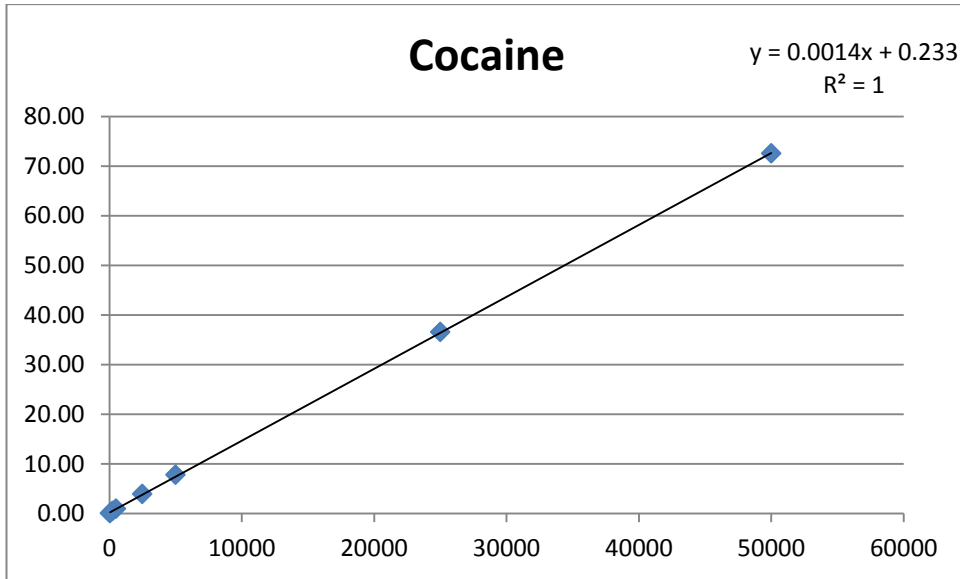
Data used to plot chart. Values in the pg column represent on-column concentrations.

pg	ratio
25000	82.67
5000	23.88
2500	10.31
500	1.66
250	1.38
50	0.24



### A4.3. Cocaine

Cocaine 304 > 182						
	COC	IS	COC/IS	Mean	St dev	%Precision
10 ug	13734351	186939	73.470			
	13698066	184558	74.221			
	13742047	193929	70.861			
	14092934	193534	72.819			
	12648576	177795	71.141	72.50	1.46	2.01
5 ug	6144711	163703	37.536			
	7372791	194805	37.847			
	6763257	183701	36.817			
	4395009	128420	34.224			
	7113854	195283	36.428	36.57	1.43	3.90
1 ug	170787	20695	8.253			
	142113	18979	7.488			
	122311	16964	7.210			
	87861	11981	7.333			
	71530	8398	8.518	7.76	0.59	7.56
500 ng	22000	5812	3.785			
	17303	4321	4.004			
	19962	4569	4.369			
	24249	6825	3.553			
	15889	4254	3.735	3.89	0.31	8.04
100 ng	1500	1987	0.755			
	1414	1846	0.766			
	1395	1117	1.249			
	1573	1613	0.975			
	1411	1585	0.890	0.97	0.20	21.11
50 ng	414	1168	0.354			
	607	1241	0.489			
	596	848	0.703			
	329	1290	0.255			
	536	971	0.552	0.47	0.17	36.93
10 ng	31	934	0.033			
	13	1240	0.010			
	80	1530	0.052			
	53	1321	0.040			
	76	913	0.083	0.04	0.03	61.00

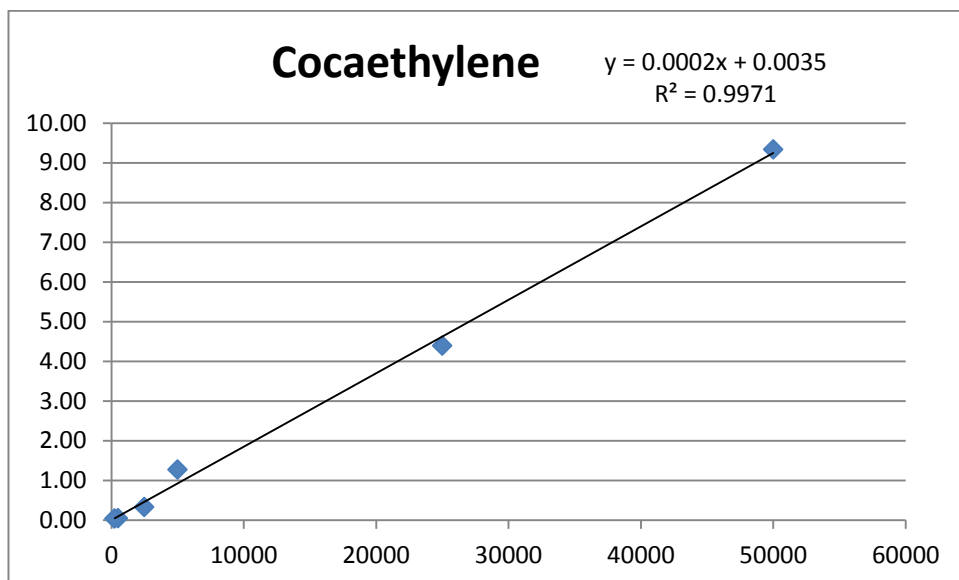


Data used to plot chart. Values in the pg column represent on-column concentrations.

<b>pg</b>	<b>ratio</b>
50000	72.50
25000	36.57
5000	7.76
2500	3.89
500	0.97
250	0.47
50	0.04

#### A4.4 – Cocaethylene

Cocaethylene 318 > 204						
	COCE	IS	COCE/IS	Mean	St dev	%Precision
10 ug	1977342	206132	9.593			
	1991518	206871	9.627			
	2131570	233677	9.122			
	2132631	226120	9.431			
	1966711	221175	8.892	9.33	0.32	3.40
5 ug	1009366	220388	4.580			
	1090379	256213	4.256			
	1089512	245541	4.437			
	806973	183881	4.389			
	1177380	273922	4.298	4.39	0.13	2.90
1 ug	27768	36129	1.301			
	25929	27110	1.046			
	23234	29802	1.283			
	17225	23979	1.392			
	14452	19071	1.320	1.27	0.13	10.35
500 ng	3555	10903	0.326			
	3633	10093	0.360			
	3136	11263	0.278			
	5116	14742	0.347			
	2515	7811	0.322	0.33	0.03	9.52
100 ng	313	1150	0.272			
	49	605	0.081			
	143	2728	0.052			
	56	3551	0.016			
	91	2741	0.033	0.05	0.03	61.29
50 ng	117	2108	0.056			
	35	1935	0.018			
	62	1967	0.032			
	129	2012	0.064			
	46	1358	0.034	0.04	0.02	46.21
10 ng	nd	nd				
	nd	nd				
	nd	nd				
	nd	nd				
	nd	nd				

