



Can phytoliths from *Sorghum bicolor*, a C₄ plant, be indicators of past water availability?

A dissertation submitted by

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A thesis submitted in partial fulfilment of its requirements for Masters
by Research in Archaeology

Bournemouth University

September, 2018

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Can phytoliths in C₄ plants indicate past water availability?

Lea Predanich

Abstract

The reconstruction of past water availability is vital to our understanding of past societies. One of the principal requirements for early state societies is a complex farming system employing irrigation. Early irrigation regimes are likely to have been ephemeral and so would no longer be visible in the archaeological record. C₃ plants have been the focus of phytolith research regarding past water availability. Research has proven that phytoliths from C₃ plants can indicate past water availability (Madella *et al.* 2009; Weiskopf *et al.* 2014; Jenkins *et al.* 2016). However there has not been a study focusing on phytoliths in C₄ plants such as sorghum. This study will determine whether or not the C₄ plant sorghum can indicate past water availability. Sorghum grown from three different sites in Jordan, Deir 'Alla, Ramtha and Salt which has been grown over a period of two years (2009-2010) was taken for phytolith processing. Statistical analyses was undertaken to assess whether variables such as climate, soil and water chemistry affect silica deposition adding further resolution to this discussion. Results show that there were unknown factors that influenced phytolith production between sites and years. However irrigation was found to be the biggest influence in determining the ratio of fixed (short) to sensitive (long) phytolith forms. Samples with a mean fixed to sensitive ratio of above 3.5 are likely to have been unirrigated while samples with a mean short to long form ratio of less than 1.7 are likely to have been irrigated. The most striking observation is that husks have a smaller ratio of fixed to sensitive forms and hence have more sensitive phytolith forms than the leaves and stems. The confidence interval is small for irrigated husks and demonstrates that anything under 0.2 is likely to have been irrigated whereas anything above 0.2 is likely to have been unirrigated. This study demonstrates that phytoliths from sorghum can be used to identify past water availability.

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Acknowledgements

I would like to first thank my thesis advisors Dr Emma Jenkins and Dr Kate Welham for their support and guidance during the research and writing of this thesis. I am gratefully indebted to their valuable comments on this thesis. Thank you to Rick Stafford for his assisting me with statistical analysis and Damien Evans for always helping when I had an issue. A final thank you to my family and loved ones, for their continual supporting me through a very challenging period in my life.

1. Introduction

Water availability and water management have been critical to the ecology of prehistoric agriculture (Madella *et al.* 2009). It has been recognised that one of the principal requirements for early state societies is a complex farming system employing labour-irrigation (Rosen and Weiner, 1994). However such systems are often difficult, if not impossible to recognise in the archaeological records, particularly in prehistoric communities when water management began (Jenkins *et al.*, 2016). This could be because of the destruction of ground features such as canals, ridged fields, and dams (Rosen and Weiner, 1994). Another factor is that early irrigation systems are likely to have been ephemeral, and as such, would no longer be visible in the archaeological record (Jenkins *et al.*, 2016). Various palaeoclimatic proxies can be used to reconstruct past rainfall (Lowe and Walker 1997; Bell and Walker 2005). It is difficult to show if climatic changes, which are reflected in palaeoclimatic indicators, are truly contemporaneous with socio-economic changes visible in the archaeological record, and even more so to demonstrate that one was the cause for the other (Flohr 2012). Therefore it is most beneficial to establish an additional palaeo-environmental proxy which could be directly linked to archaeological evidence.

Previous studies have shown that phytoliths can be used to detect the level of past water availability in C₃ plants (Madella *et al.* 2009; Flohr 2012; Weisskopf *et al.* 2014; Jenkins *et al.* 2016). However there has not been a study focusing on phytoliths in C₄ plants such as maize, sorghum and pearl millet. Mixed plant assemblages on an archaeological site specific features or contexts are the norm. Many societies use both C₃ and C₄ plants which could cause a confused and diluted record.

Phytoliths are microscopic, crystal-like structures composed mostly of silica dioxide (SiO₂) (Hart 2015) which is absorbed by the plant in a soluble state from the ground water (Piperno 2006). In solution, silica exists as monosilicic acid (H₄SiO₄) that is transported upwards in the vascular system and becomes concentrated during transpiration around the leaf stomata (Carter 2007). The supersaturated solution begins to gel, solidify and form into solid opaline silica (SiO₂:nH₂O) bodies (phytoliths) within and between some of the plant cells (Carter 2007, p.7). Other

elements of phytolith composition include small amounts of aluminium, iron, titanium, magnesium, phosphorus, copper, nitrogen and carbon (Hart 2015). The term Phytolith is derived from the Greek words *phyto* meaning plant and *lith* meaning stone (Hart 2015). Phytoliths have been researched under various names such as opal phytoliths, silica phytoliths, plant opal, biogenic opal or simply phytoliths (Piperno 2006). Since phytoliths are inorganic they are highly resistant to diagenesis making them useful to the archaeologist and palaeoecologist.

1.1 Aims and objectives

The aim of this research is to:

- Validate whether or not phytoliths in sorghum, a C₄ plant, can be indicators of past water availability.
- Explore how valid this method is when comparing the results with C₃ plants

These aims are reached by:

- To determine the relationship between fixed (short) to sensitive (long) phytolith forms and water availability in relation to various other environmental factors such as temperature, evaporation and relative humidity from sorghum.
- Run statistical analysis to determine whether irrigation and environmental variables affect the results.
- Analysis of the fixed to sensitive phytolith forms and water availability from Deir 'Alla, Salt and Ramtha. All three sites are located in Jordan, (1) Deir 'Alla, which is in the Jordan valley; (2) Salt, which is located 30km south of Deir 'Alla; and (3) Ramtha, which is in the north of Jordan, 5 km from the Syrian border.
- Comparing results to previous studies of this relationship in C₃ plants.

2. Literature Review

2.1 Phytolith Composition and Characteristics

Phytoliths are present in almost all plant parts such as the stems, leaves, roots and inflorescences (Carter 2007, p.8). In general, more phytoliths form in the above ground tissue than below, but in some plants phytoliths are spread equally throughout. In the case of silica accumulating herbaceous plants, such as sedges and grasses, studies have shown that a higher phytolith content may occur in the fruits and the epidermis of bracts which surround and protect the grass seeds (Bozarth 1992; Lentfer 2003; Piperno 1988). Lanning and Eleuterius (1981) study demonstrates that leaves accumulate more than twice as much silica as stems, and the roots of the plants are found to accumulate the least amount of silica in vegetative parts of herbaceous plants. Silica uptake and deposition is influenced by the rate of transpiration which in turn is dependent on water availability. There are substantial differences in the silica concentration amongst plants. Hutton and Norrish (1974) found a correlation between water transpired by barley grown in controlled greenhouse conditions and the silica content of the plant. Results of that study included wheat and further showed that the amount of silica found in the husks was proportionate to water transpired. Jones et al. (1963) established that silica in oat plants could be calculated from the concentration of Si in the amount of water transpired. Patterns of phytolith formation appear to be consistent within families and species though some species produce more phytoliths than others, for example rice (Lentfer 2003). Only a fraction of the global flora has been investigated for the presence of phytoliths (Carter 2007).