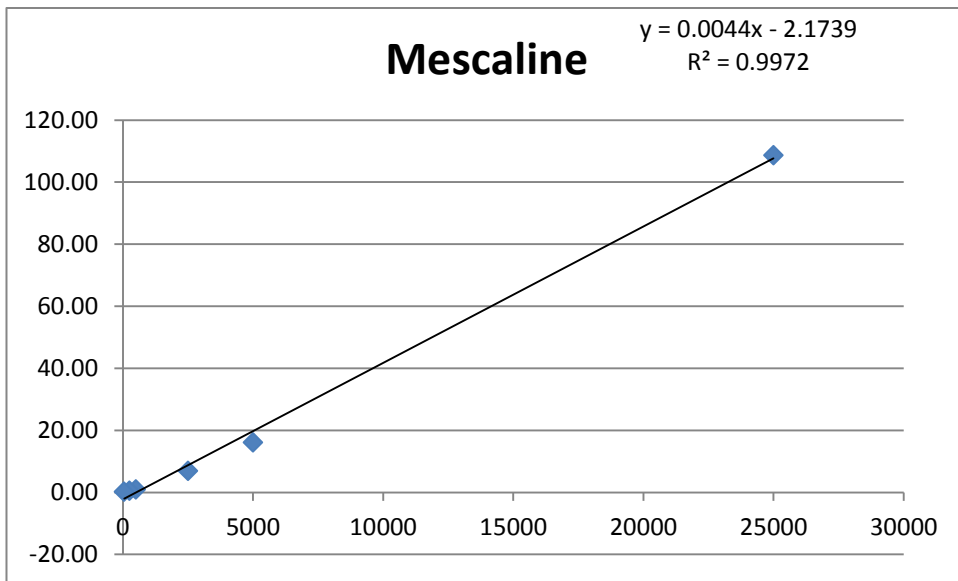


Data used to plot chart. Values in the pg column represent on-column concentrations.

<b>pg</b>	<b>ratio</b>
50000	9.33
25000	4.39
5000	1.27
2500	0.33
500	0.05
250	0.04

#### A4.5. Mescaline

Mescaline						
	MES	IS	MES/IS	Mean	St dev	%Precision
10 ug	6533136	30643	213.202			
	6929166	32301	214.519			
	7211650	35528	202.985			
	7206153	36546	197.180			
	6651676	30917	215.146	208.61	8.08	3.87
5 ug	2971756	27589	107.715			
	3460063	33016	104.800			
	2781381	24596	113.083			
	3035824	29186	104.016			
	3469963	30573	113.498	108.62	4.48	4.13
1 ug	87774	4748	18.487			
	61891	3399	18.209			
	44205	3075	14.376			
	34231	2610	13.115			
	30840	1888	16.335	16.10	2.35	14.59
500 ng	9965	1357	7.343			
	14913	1762	8.464			
	9075	1258	7.214			
	7811	1389	5.623			
	11193	1824	6.137	6.96	1.11	15.97
100 ng	604	436	1.385			
	517	576	0.898			
	390	499	0.782			
	675	542	1.245			
	502	620	0.810	0.93	0.21	22.89
50 ng	180	353	0.510			
	158	297	0.532			
	112	225	0.498			
	142	284	0.500			
	176	314	0.561	0.52	0.03	5.07
10 ng	51	347	0.147			
	47	365	0.129			
	16	296	0.054			
	74	292	0.253			
	96	190	0.505	0.22	0.18	80.77

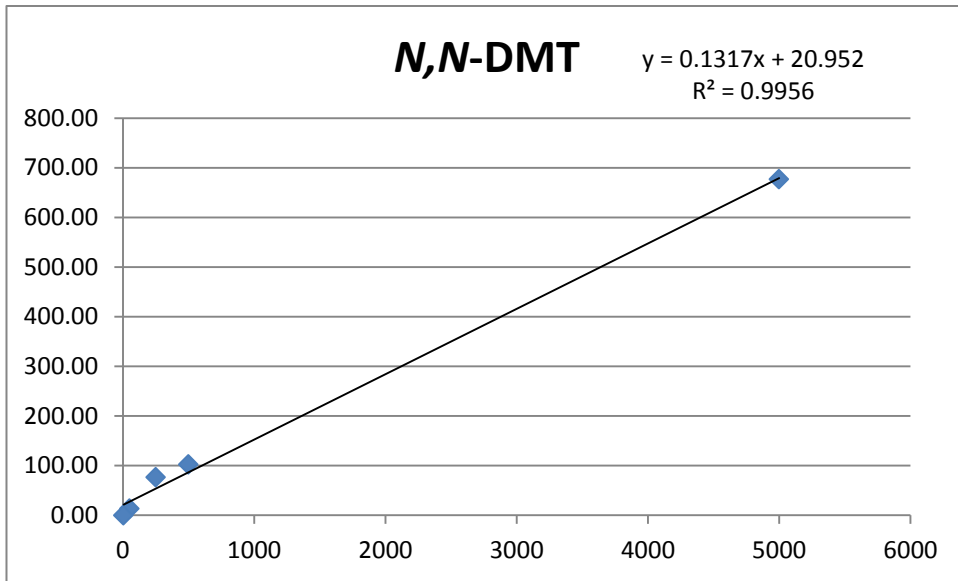


Data used to plot chart. Values in the pg column represent on-column concentrations.

pg	ratio
25000	108.62
5000	16.10
2500	6.96
500	0.93
250	0.52
50	0.22

#### A4.6. *N,N*-dimethyltryptamine

DMT						
	DMT	IS	DMT/IS	Mean	St dev	%Precision
10 ug	11707579	30643	382.064			
	11929183	32301	369.313			
	13226246	35528	372.277			
	13529271	36546	370.198			
	13310000	30917	430.507	384.87	26.01	6.76
5 ug	9455325	27589	342.721			
	10538802	33016	319.203			
	10054221	24596	408.775			
	9073563	29186	310.888			
	10796029	30573	353.123	346.94	38.56	11.12
1 ug	2568236	4748	540.909			
	2085635	3399	613.603			
	1985009	3075	645.531			
	1806542	2610	692.162			
	1684159	1888	892.033	676.85	132.29	19.54
500 ng	738821	1357	544.452			
	738317	1762	419.022			
	743683	1258	591.163			
	987187	1389	710.718			
	820688	1824	449.939	543.06	116.71	21.49
100 ng	56051	436	128.557			
	56871	576	98.734			
	59550	499	119.339			
	56988	542	105.144			
	53318	620	85.997	102.30	13.87	13.56
50 ng	20917	353	59.255			
	21054	297	70.889			
	19229	225	85.462			
	24465	284	86.144			
	20211	314	64.366	76.72	10.83	14.12
10 ng	3887	347	11.202			
	3766	365	10.318			
	3404	296	11.500			
	3833	292	13.127			
	3759	190	19.784	13.19	3.83	29.01
1 ng	5217	7969	0.655			
	1038	6155	0.169			
	692	4699	0.147			
	614	4969	0.124			
	542	4753	0.114	0.14	0.02	17.74