There are some plants that produce either few or no identifiable phytoliths (Piperno 1988). Some species have been identified as silica rejectors such as tomato and faba bean which actively prohibit the uptake of silica (Jenkins *et al.* 2011). Tsartsidou et al. (2007) found that woody species, legumes and fruits of dicots produced only small amounts of phytoliths per unit tissue dry weight, while leaves of trees and shrubs produce significant amounts of phytoliths and that grasses are prolific phytolith producers. It was also found that much of the bark phytoliths are clearly contaminants (Tsartsidou *et al.* 2007). In addition to this phytoliths produced by

woody species have irregular or variable morphologies (Tsartsidou *et al.* 2007). Schiegl *et al.* (1996) deduced that the lack of phytoliths from calcitic ash (often found in hearths) in prehistoric caves is the result of an alkaline environment produced by calcite. At high pH levels the phytoliths are less stable and may have dissolved (Schiegl *et al.* 1996).

2.2 Understanding the difference between C₃ and C₄ plants

Phytoliths form in both C₃ and C₄ plants. Both use the process of photosynthesis to convert light to energy and atmospheric CO₂ into plant food energy (carbohydrates) (Strömberg and Mclerney 2011). The plants differ in the leaf anatomy and enzymes used to carry out photosynthesis (Strömberg and Mclerney 2011). These differences are important with respect to their optimal growing conditions, nitrogen and water-use efficiency, forage quality, and seasonal production profile (Strömberg and Mclerney 2011).

C₃ plants are associated with temperate or cool-season plants (Betts, n.d). C₄ plants dominate the warm climate and are relatively rare in cool climates characteristic of high altitudes or high elevations (Kubien *et al.* 2003). C₄ plants such as maize, sorghum, and sugarcane, approximately have 50% higher photosynthesis efficiency than those of C₃ plants such as rice, wheat and potato (Wang *et.al*, 2012). This is because there is a different mechanism of carbon fixation by the two types of photosynthesis (Wang *et.al*, 2012). C₃ photosynthesis uses the Calvin cycle for fixing CO₂ catalysed by ribulose-1,5-bisphosphate carboxylase (RuBisCo), this takes place inside of the chloroplast in mesophyll cell (Wang *et.al*, 2012).

In all plants CO₂ is fixed by the enzyme RuBisCo (Gowick and Westhoff, 2010). The first step is the reaction between CO₂ and RuBisCo forms two molecules of phosphoglycerate which is a 3-carbon molecule (Ehlereinger and Cerling 2002). This explains why plants that use this pathway are called C₃ plants. The C₄ plants, in addition to the C₃ cycle, use an additional reaction pathway called the C₄ cycle (Gowick and Westhoff, 2010). They reduce carbon dioxide captured during photosynthesis to useable components by first converting carbon dioxide to

oxaloacetate, a 4-carbon acid, hence their name - C₄ plants (Betts, n.d). At moderate temperatures of 20-30°C, RuBisCo in C₄ plants encounters CO₂ concentration close to saturation, and the potential for photorespiration is low (Sage 2002). By contrast, CO₂ concentrations in C₃ plants at 20-30°C are near the K_m for CO₂ of RuBisCo, and photorespiration can be high (Sage 2002). As a result of the high CO₂ environment that allows RuBisCo to function near CO₂ saturation, C₄ species at 20-30°C require one-third to one-quarter as much RuBisCo as C₃ species to maintain a given CO₂ assimilation rate (Sage 2002). This reduced requirement explains the 60-80% lower RuBisCo content of C₄ plants relative to C₃ species of similar life form (Sage 2002). Even though the C₄ photosynthetic system is more complex than the C₃ system, the ultimate limitation to CO₂ fixation for both photosynthetic types is the activity of RuBisCo (Kubien *et al.* 2003).

C₃ plants have an optimum temperate range of 18-24°C. The plant starts to grow with the soil temperature reaches 4-7°C (Strömberg and Mclerney 2011). As the temperature increases C₃ plants become less efficient (Strömberg and Mclerney 2011). They do however provide a higher percentage of crude protein than C₄ plants (Strömberg and Mclerney, 2011). C₃ grasses are more efficient in the cool temperature of spring and fall with shorter photoperiods and higher soil moisture (Strömberg and Mclerney 2011). When summer comes there is a decrease in growth and dormancy is caused by high temperatures and low precipitation (Strömberg and Mclerney 2011).

C₄ plants grow best at 32-35°C. They begin to grow when the soil temperature is 15-18°C (Strömberg and Mclerney 2011). The unique biochemical and structural differences found in leaves of C₄ species allow for more efficient use of nitrogen (N) when compared to C₃ species due to increased catalytic efficiency of RuBisCo (Byrd *et.al*, 1992). Though C₄ leaves have lower levels of nitrogen (N) and especially RuBisCo it is suggested that there are no obvious differences in maintenance requirement between C₃ and C₄ plants (Byrd *et.al*, 1992). It is suggested that the growth and maintenance of tissue is more dependent on the composition of biomass than on the species or photosynthetic pathway (Byrd *et.al*, 1992).

Both C₃ and C₄ plants can be annual or perennial (Strömberg and McIerney 2011). Annual C₃ plants include wheat, rye and oats while perennial C₃ plants include orchardgrass, fescues, and perennial ryegrass (Strömberg and McIerney 2011). Annual C₄ plants include corn, sudangrass, and pearl millet while perennial C₄ plants include big bluestem, indiagrass and switchgrass (Strömberg and McIerney 2011).

2.3 Silica in plants

Silica (Si) is the second largest element after oxygen in soil (Ma and Yamaji, 2006). The Si content of soils can vary dramatically from <1 to 45% dry weight (Sommer *et.al*, 2006 as cited in Currie and Perry, 2007). Silica dioxide comprises 50-70% of the soil mass (Ma and Yamaji, 2006). There is no evidence that shows Si is involved in the metabolism of plants, as a result of this, Si has not been recognised as an essential element for plants (Ma, 2009). However, studies have shown that Si deficiency in soil is a factor for poor crop production, especially in soils that are regarded to be low or limiting in plant available Si and for known Si-accumulating plants such as rice and sugarcane (Ma and Yamaji, 2006). Si in plants has also shown an enhanced plant resistance to various abiotic stresses, including drought, salinity, heat, cold, metal toxicity, lodging and nutrient imbalance (Ma and Yamaji, 2015). The beneficial effects of Si characteristically differ with the plant species (Ma *et.al*, 2001). Furthermore Si is the only element that does not damage plants when accumulated in excess due to its properties of un-dissociation at physiological pH and polymerization (Ma *et.al*, 2001).