Data used to plot chart. Values in the pg column represent on-column concentrations.

pg	ratio
50000	384.87
25000	346.94
5000	676.85
500	102.30
250	76.72
50	13.19
5	0.14

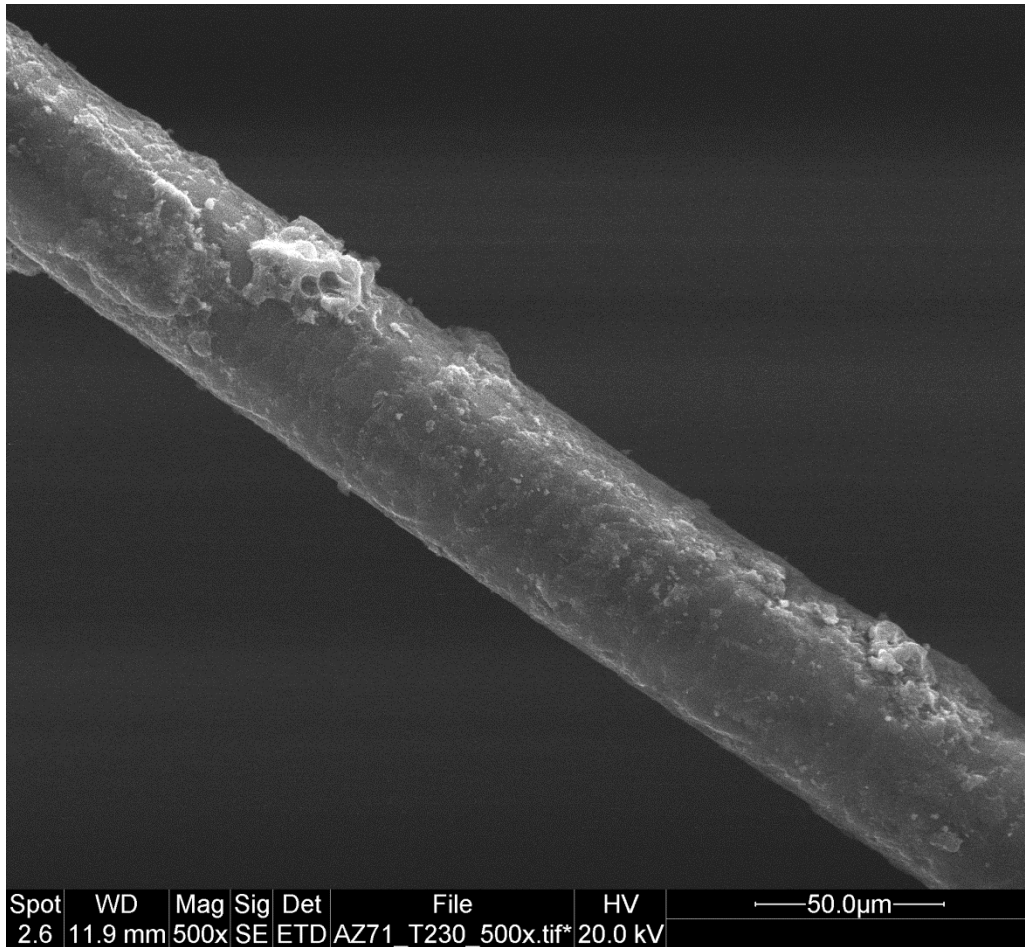


## Appendix 5: SEM Images

### A5.1. Introduction

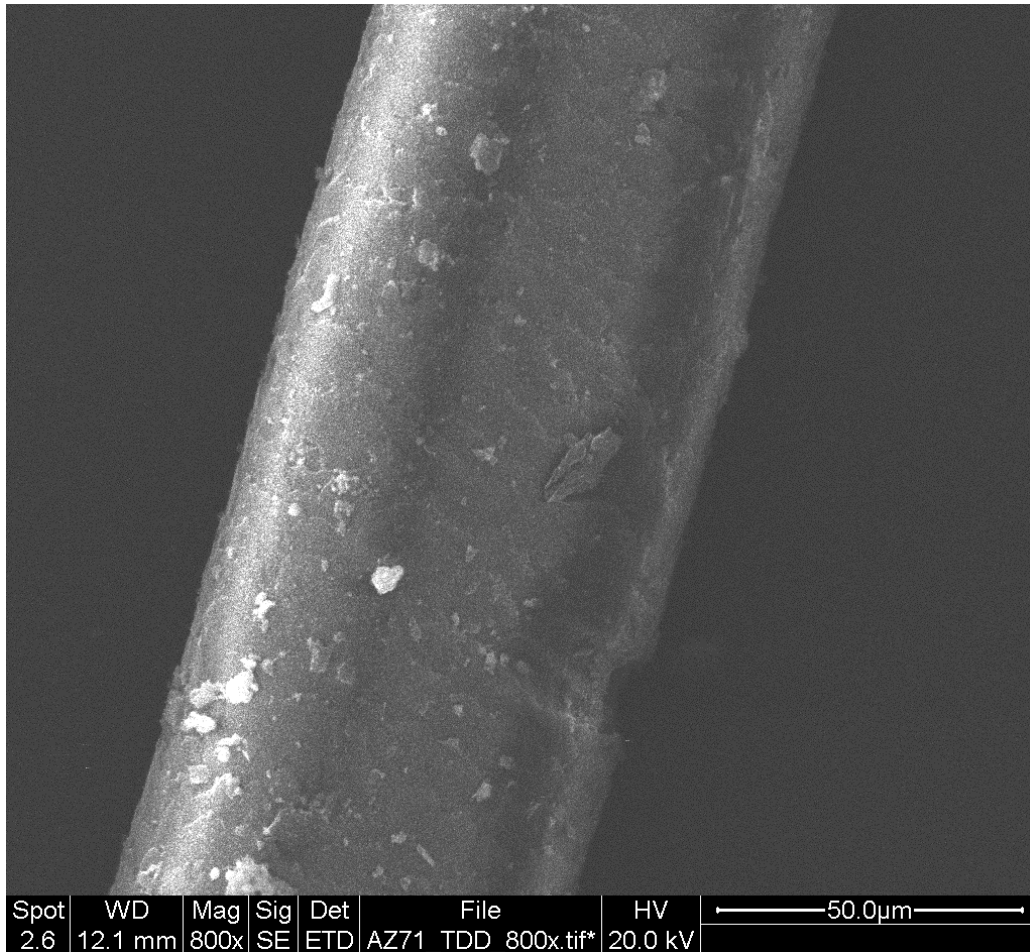
This Appendix details some of the images generated using scanning electron microscopy (SEM) to examine the state of preservation of hair from the Azapa Valley. A representative sample of hair from all three Azapa sites was selected for SEM imaging. No cleaning was performed prior to SEM imaging. Images were obtained using an FEI Quanta 400 scanning electron microscope (Eindhoven, Netherlands).