Since phytoliths are composed of amorphous silica and organic matter in varying amounts, when removing the organic matter (e.g. through acid extraction, burning or breakdown processes in the soil) the remaining structure is likely to be porous (Hodson, 2016). There is now a question of the porosity of the lumen deposits as they do not appear to be laid down onto a carbohydrate matrix in the same way as the wall deposits (Hodson, 2016). Though studies show that lumen phytoliths show obstructions within their structure (Hodson, 2016). If cell wall phytoliths do show greater porosity this also suggests that they may breakdown more easily in the soil, sediments and archaeological contexts (Hodson, 2016). It has also been suggested

that greater porosity leads to greater fragility which is possibly a more important factor than dissolution rate (Hodson, 2016).

2.3.1 Silica Uptake in plants

The form of Si that is taken up by plants in soil waters is silicic acid [Si(OH)₄], a very weak acid (pka >.9.5) (Currie and Perry, 2007). The entry of Si(OH)₄ into the roots most likely takes place following water, via either an apoplastic (space outside the plasma membrane) or symplastic (inner side of plasma membrane) route (Geurriero *et al.* 2016). The ability of a plant to accumulate Si varies greatly between species, ranging from 0.1 to 10.0% Si (dry weight) (Currie and Perry, 2007). Cornelis *et al.* (2011) described the different mechanisms by which the Si is absorbed by plants, i.e active, passive and rejective (Tubuna and Heckman, 2015).

The amount of uptake of silica by the active mechanism is typically larger than that predicted based on the mass flow and is attributed to the density of silica transporters in the roots and shoots that facilitate the absorption process across the membranes of root cells (Tubuna and Heckman 2015). This is seen in the study by Ma and Yamaji (2006) who used plants which accumulate different levels of Si; rice (high); cucumber (medium) and tomato (low). It was suggested that the density of the transporter differs amongst plant species because the transport process is energy dependent (Ma and Yamaji, 2006). The next step in this process is the transportation of Si from cortical cells to the xylem (Ma and Yamaji, 2006). Results found that the xylem holds a considerable higher amount of Si concentration in rice than it did in cucumber and tomato (Ma and Yamaji, 2006). In addition, unlike in rice, where xylem loading of Si is achieved by a transporter, xylem loading is achieved by diffusion in cucumber and tomato (Ma and Yamaji, 2006). Results show that the most important determinant for a high level of Si to accumulate in rice shoots is xylem loading (Ma and Yamaji, 2006). The lower density of the transporter to transport Si from the external solution to the cortical cells, and a defective or absence of transporter to transport Si from cortical cells to the xylem could explain the much lower accumulation of Si in cucumber and tomato (Ma and Yamaji, 2006). The amount of uptake of silica by the plant via the passive mechanism is likely entirely driven by

mass flow (Tubuna and Heckman, 2015). In the rejective mechanism, the build-up of the concentration of H_4SiO_4 in the soil solution typically results from the low concentration of silica that are absorbed by plants (Tubuna and Heckman, 2015).

While in the shoot, with water loss through transpiration, silicic acid is concentrated and condensed into a hard polymerized silica gel ($SiO_2 \cdot nH_2O$), also known as a phytolith, and finally deposited in the different tissues (Ma, 2009). The phytoliths are found in specific cells, e.g. the silica cells, which are in vascular bundles and in silica bodies in bulliform cells, fuscoid cells or prickly hairs in rice, wheat and bamboo (Tubuna and Heckman, 2015). It is known that there are two Si transporters, Lsi1 and Lsi2 (Hodson, 2016). Ma and Yamaji (2015) documented a number of other Si transporters that are involved at the nodes of plants, they also point out that there should be transporters, as yet unknown, that are involved in the concentration of silica in cells where deposition takes place (Hodson, 2016).

The plants that rely primarily on active, passive or rejective mechanisms are classified as high, intermediate, or non-accumulators (Tubuna and Heckman, 2015). The plants in the high-accumulation category in the Tubuna and Heckman (2015) study had a Si content in the shoot that ranged from 1.0% to 10% dry weight and were primarily monocotyledons such as bamboo (*Bambuseae*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), and wheat (*Triticum aestivum*) (Tubuna and Heckman, 2015). The intermediate-accumulation category were mostly dryland Gramineae with shoot contents that ranged between 0.5% and 1.5% dry weight (Tubuna and Heckman, 2015). The low-accumulation group were dicots which accumulated <0.2% shoot dry weight silica (Tubuna and Heckman, 2015).

2.4 Phytolith Function in Plants

Phytoliths were treated as waste products by many scientists until recently, solid secretions of soluble silica that was taken up without a purpose by plants (Piperno 2009, p.2). However this view changed when studies of other different kind of plants such as grasses, oats and bamboos, showed that there was a purpose to the active

uptake of silica (Kaneko 1995; Jones *et al.* 1963; Ueda 1961). Now it is believed that silica in plants has a variety of important functions. Phytoliths might serve protective, structural, and physiological functions. Phytoliths are an energetically inexpensive form of structural support when compared to carbon-based compounds, this creates opportunity for silica to produce robust foliage more quickly and compete more efficiently for light or space (Stromberg *et al.* 2016). The same genes that regulate the production of lignin also regulate phytolith development. Lignin is a plant tissue that serves as a defensive mechanism by giving the plants a rigid silica structure it makes them more difficult to consume and digest, (Hart 2015). If there is a high concentration of phytoliths in a plant it could make them harder for herbivores and pathogens to break down and digest (Hart 2015). The silica-enforced cell walls may also prevent cell collapse during droughts which would make phytolith-based support especially beneficial in seasonally arid environments (Coughernour 1985). They also play an important role in physiology by mitigating the toxic effects of aluminium and other heavy soil minerals, which would otherwise cause damage to the plant (Piperno 2009).

2.5 Phytolith deposition in soils and sediments

Phytoliths are deposited into the soil in one of three ways. The first is that a large percentage of phytoliths are deposited directly into the ground after a plant dies and decays (Hart 2004). Secondly, some phytoliths are deposited when plants are burned (Hart 2004); this process sometimes allows phytoliths to be transported great distances because of the ash and air currents (Rapp and Mulholland 1992). Finally, some phytoliths are deposited into the soil with the waste products of the original digested plant material by animals (Hart 2004). These depositional patterns, in conjunction with other phytolith characteristics, make silica bodies extremely useful in archaeological studies (Hart 2004). Since phytoliths are inorganic and are highly resistant to diagenesis they remain in the sediment for tens of thousands of years (Rosen and Weiner 1992). Released phytoliths become microfossils of the plant that formed them. Phytoliths can be collected from a variety of sources such as paleosols, tooth tartar, coprolites, ceramics, tools and bricks (Portillo *et.al*, 2006, p).

Phytoliths are typically found in archaeological deposits situated in many environmental settings that range from rain forests to deserts (Rosen and Weiner 1992).

2.6 Classification

The basis of phytolith analysis is the understanding of how the shapes of phytoliths extracted from sediments and seen under a microscope can be related to the species or family of plants in which they formed (Carter 2007). Phytoliths can be very hard to identify taxonomically because typically, when produced by closely related taxa, they are very similar to each other (Portillo *et.al*, 2006). The lack of representation in some phytolith assemblages is another problem, because as mentioned above some plants produce few to no phytoliths. It has been shown that morphometric analysis is an effective tool for discriminating among phytoliths produced by closely related taxa (Portillo *et.al*, 2006). Morphometric analysis has successfully been used to distinguish between rice and wild *Oryza* phytoliths (Pearsall *et al.* 1995; Zhao *et al.* 1998). Paradigms were developed using morphometric analysis to distinguish between inflorescence phytoliths produced by several species of wheat and barley although this method has not been widely adopted in the phytolith community (Ball *et al.* 1999).

There was a problem in concern to the discipline of phytolith analysis which was the lack of a universally applicable key that allowed for the description, identification and classification of phytoliths (Dominique Meunier and Colin, 2001). So different researchers would call the same phytolith form by a different name and this was exacerbated by the fact that researchers often did not include images of the phytoliths in their assemblage. The attempt to correlate the multitude of keys thus produced was proving to be difficult as there was a lack of standardised nomenclature (Dominique Meunier and Colin, 2001). As more researchers entered the field of phytolith research the need to develop a standardised phytolith key become more urgent (Dominique Meunier and Colin, 2001). A discussion on the terminology and classification of phytoliths arose in August 2000 during the 3rd International Meeting on Phytolith Research (IMPR) in Brussels (Madella *et.al.* 2005,

p.253). It was agreed between delegates that there was a need to harmonise and standardise the naming and description of phytoliths which would improve communication between researchers (Madella *et.al.* 2005, p.253). The result of this is the “International Code of Phytolith Nomenclature 1.0” (Madella *et.al.* 2005).

The code proposes that a particular phytolith type should be formed by a maximum of up to three descriptors (Madella *et.al.* 2005). The first descriptor describes the shape which can be 2D or a 3D, whichever is more indicative. The second should describe the texture and/or ornamentation and whether it is characteristic or diagnostic. The third descriptor should be the anatomical origin when this is clear and beyond doubt (Madella *et.al.* 2005, p.254).

2.7 History of Phytolith Research

Piperno (1988) divides the history of phytolith research into four phases. Phytolith research began with the discovery of calcium phytoliths during the early years of light microscopy in 1675 by Loeuwenhoek (Rapp and Mulholland 1992). Many years went by without any advance. It was in 1835 when the “discovery and exploratory stage” happened. The first published report on phytoliths, which was the first to observe silica phytoliths in living plant tissue, was written by a German botanist named Struve (Piperno 2006). One year later, Christian Gottfried Ehrenberg, another German scientist, who was one of the leading figures of phytolith analysis developed the first classification system for phytoliths (Piperno 2006). Aside from these initial discoveries, phytolith research was only used intermittently until the turn of the 19th century.

The time between 1895 to 1936 phytolith research was going through a “botanical phase” were it centred on understanding comparative plant physiology and phytolith formation (Hart 2016). From 1900 until 1936, German scientists such as Netolitzky dominated the field of phytolith studies and produced many reports relating to production, taxonomy, intraspecific variation, and dispersion techniques (Hart 2004). Most of their studies involved analysis of phytoliths in the grass family as well as a few other monocotyledons (Hart 2004). It was also during this time that large

numbers of tables and charts of silica producing plants were tabulated. However, the onset of World War Two halted phytolith research in Germany and the rest of Europe (Hart 2004). The third phase of phytolith research did not begin until sometime after the end of the Second World War. (Hart 2004).

From the 1950's, the quantity of published research articles on phytolith research and volume of new applications for phytolith data expanded rapidly (Pearsall and Piperno 1993). During this period the focus of phytolith research had expanded into the United Kingdom, the United States and Japan. The period between 1955 and 1975, the "Ecological Research" phase, researchers used phytoliths in soils to index past environmental histories (Hart 2016). There was a renewed interest in phytoliths when a number of studies disproved the previous view that phytoliths lasted only 1000 years and can only be found in certain contexts. This increased recognition led to the discovery of phytoliths in other contexts, including calcareous Wisconsin-age loess and till, sedimentary rocks and other geologic materials of Cretaceous, Tertiary, and Quaternary age, deep sea cores and atmospheric dusts (Piperno, 1988). In an attempt to investigate past ecosystems, researchers expanded their research into nonmonocotyledonous species such as those of the coniferous and deciduous trees (Hart 2014). A paper by Rovner published in 1971 is widely credited with bringing wider attention to the use of phytoliths for archaeological research (Hart 2016).

Between 1978 and 2000 is the "modern period of archaeological phytolith research" when phytolith research was established as an independent and important area of archaeology (Piperno 1988). A large number of projects using phytoliths started to focus on reconstructing past environments and discovering the origins and escalation of agriculture in societies (Hart 2016). This research was popular across the globe resulting in the creation of the International Phytolith Society in 1996 (Hart 2016). As mentioned above (p.17) were conferences focused on phytolith method and theory (e.g Madella *et.al.* 2005).

The number of phytolith studies continues to rise. It has developed into an established methodological tool for answering numerous archaeological and paleoenvironmental questions (Neumann et al. 2016). Phytolith papers appear

regularly in a wide variety of journals that range from publication with broad audiences to niche specialist papers like *The Phytolitharian* (Hart 2016). In a special volume of the journal 'Vegetation History and Archaeobotany' (VHA) emphasis was put on the rapidly increasing importance of phytoliths in archaeology (Neumann et al. 2016). It was one of the outcomes of the 9th International Meeting for Phytolith research (IMPR) (Neumann et al. 2016) whose main theme was "Towards integrative phytolith research" (Neumann et al. 2016). Scholars have broadened the diversity of research topics that can be studied through phytolith analysis by examining phytolith production in previously untested, yet potentially important plant taxa (Hart 2016). Prior to this period some of the most important research focused on the origins and intensification of agriculture which was largely limited to the Lowland Neotropics, Southwest Asia and to a smaller extent Southeast Asia and Africa (Hart 2016). In the new millennium research has come to refine some of these identifications and expanded to new crops such as *Setaria* and *panicum millets* (Lu et al. 2009). This research will further our understanding of the human past.