The specimens were mounted onto a stub, then coated with a thin layer (~ 20 - 30 nm) of gold, using a technique called sputtering. The coating prevents the build-up of high-voltage charges when using the high-vacuum mode on the SEM. Charging occurs where a negative charge builds up in a specimen. It is a particular problem with biological samples (Dykstra and Reuss 2003: 376). The resulting charges cause image artefacts, such as variations in brightness, blurring and movement of the specimen (Bozzola and Russell 1999: 65).



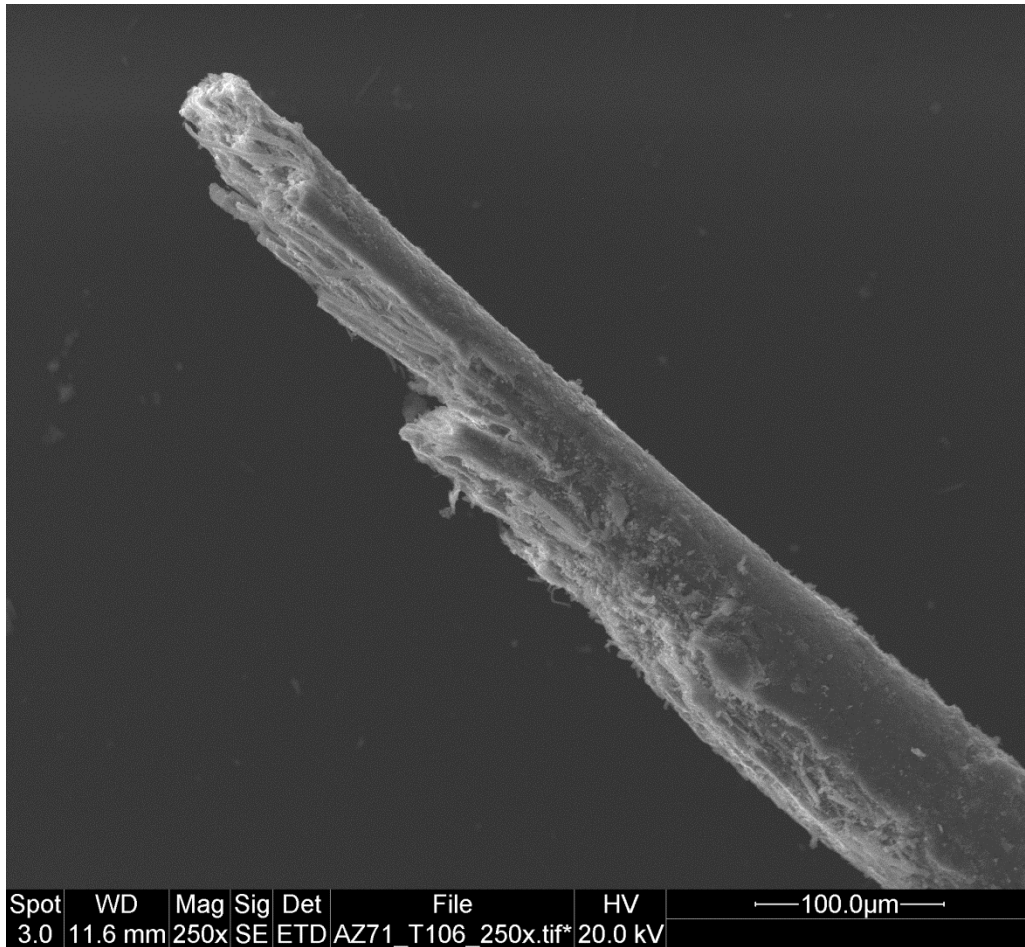
**Figure A.5.1** – Scalp hair from AZ-71 T230 (male, subadult).

This electron micrograph shows an example of scalp hair that has retained much of its structure. The cuticle is intact and the scales are visible. The proximal end of the hair is to the left of the image. There is some adherent material, mostly likely products of decomposition adhering to the hair. This image is representative of most of the hairs observed from all three Azapa sites.



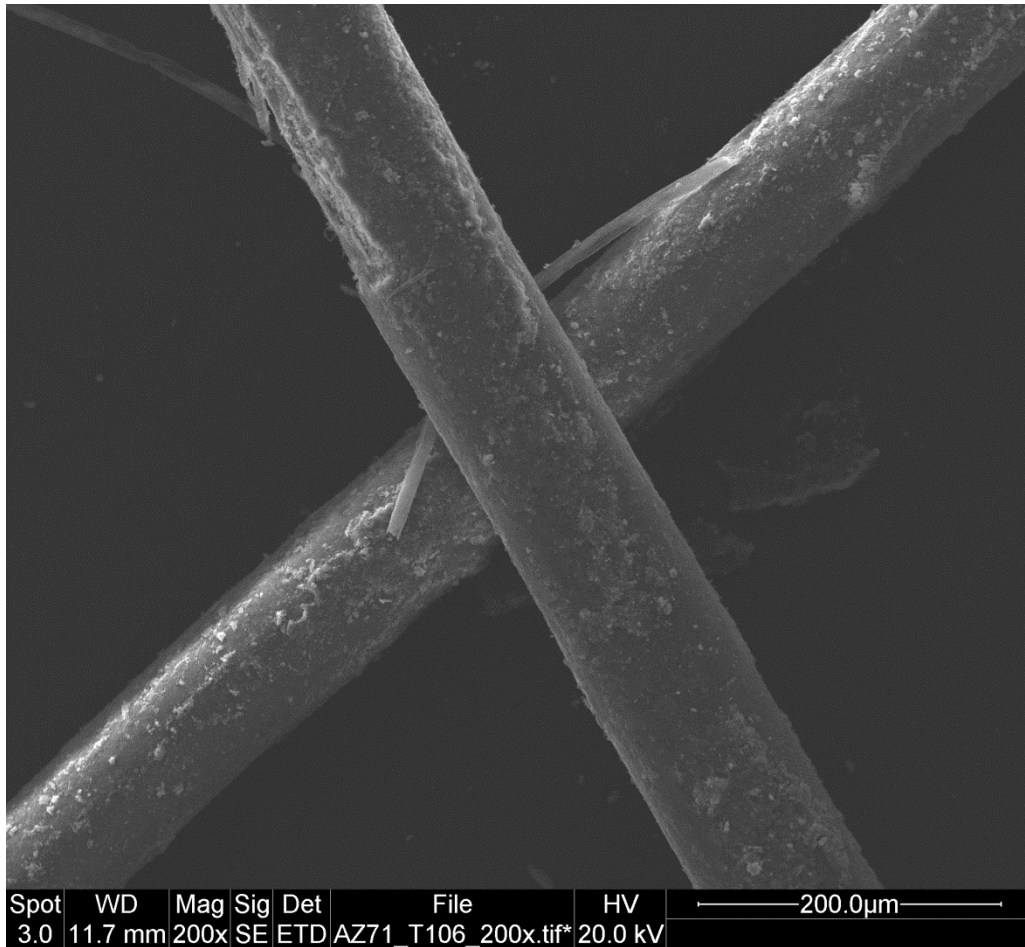
**Figure A.5.2** – Scalp hair from AZ-71 TDD (female, adult aged c 40 years)

This example illustrates hair from an adult female buried at AZ-71. The cuticle of the hair is intact. It is possible to ascertain from the cuticle pattern that the proximal end of the hair is towards the bottom of the image. There is some material adhering to the hair, most likely decomposition products and material from the burial environment.