2.8 Phytoliths used in Paleoenvironmental Reconstruction

Phytolith analysis offers the potential to reconstruct paleoenvironments in all continents and most regions of the world (Barboni et al. 1999). Since phytoliths are made from silica they are deeply resistant to decomposition in most environments and therefore are especially beneficial where there is a paucity of alternative climate and environmental proxies (Carter 2015). Environments where phytoliths are not resistant to decomposition are areas with heavily alkaline soils which severely affect opal phytolith preservation leading to decay (Rovner 1971). While it is difficult to distinguish pollen produced by different grass species, phytoliths are able to determine the composition of paleoenvironments dominated by grasses (Carter 2015). Phytoliths can be found in sedimentary deposits from many differing environments, providing the method of extracting proxy environmental and climatic information from Quaternary sediments (Carter 2001).

Phytoliths have been used to record changes in precipitation. Alexandre et al. (1997) reconstructed the African tropical grassland history during the late Holocene with

phytolith analysis. The samples were taken from three sites and interpreted on the basis of modern phytolith assemblages from the same regions and compared to pollen data obtained previously (Alexandre *et.al.* 1997). The results showed that in one of the sites it had its driest phase of the Holocene era between 12 000 and 4000 B.P., initiating the opening of the dense forest and its replacement of short grass savannah (Alexandre *et.al.* 1997). Wetter climatic conditions developed from ca. 1000yr B.P., as presented by the establishment of tall grass savannah woodland (Alexandre *et.al.*1997).

Barboni *et.al.* (1999), took Holocene and Pleistocene sediments from an arid tropical region in Ethiopia and analysed them for their phytolith content. The study was a test on the usefulness of phytolith assemblages to indicate paleoenvironments at Middle Awash in Ethiopia, where fossil pollen grains are poorly preserved in sediments that produced abundant archaeological remains (Barboni *et.al.* 1999). The results showed that Holocene and Pleistocene sediments contained well-preserved and different phytolith assemblages which showed that no movement occurred between the modern soil and geological strata therefore proving that phytolith assemblages are valid tools for investigating past environments (Barboni *et.al.* 1999). The study demonstrated that modern phytolith assemblages enable the differentiation between sub-desertic steppe from wooded riparian forest, and characterise the composition of the C₄ grass associations (Barboni *et al.* 1999). It was found that the phytolith assemblage of the Holocene sample records C₄ *Chloridoideae* grassland close to the modern sub-desertic shrub steppe, where C₃ *Pooideae* would have similarly covered the highlands (Barboni *et al.* 1999). The phytolith assemblage from the Pleistocene sediment indicated an open grassland with more trees and shrubs, developed under humid edaphic conditions or under climate more humid than today (Barboni *et al.* 1999). C₃ *Pooideae* grasses were actually more abundant in the fossil than the modern samples which could indicate that there was stronger wind or runoff under wetter climatic conditions in the past (Barboni *et al.* 1999).

Carter (2001), as part of a multiproxy investigation, extracted phytoliths from sediments in a 197-m core in Hawkes bay, New Zealand. They provided a continuous vegetation – climate record spanning the time period from at least the

last interglacial to the present. The phytolith record demonstrated that grass/cyperaceae grew during warmer periods and woody taxa dominated the site during colder periods. During the present interglacial, the Poukawa basin is occupied by a shallow lake surrounded by an extensive fen (Carter 2001). During colder – drier periods, the floor of the basin dried out and woody taxa occupied the basin floor (Carter 2001). This contrasted with the pollen record, which demonstrated a converse pattern. The apparent discrepancy reflects the purely local provenance of the phytolith assemblage (Carter 2001). Significant changes in phytolith assemblages occur at the same depth as major tephras (fragments and particles ejected by a volcanic eruption), indicating a sharp decline in trees and shrubs and a surge in grass and cyperaceae (family of graminoid (grass-like) plants) (Carter 2001). A series of successional changes follow each major tephra fall. Initially, the woody taxa are killed off and replaced by grass and cyperaceae that rapidly colonise the fresh surface (Carter 2001). Trees and or shrubs succeed the grass and cyperaceae after a significant lag (Carter 2001). It confirms the usefulness of phytolith analysis as a method of determining environmental and climatic information. It is especially useful as a compliment in the interpretation of long multiproxy records.

In 2003, Abrantes (2003), used phytoliths to derive a 340,000 year continental climate record on tropical Africa from an eastern equatorial Atlantic site. Total phytoliths as well as C₃ and C₄ grass phytoliths were used to compare the distribution pattern on other continental climate proxies such as diatoms (Abrantes, 2003). In this case the phytolith accumulation rate dominated by C₄ grasses and their variability validates the cold stages and interstadials as times of arid conditions in the southern Sahara and Sahel regions, and marks an increase in aridness from Termination II to Termination I (Abrantes 2003). Termination is the period of time during a glacial cycle when there is relatively rapid transition from full glacial climates to full interglacial climates (Paillard 2009). Termination II is the end of Marine isotope stage 6 which dates to around c.191,000 to c.130,000 years ago and Termination I, known as the Last Glacial Termination, is the end of Marine isotope stage which dated to around c.31,000 to c.16,000 years ago (Paillard 2009). The total and arid phytolith frequency spectrum showed how major alterations in continental aridity

and/or wind strength conditions over North Africa were clearly determined by global ice volume and the 100,000 year cycle characteristic for high-latitude climate change (Abrantes, 2003). This study confirmed that with the joint use of diatoms, both freshwater diatoms and limnobiote, and C₃ and C₄ grass phytoliths can be an important tool for identifying different transporting agents in assessing the different contributions of vegetation and dust sources in terms of aridity, wind direction and strength (Abrantes 2003).

Phytolith analysis of selected native plants and modern soils from southeast Uruguay demonstrated the potential that phytolith analysis has on paleoenvironmental and archaeological reconstruction of the region (Iriarte and Paz, 2009). The modern phytolith reference collection was established based on 60 Poaceae species and out of 50 of them, 25 contribute diagnostic phytoliths at different taxonomic levels coinciding with all the major ecological zones of south-eastern Uruguay (Iriarte and Paz, 2009). The modern soil analysis revealed significant patterns that differentiate a number of specific habitats, showing that distinct vegetational units may be discriminated by the phytolith signatures they produce (Iriarte and Paz, 2009). Overall the study demonstrated that phytoliths are very useful in showing vegetational units dominated by specific plant species, and illustrated the potential of phytolith analysis for paleoenvironmental and archaeological reconstruction in the region.