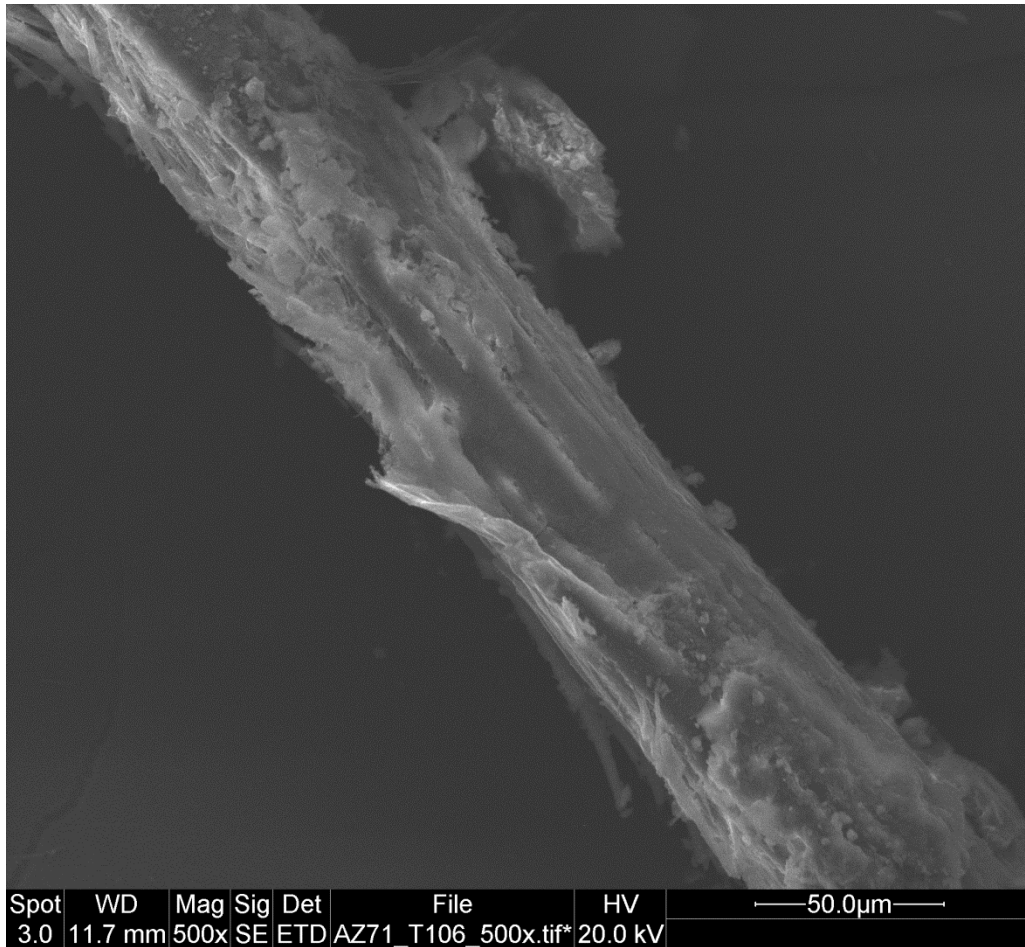
**Figure A.5.3** – Scalp hair from AZ-71 T105 (male, adolescent).

This image shows a hair that has broken. There is marked loss of cuticle, exposure of the cortex and separation of various ultrastructural components of the hair fiber. There is some adherent material on the outer layers of the hair.



**Figure A.5.4** – Scalp hair from AZ-71 T105 (male, adolescent).

The image above shows scalp hairs from the same individual as shown in Fig. A.5.2. This image shows some localised loss of cuticle and adherent material on the outer surface of the hair fiber. However, the majority of the hair structure is intact.



**Figure A.5.5** – Scalp hair from AZ-71 T105 (male, adolescent).

This image is representative of the worst damage observed in scalp hair from Azapa individuals. There is a complete loss of cuticle with exposure of the cortex. There is also adherent material on the exposed areas of the hair fiber.

## Appendix 6: GC-MS data

### A6.1. Introduction

It was noted that after 50 mg hair samples were processed for drug analysis using the acid extraction technique suggested by Valente *et al.* (1981), some Cabuza samples produced a pungent yellow-brown substance in the preparation vials. It was decided to screen the substance using gas chromatography-mass spectrometry to see what the substance was composed of. GC-MS was chosen as it was likely the substance was composed of human decomposition products, containing a range of lipids and volatile organic compounds, as well as decomposition products of carbohydrates and proteins (Dent *et al.* 2004; Forbes *et al.* 2005).

### A6.2. Sample preparation

Vials used in the preparation on the Cabuza hair samples were retained, as a number of them contained a pungent yellow-brown substance (see fig, A6.2).

The waxy substance was redissolved in 100  $\mu$ l dichloromethane (DCM) then trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA; 50  $\mu$ l) at 60 °C for 20 min before being dried under nitrogen and redissolved in 100  $\mu$ l of



**Fig. A6.1** – Yellow-brown waxy substance produced by sample processing (Image: Andrew Holland).

dichloromethane (DCM) prior to gas chromatography–mass spectrometry.

### A.6.3. GC-MS conditions

Analysis was carried out by combined gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890A Series GC connected to an 5975C Inert XL mass selective detector. The splitless injector and interface were maintained at 300 °C and 340 °C, respectively. Helium was the carrier gas at constant flow rate. The temperature of the oven was programmed from 50 °C (2 min) to 350 °C (10 min) at 10 °C/min. The GC was fitted with a 15 m × 0.25 mm, 0.25 µm HP-5ms 5% phenyl-methylpolysiloxane phase fused silica column. The column was directly inserted into the ion source where electron impact (EI) spectra were obtained at 70 eV with full scan from  $m/z$  50 to 800 (Heron *et al.* 2010). The total run time was 42 minutes.

Peaks were identified using the NIST mass spectral library.