In the last decade there has been a surplus of researchers taking advantage of the information contained within these microfossils. Included in this is the development of statistical methods, as well as chemical, and isotopic microanalytical methods (Carter 2007).

2.9 Phytoliths in Archaeology

Phytoliths in archaeological research can be useful in determining past diet, agricultural practices, plant processing, the use of domestic space and non-dietary plant exploitation (Neumann *et.al*, 2016). Phytolith analysis of each plant part such as the leaf or stem, of cereals or wild grasses have the potential to be used for interpreting the use of a room or understanding past cultural practices, such as

stabling, storing or grain processing (Rosen, 2000, 2005). An example of this would be if there were no husk phytoliths in a room but there were lots of stem phytoliths then it could suggest that the space was used as a stable or barn storing fodder.

Phytoliths are used to understand the agricultural origin and dispersal of key economic plant species which are of fundamental importance to archaeology (Ball *et.al*, 2016). The domestication of plants and the development and spread of agriculture were transformative events in human and ecological history (Ball *et.al*. 2016). Phytoliths have been integral in aiding our understanding of this. For example squashes and gourds of *Cucurbita* and other *Cucurbitaceae*, major early cultivars and domesticates of the Americas, spread considerably outside their areas of origin, and produced diagnostic phytoliths which showed an archaeological documentation of their history (Ball *et al.* 2016). Six different species ranging from eastern North America to southern South America were domesticated in prehistory, and phytolith research points to an early domestication of species native to the lowland Neotropics of Mesoamerica (*C. argyrosperma*) and northern South America (*Cucurbita moschata* and *C. ecuadorensis*) (Ball *et al.* 2016).

Phytoliths are valuable in recording the early domestication of wheat (*Triticum sp.*), barley (*Hordeum spp.*), rice (*Orzya satvia*), maize (*Zea mays*) and millet (*Panicum spp.*) To distinguish between domestic crops and their wild ancestors, phytolith morphometric methods are used; this allows a more accurate timing of the onset of agriculture (Carter 2007). Phytolith evidence indicated that Asian rice, *Oryza sativa L.*, was domesticated in the middle of Yangtze River Valley of southern China about 10,000 radiocarbon years ago during the Late Pleistocene and Holocene periods (Zhao and Piperno, 2000).

Analysis of phytoliths from sediments has also proved to be useful in describing the early cultivation of non-grain crops such as bananas and taro (Carter 2007, p.21). Although the hybridisation of bananas has made them nearly sterile, resulting in the production of little to no seeds, they do produce distinctive phytoliths and starches (Carter 2007). These can be extracted from sediments as was the case for the site

of Kuk in New Guinea. In this study, pioneered by Denham et al. 2003, phytoliths and starches were extracted from pre-historic sediments and used to document the intentional planting of taro (*Calocasia esculenta*) and banana (*Musa* spp.). Results showed that high banana phytolith percentages reflect large plant populations rather than high phytolith production relative to other species (Denham et al. 2003). Secondly *Musa* bananas would not be expected in a grassed landscape subject to periodic burning. Other banana species, such as *Ensete glaucum*, are more fire-tolerant and might be expected to thrive in repeatedly burned landscapes; however, they account for only a minor component of Musaceae phytoliths during the Holocene at Kuk (Denham et al. 2003). The large percentages of bananas within a managed grassed landscape beginning at 6950 to 6440 cal yr B.P. are interpreted to be diagnostic of deliberate planting (Denham et al. 2003). This explains that agriculture arose independently in New Guinea between 6950 to 6440 BP. In Africa there is no evidence of wild ancestors of *Musa* so the appearance of it in the archaeological record shows that it must have been introduced by humans (Mbida et.al, 2000). In the site of Nkang the occurrence of *Musa* in phytolith form is the first archaeological indication of a cultivated crop for such an early period in Central Africa (Mbida et.al, 2000).

Garcia-Granero et al. 2017 showed the potential of phytoliths for reconstructing plant use at sites where macro-remains are absent or poorly preserved. Phytoliths and starch remains on grinding stones from archaeological sites in northern India were studied. Results showed that the low number of phytoliths at the site of Datrana IV suggests that at least some of the grinding tools might have been used for activities other than plant processing, such as polishing stone beads (Garcia-Granero et al. 2017). The absence of phytoliths and excess of starch grains in some samples at Loteshwar suggest that cereals were de-husked and well cleaned prior to grinding (Garcia-Granero et al. 2017).

Peto et al. (2013) used phytolith analysis from a sub-terranean building excavated at the site of Gyor-Menfocsanak, western Hungary, to try and identify the inner use of space in the building. Results showed that the visible separation of anatomically non-

identical micro-indicators might refer to conscious space use in the deposition of different plant materials in different parts of the site (Peto *et al.* 2013). Furthermore it can be fairly certain that chaff from dehusking activity was deposited or stored in a well-defined section in the south-western corner of the building. (Peto *et al.* 2013). Peto *et al.*'s. (2013) results provide us with the possibility to compare and discuss phytolith signals over a broader geographical and time scale, since the management of cereals leaves similar patterns regardless of the culture and chronology of the site in question.

The waterlogged site of Győr-Ménfőcsanak dating back to the 14th-15th century AD in southeast Hungary which supplied exemplary preservation conditions, allowed the observation of phytoliths in their original anatomical context, as well as aDNA analysis (Peto *et al.* 2015). The aDNA analysis proved the Asian origin of a crop used at the site although it is not clear it is had been introduced or locally grown (Peto *et al.* 2015). This approach demonstrates the potential of phytolith analysis as part of a multi-proxy approach, from macro- to microscale and further down the molecular level.

Phytolith analysis has shown that it can be very helpful for reconstructing agricultural activities and crop-processing. This relevance is related to the possibility of distinguishing different parts of grasses and cereals that derive from the various processing stages and are more commonly preserved when macro-remains and unavailable or uninformative (Dal Corse *et al.* 2016). Light leaves and husks do not usually survive fire and culms are rarely preserved through carbonization (Dal Corse *et al.* 2016). Phytoliths do not have the same preservation problems as organic plant remains because they are inorganic (Harvey and Fuller, 2005). Burnt plant materials will leave behind phytoliths in ash, but equally unburnt plants that decompose will leave phytoliths in archaeological sediment (Harvey and Fuller, 2005). Therefore there is a higher chance of recovering evidence for any particular species, or plant part from phytoliths than carbonized evidence (Harvey and Fuller, 2005).

Dal Corso et.al (2016) study demonstrates the potential of phytolith research for studying archaeological contexts outside of domestic structures. The study focused on Fondo Paviani, a Bronze Age site in northern Italy dating to (ca. 1650-1350 yr B.P). The study aimed to use phytoliths to distinguish between different grass subfamilies in the cereal economy to possibly provide some clues about local crop processing activities (Dal Corso et.al, 2016). To manage this, samples from a shallow ditch at the edge of the site filled with domestic waste and samples from a near-site fen with natural infilling were taken for analysis (Dal Corso et.al, 2016). Silica skeletons from the inflorescences of panicod grasses are thought to have originated from *Panicum* or *Setaria* which could indicate that small hulled millets were processed at the site (Dal Corso et al. 2016). There were peculiar sharp cuts on these silica skeletons from the inflorescences of possible cereals which have been interpreted as threshing remains that had entered the ditch together with domestic waste from the sites (Dal Corso et al. 2016). Threshing remains found in the fen deposits imply that a cereal processing area existed near the site enclosure on in the field outside the enclosure (Dal Corso et al. 2016). The results indicate that threshing was a local activity and probably aided by animal trampling (Dal Corse et al. 2016).

Phytoliths from secure, non-mixed contexts, such as *in situ* burning, are an important source of information on fuel use, especially where monocotyledonous plants have been used as fuel (Shillito, 2013). Plants such as grasses and sedges combust quickly compared to wood, and do not tend to preserve in the charred macrobotanical record, but can survive in phytolith form (Shillito, 2013). The combination of a secure context and ease of relative quantification that makes ash deposits particularly well suited to phytolith analysis, means that clear distinctions can be made between different fuel inputs (Shillito, 2013). By further combining phytolith analysis with micromorphology, the specific context of the phytoliths at the microscale can be observed (Shillito, 2013). Micromorphology has become an increasingly important analytical tool in understanding site formation processes and the use of space particularly within settlements (Matthews et.al, 2011). These have

been observed as a significant component of ash deposits for example at Çatalhöyük, Turkey (Matthews, 2005).

Ryan and Rosen (2016) discuss how phytoliths can indicate changing patterns of plant exploitation. At Çatalhöyük sediment samples were collected from floors and features within buildings, such as storage bins and hearths, as well as rubbish areas (middens) and external fire-spots (Ryan and Rosen, 2016). Samples were taken from various excavated areas across the East Mound including the 4040 Area designed to investigate social geography through the excavation of adjacent houses and middens, as well as the South Area, which yielded information on temporal trends in plant use (Ryan and Rosen, 2016). Overall the phytolith data collected suggests that both wetland and dry-land plants were routinely exploited at Çatalhöyük throughout the Neolithic occupation, and that overall there was a very consistent pattern of plant and land use (Ryan and Rosen, 2016).

Phytoliths have been further used to identify non-food use of plants. *In situ* phytolith impressions of basketry and matting in buildings at Çatalhöyük have provided important insights into the use of plants such as reeds and sedges in craft activities (Rosen 2005, Ryan 2011 as cited in (Shillito, 2013)). Visible layers of phytoliths on skeletal remains have also provided direct evidence for the use of bindings in burial contexts (Boz et al 2007). These phytolith demonstrate the kinds of new perspectives this microfossil can provide in archaeology.

Ramsey et al. (2017) studied new phytolith evidence of plant use from the 23,000 year old site of Ohalo II in Israel. Emphasis on wetland-type resources from the perspective of the phytolith assemblage at Ohalo II reflects an intense use of phytolith-rich reed and sedge (*Cyperaceae*) stems and leaves were likely used for craft and construction purposes, as well as byproducts of food-plant collection (Ramsey *et al.* 2017). Ramsey et al. (2017) notes that these types of plant parts are largely invisible when using macro-botanical methods. Macro-botanical remains and an area of open-air hearths, show that there was an emphasis on steppe/parkland grasses however woodland-type resources almost exclusively dominate the

phytoliths remains (Ramsey *et al.* 2017). The lack of steppe/parkland grass-type evidence from the phytolith assemblage suggests that the grains found in the hearths were already de-husked, since phytoliths form in the husk surrounding the grain (Ramsey *et al.* 2017). The grains from this hearth area may result from a later processing stage in which inhabitants had already discarded the inedible husk (Ramsey *et al.* 2017). The discord between the macrobotanical and phytolith evidence in this context provides interesting insights into the use of hearths. Similar results to these throughout the site show that grass and cereal processing may have been a largely “indoor” activity (Ramsey *et al.* 2017). The comparison of the phytolith assemblage and macro-botanical assemblage provide a baseline to help inform the interpretation of phytolith assemblages at other sites in Southwest Asia. The results also helped highlight patterns of plant use at the site.

2.10 Phytoliths as indicators of ancient water availability

As outlined in the introduction, phytoliths have been used as indicators of past water availability. An experimental study of wheat and barley by Rosen and Weiner (1994) suggested conjoined phytoliths with greater than 300 cells were an indicator of growth under conditions of high water availability in arid and semi-arid regions. This hypothesis is based on the idea that greater water availability means that plants have a greater rate of water and silica uptake, which allows formation of larger multi-celled phytoliths (Rosen and Weiner 1994). The size criteria established by Rosen and Weiner (1994) have been applied to archaeological sites to suggest the presence or absence of irrigation agriculture (Katz *et al.* 2007). At the Neolithic site of Çatalhöyük, Turkey, believed to have been situated in a wetland area, the method has been used to suggest wheat was growing under dryland conditions, and thus must have been brought to the site from several kilometres away (Roberts and Rosen 2009).

However, subsequent to this it was discovered that conjoined phytolith forms are more prone to mechanical breakage than was previously thought (Jenkins, 2009). This means that while more conjoined cells may form with increased water availability, taphonomic processes in the archaeological record-particularly over a long period of time, can cause these cells to disaggregate making this method

problematic unless your phytolith assemblage has remained undisturbed through time (Jenkins *et al.* 2016).

A study by Madella *et al.* (2009) researched a method that does not rely on conjoined forms for using phytoliths to indicate past water availability. Five cereals were grown in a growing chamber under controlled conditions of light, heat and day length, and in experimental fields in Cambridge (Madella *et al.*, 2009). The five cereals used were bread wheat (*Triticum. aestivum*), emmer wheat (*T. dicoccum*), spelt wheat (*T. spelta*), two row barley (*Hordeum vulgare*) and six row barley (*H. distichon*) grown under two different climatic regimes: Middle Eastern and Northern European. The climatic area chosen for the Middle Eastern regime is that of central Iraq. The study looked at the ratio of fixed (short) to sensitive (long) phytolith forms and the percent of sensitive forms in the cereals they analysed. The results showed that in bread and emmer wheat, the production of sensitive (long) forms is remarkably enhanced by a higher availability of water to the plant (Madella *et al.*, 2009). Morphological analysis showed that sensitive forms in the leaf are influenced by the water regime while the stem do not differ much in sensitive form production (Madella *et al.* 2009). Therefore the variability of sensitive form is not equal in the entire plant and depends of the level of evapo-transpiration of the involved plant part (Madella *et al.* 2009).