### A.6.4. Summary of GC-MS data

Individual	Compounds present	Possible source of compound	Reference
AZ-6 TMCA3	C4 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).
	C5 dicarboxylic acid		
	C6 dicarboxylic acid		
	C7 dicarboxylic acid		
	C8 dicarboxylic acid		
	C9 dicarboxylic acid		
	Benzoic acid		



		material; putrefaction of the amino acid phenylalanine. Possible analytical artefact.	Swann <i>et al.</i> (2010).
<b>AZ-71 T127</b>	C4 dicarboxylic acid C5 dicarboxylic acid C6 dicarboxylic acid C7 dicarboxylic acid C8 dicarboxylic acid C9 dicarboxylic acid C10 dicarboxylic acid Malic acid Hydrocinammic acid  Benzoic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.  Produced by decomposing plant materials  Ubiquitous; produced by decomposing plant material; putrefaction of the amino acid phenylalanine. Possible analytical artefact.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).  Linderman (1970).  Haider and Martin (1975); Swann <i>et al.</i> (2010).
<b>AZ-71 T131</b>	C4 dicarboxylic acid C5 dicarboxylic acid C7 dicarboxylic acid C8 dicarboxylic acid C9 dicarboxylic acid C10 dicarboxylic acid Malic acid Sterols?  Indole related compound?	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.  Sterols are a component of animal fats and plant oils.  Product of proteolysis	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).  Evershed (1993).  Dent <i>et al.</i> (2004); Statheropoulos

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*et al.* (2005).

<b>AZ-71 T470</b>	C3 carboxylic acid C3 dicarboxylic acid C7 dicarboxylic acid C8 dicarboxylic acid C9 dicarboxylic acid Piperidone  Hydrocinnamic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.  Decomposition product of amino acids (lysine)  Produced by decomposing plant materials	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).  Swann <i>et al.</i> (2010).  Linderman (1970).
<b>AZ-71 T504</b>	C4 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).
<b>AZ-141 T12</b>	C4 dicarboxylic acid C5 dicarboxylic acid C6 dicarboxylic acid C7 dicarboxylic acid C8 dicarboxylic acid C9 dicarboxylic acid C10 dicarboxylic acid Benzoic acid  Hydrocinnamic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.  Ubiquitous; produced by decomposing plant material; putrefaction of the amino acid phenylalanine. . Possible analytical artefact.  Produced by decomposing plant materials	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).  Haider and Martin (1975); Swann <i>et al.</i> (2010).  Linderman (1970).
<b>AZ-141 T13</b>	C5 dicarboxylic acid C7 dicarboxylic acid C8 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).

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	C9 dicarboxylic acid	into dicarboxylic acids.	
	Benzoic acid	Ubiquitous; produced by decomposing plant material; putrefaction of the amino acid phenylalanine. Possible analytical artefact.	Haider and Martin (1975); Swann <i>et al.</i> (2010).
	Monosaccharides	Breakdown of glycogen by micro-organisms	Dent <i>et al.</i> (2004).
<b>AZ-141 T28</b>	Benzoic acid	Ubiquitous; produced by decomposing plant material; putrefaction of the amino acid phenylalanine. Possible analytical artefact.	Haider and Martin (1975); Swann <i>et al.</i> (2010).
	C6 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).
	C7 dicarboxylic acid		
<b>AZ-141 T34</b>	Acetamide	Plasticiser, probably from plastic sample bags	Ma and Yu (2004).
	C5 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).
	C6 dicarboxylic acid		
	C7 dicarboxylic acid		
	C8 dicarboxylic acid		
	C9 dicarboxylic acid		
<b>AZ-141 T54</b>	Formamide	Plasticiser	Ma and Yu (2004).
	Acetamide	Plasticiser	
	C18 saturated fatty acid	Major component of adipocere; produced by hydrogenation of oleic acid.	Dent <i>et al.</i> (2004); Forbes <i>et al.</i> (2005).
	C9 dicarboxylic acid	Breakdown of long fatty	Dent <i>et al.</i>

	C10 dicarboxylic acid	acid chains into shorter chains, then oxidation into dicarboxylic acids.	(2004); Heron <i>et al.</i> (2010).
	11-Eicosenoic acid	Naturally occurring monounsaturated fatty acid found in plant oils and human adipose tissue.	Radwan (1976); Satil <i>et al.</i> (2003).
<b>AZ-141 TTOL</b>	Formamide	Plasticiser	Ma and Yu (2004).
	Acetamide	Plasticiser	(2004).
	C2 carboxylic acid	Structurally similar compounds detected in decaying human remains	Statheropoulos <i>et al.</i> (2005).
<b>AZ-141</b>	C5 dicarboxylic acid	Breakdown of long fatty acid chains into shorter chains, then oxidation into dicarboxylic acids.	Dent <i>et al.</i> (2004); Heron <i>et al.</i> (2010).
<b>TDESC2</b>	C6 dicarboxylic acid		
	C7 dicarboxylic acid		
	C8 dicarboxylic acid		
	C9 dicarboxylic acid		
	Piperidine	Decomposition product of amino acids (lysine).	Swann <i>et al.</i> (2010).
	Benzoic acid	Ubiquitous; produced by decomposing plant material; putrefaction of the amino acid phenylalanine. Possible analytical artefact.	Haider and Martin (1975); Swann <i>et al.</i> (2010).
<b>AZ-141</b>	Formamide	Plasticiser	Ma and Yu (2004).
<b>TDESC8</b>	Acetamide	Plasticiser	(2004).
	Piperidone	Decomposition product of amino acids (lysine)	Swann <i>et al.</i> (2010).

### A. 6.5. Comment on results

The results from the GC-MS analysis indicate the presence of compounds entirely consistent with the decomposition of human and plant remains. Figures A6.1, A6.2 and A6.3 illustrate examples of TIC chromatograms from this set of analyses.

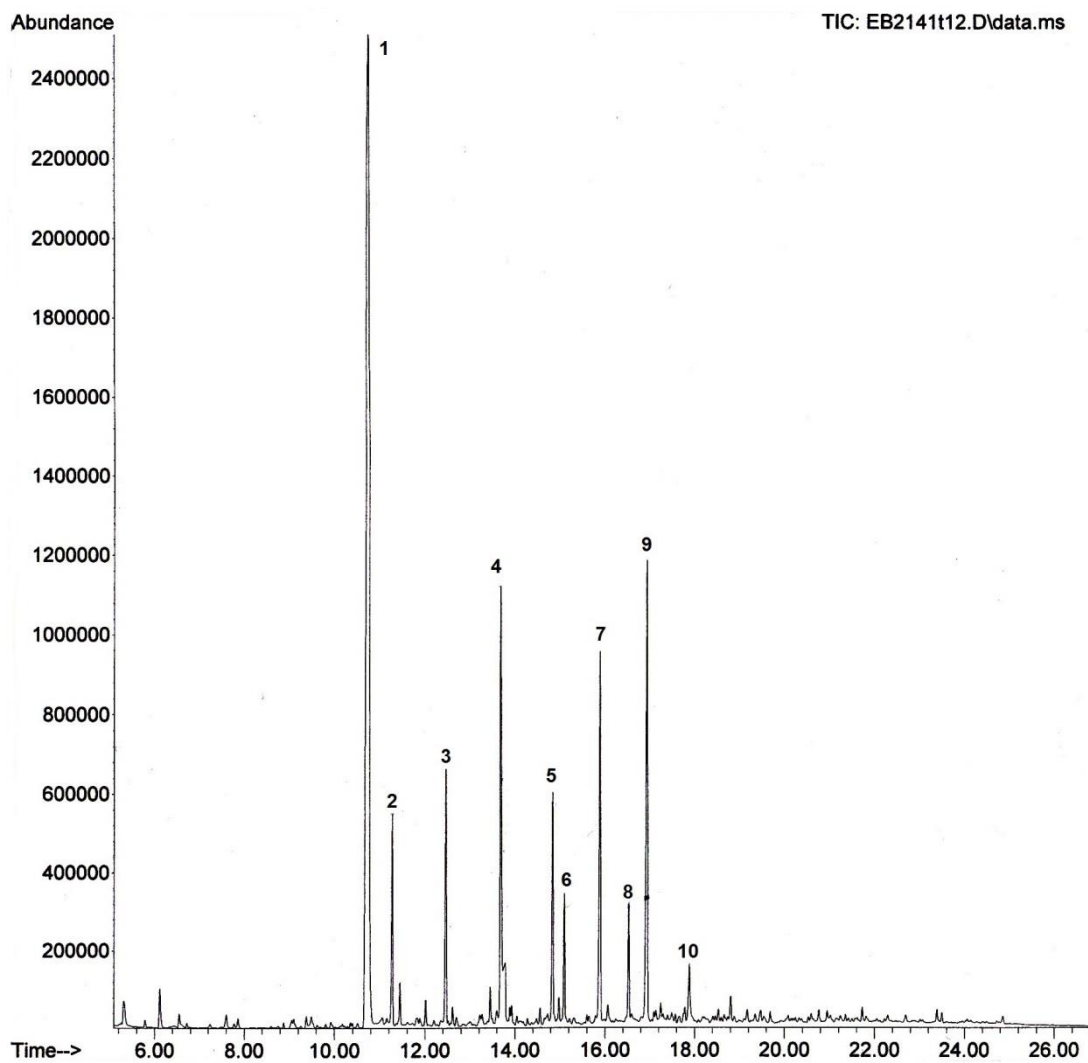
The most common compounds detected in these samples are dicarboxylic acids. These are predominantly shorter chain molecules (range from 3-10 carbon atoms) probably formed from the breakdown of longer unsaturated fatty acids. The unsaturated fatty acids oleic acid, linoleic and palmitoleic acid are major components of human adipose tissue (Dent *et al.* 2004). Oxidation of carbon-carbon double bonds in these unsaturated fatty acids produces dicarboxylic acids (Regert *et al.* 1998; Heron *et al.* 2010). Temperature accelerates this reaction. Given that these individuals were buried in a hyperarid climate, it is likely that environmental conditions, relatively high ambient temperatures over a very long period of time, contributed to the formation of these compounds Regert *et al.* (1998). In addition, the sample from AZ-71 T131 yielded two sterols. Sterols, such as cholesterol, are components of human and animal fats. Plants also contain sterols, the major sterols being campesterol and sitosterol (Evershed 1993). A saturated C18 fatty acid (18:0) (Stearic acid) was detected in AZ-141 T54. Stearic acid is a major component of adipocere, and is a well-documented product of the hydrolysis of triglycerides (Forbes *et al.* 2005; Pinheiro 2006). In addition, 11-eicosenoic acid, a monounsaturated fatty acid (20:1) was also detected in this sample. This fatty acid is present in oils and fats from plants (Radwan 1976; Satil *et al.* 2003), but is also present in trace amounts in human adipose tissue (Notter *et al.* 2009). It has also been detected as a component of adipocere (Nushida *et al.* 2008).

Decomposition products of proteins were also detected in some of the samples. Benzoic acid was detected in five samples. Benzoic acid (as well as phenylacetic acid and phenylpropionic acid) is produced when the amino acid phenylalanine undergoes putrefaction (Swann *et al.* 2010). However, the presence of this components is sometimes as an analytical artefact. Piperidone and piperidine have been found to be decomposition products of cadaverine (Swann *et al.* 2010). Cadaverine is a foul smelling volatile amide produced by the decarboxylation of the amino acid lysine (Dent *et al.* 2004; Swann *et al.* 2010). An indole-related compound was detected in AZ-71 T131. Indole is produced in advanced stages of proteolysis, after proteins have been broken down into shorter polypeptides and amino acids. These compounds are broken down further into indole and skatole (Dent *et al.* 2004; Statheropoulos *et al.* 2007).

Benzoic and cinnamic acids are produced by decomposing plant materials (Linderman 1970; Haider and Martin 1975). Many of the individuals buried in the Azapa Valley would have had some plant materials (e.g. tubers, maize, squashes/gourds, coca leaves) included as part of their grave goods, and other plant materials may have been used in grave construction, for example reeds (Focacci 1990). The decomposition of these materials may be responsible for the presence of benzoic and cinnamic acids.

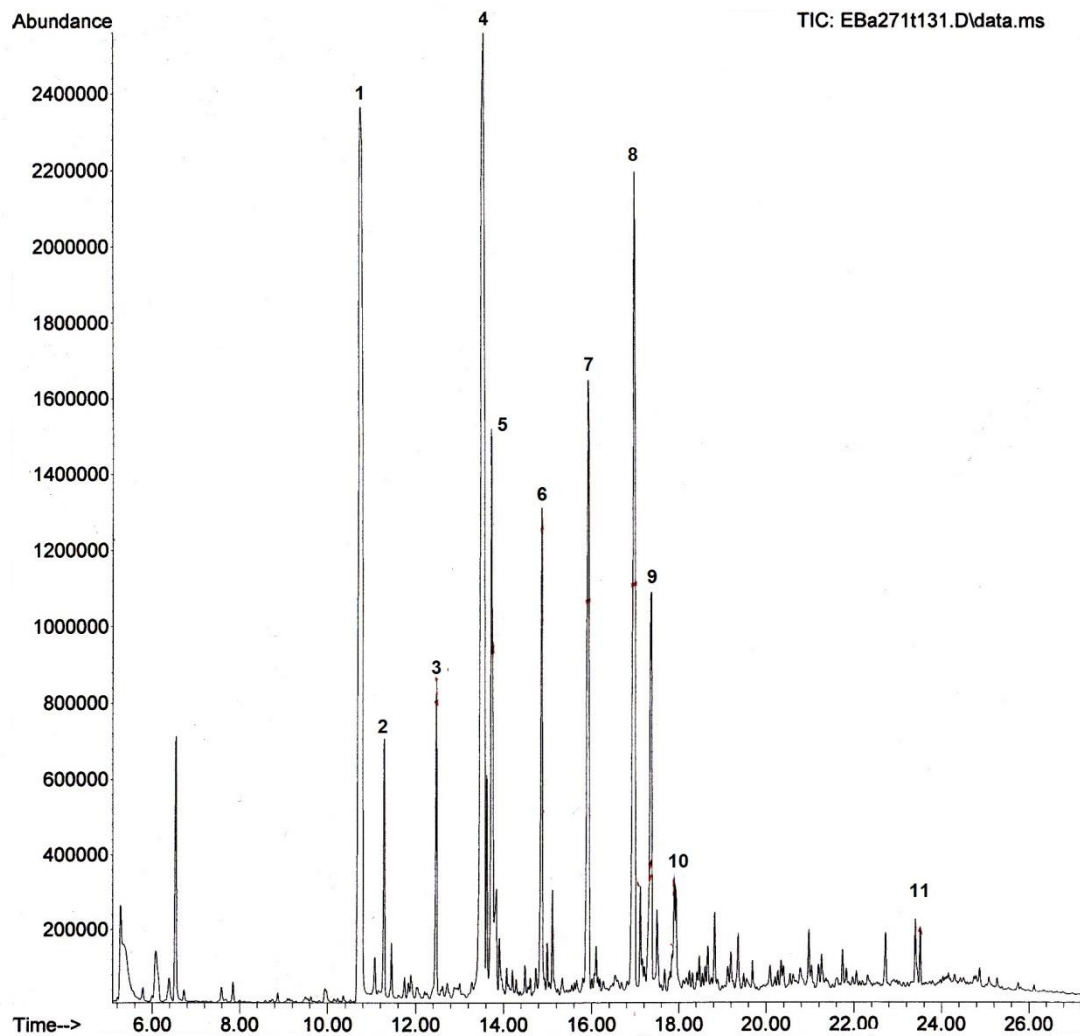
Monosaccharides were detected in the sample from AZ-141 T13. These compounds are probably a result of the breakdown of carbohydrates into smaller sugar molecules. This could be a result of the breakdown of carbohydrates such as glycogen from body tissues or decomposing plant material (Dent *et al.* 2004).

The plasticisers present, formamide and acetamine, are present probably as a result of the hair samples being stored in plastic packaging for almost 30 years.

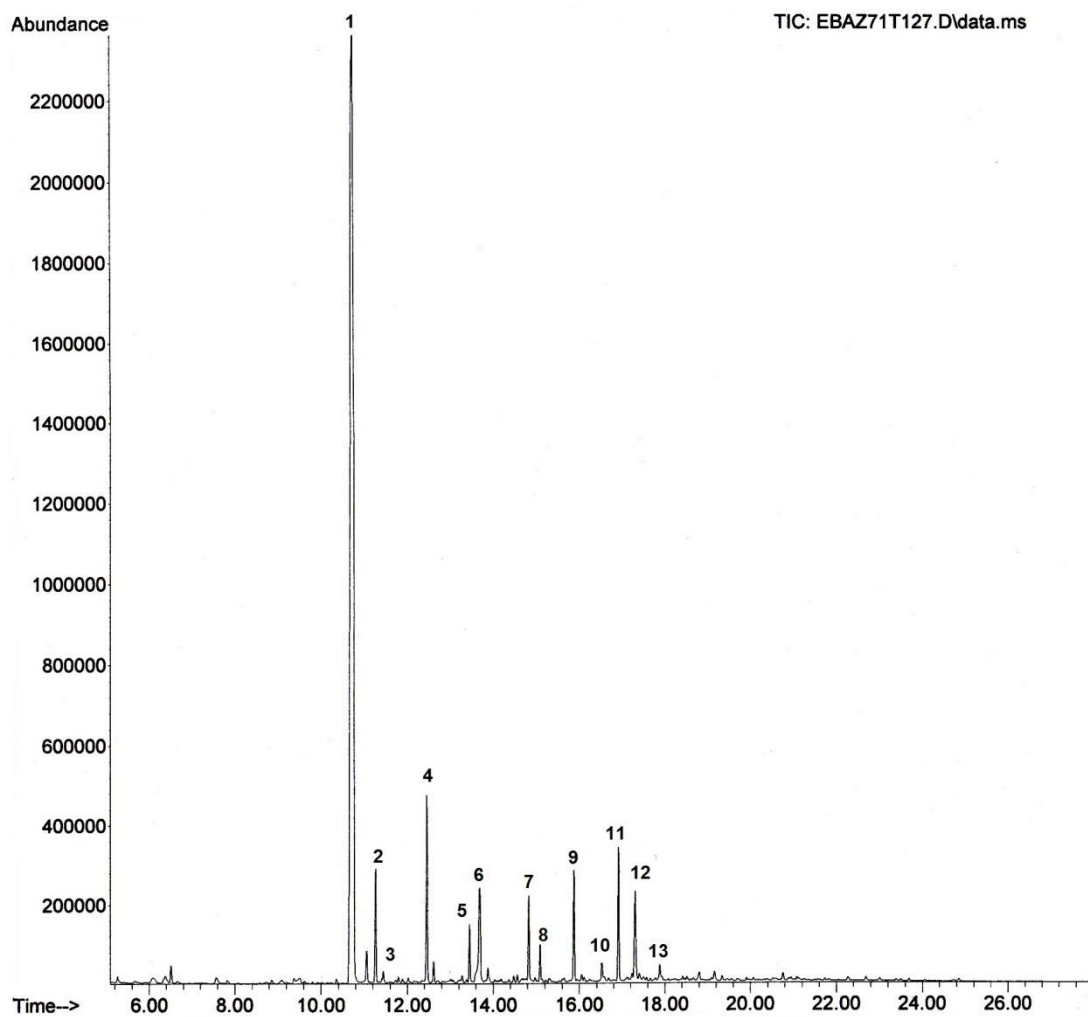


**Fig A6.1** – TIC for AZ-141 T12. Peaks identified: 1 – plasticiser; 2 – C4 DCA; 3 – C5 DCA; 4 – C6 DCA; 5 – C7 DCA; 8 – hydroxycinnamic acid; 9 – C9 DCA; 10 – C10 DCA





**Fig A6.2** – TIC for AZ-71 T131. Peaks identified: 1 – plasticiser; 2 – C4 DCA; 3 – C5 DCA; 4 – malic acid; 5 – C6 DCA; 6 – C7 DCA; 7 – C8 DCA; 8 – C9 DCA; 9 – indole-related compound; 10 – C10 DCA; 11 – sterols



**Fig A6.3** - TIC for AZ-71 T127. Peaks identified: 1 – plasticiser; 2 – C4 DCA; 3 – C5 DCA; 4 – C6 DCA; 5 – malic acid; 6 – C6 DCA; 7 – C7 DCA; 8 – benzoic acid; 9 – C8 DCA; 10 – hydroxycinnamic acid; 11 – C9 DCA; 12 – indole related compound; 123 – C10 DCA.