Another case study on ancient rice farming practices by Weisskopf *et al.* (2014), focused on Neolithic China and India. Phytolith analysis was used to establish patterns identifiable to specific cultivation systems (Weisskopf *et al.*, 2014). The study used modern analogue phytolith assemblages of associated crop weeds found within cultivation regimes, as well as in wild rice stands (*Orzya nivara* or *Orzya rufipogon*), to interpret the archaeobotanical assemblages (Weisskopf *et al.* 2014). Rice weeds and sediment samples were recorded and collected from a range of arable systems and wild stands in India (Weisskopf *et al.* 2014). The preliminary results showed that there was a correlation with variation in rice cultivation systems on the basis of differences in environmental conditions and regimes, with wetness being the main factor (Weisskopf *et al.* 2014). The Chinese samples contain rice alongside phytolith morphotypes from hydrophilic species which are indicative of wet rice farming (Weisskopf *et al.* 2014). The Indian samples on the other hand have high proportions of rice mostly accompanied by Poaceae husk with very low levels

or no phytoliths from hydrophilic species which indicate rainfed cultivation systems (Weisskopf *et al.* 2014). These results illustrate that this method using phytoliths can be utilised for different archaeological regions and periods.

Weisskopf *et al.* (2015) results further demonstrate that plant remains, including weed assemblages, can reveal wetter or drier growing conditions. The evidence from the phytolith assemblages suggested that cereal crop choices changed over three cultural periods in China (Weisskopf *et al.* 2015). The archaeological samples come from three Neolithic sites in the Lower Yangtze: Tianluoshan (wetland decrue) (4800-4300 B.P), Caoxieshan (small, frequently drained fields) (3950-3700 B.P) and Maoshan (large, intensive irrigated fields) (3000-2300 B.P). The percentage of sensitive forms vs the percentages of fixed morphotypes illustrate the distinctive patterns in modern analogue rice fields, and the wild rice stands. Results showed that phytolith ratios decrease according to the decrease in water abundance in each arable system and they are wettest in conjunction with wild rice stands (Weisskopf *et al.* 2015). Despite the environmental fluctuations that changed from favourable conditions to less favourable conditions rice continued to be farmed which suggests its possible role as an elite product (Weisskopf 2015). The study shows that phytoliths can be used to differentiate between arable field systems and is a useful tool for exploring and understanding developments in early rice farming.

Jenkins *et al.* (2016) cultivated crops in Jordan over a period of three years at three different crop growing sites, two of the sites (Deir 'Alla and Ramtha) are the same crop growing stations that were used to grow the sorghum used in this study. Native land races of six-row barley (*Hordeum vulgare*) and durum wheat (*Triticum durum*) were grown. The crops were subjected to different irrigation regimes: (1) no irrigation- 0% of crop water requirements; (2) under irrigated – 80% of crop water requirements; (3) irrigated – 100% of crop water requirements; and (4) over irrigated – 120% of crop water requirements. A fifth regime was added in the second and third season which an under-irrigated plot was given 40% of crop water requirements (Jenkins *et al.* 2016). Only the husks of the plants were processed. This was the first large scale experimental project to test this method and take into account multiple variables that can affect phytolith production such as soil composition and chemistry,

location, climate and evapotranspiration rates. Results showed that wheat has a higher Si uptake than barley. Analysis found that irrigation was the most significant factor in the increased ratio of fixed to sensitive forms (Jenkins *et al.* 2016). Results show that phytoliths can provide a strong indication of past water availability, and of irrigation if it is known that the study region was arid or semi-arid during the period of occupation, when the ratios of fixed to sensitive forms are high (Jenkins *et al.* 2016).

3. Methodology

3.1 Crop growing method

The modern sorghum analysed in this thesis was experimentally grown as part of the University of Reading's Water, Life, and Civilisation project in collaboration with NCARE (National Centre for Agricultural Research and Extension, based in Jordan). The sorghum was grown specifically to test the relationship between water availability and both phytolith and stable isotope ratios in modern cereals (Jenkins *et al.* 2011b). The sorghum was purchased at an Amman market and was grown at Deir 'Alla, Ramtha and at the farm of Sameeh Nuimat (on behalf of NCARE) near Salt (Figure 1). In the first year there were not enough sorghum grains therefore new seeds had to be acquired the next year which appeared to belong to a different, taller variety of sorghum (Flohr, 2012).

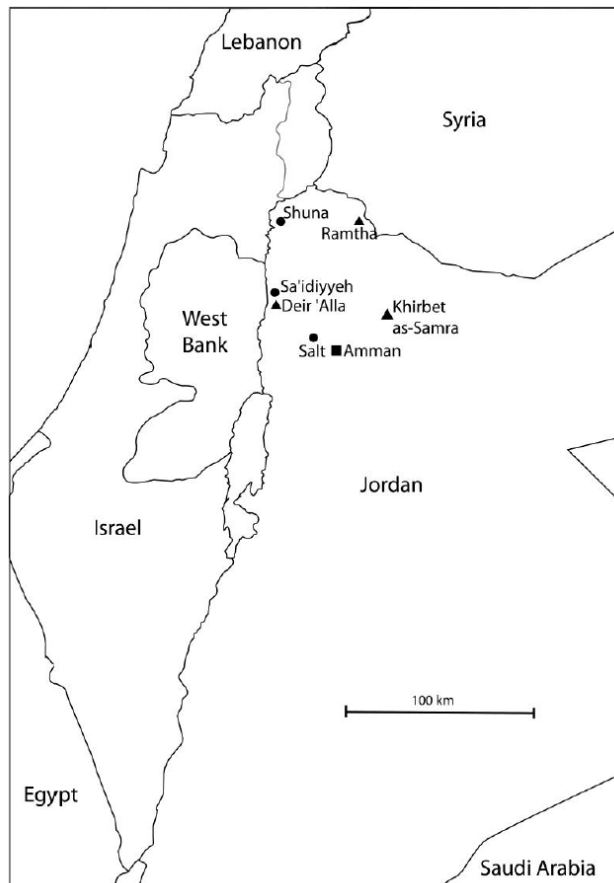


Figure 1: Map of the Southern Levant with the crop growing stations (triangles including Salt) and the surrounding archeological sites (circles). (Flohr *et al.* 2011).

All the sites differ significantly in their micro-environments despite all being located in the north of Jordan. Deir 'Alla, located in the Jordan Valley, is 200m below sea level whereas Ramtha, situated on the Jordan Plateau, is at 510m above sea level, (Flohr, 2012). Ramtha consistently receives the most annual rainfall (Flohr *et al.* 2011). Except for the crops at the farm near Salt, conditions such as rainfall, evaporation, temperature, and relative humidity were closely monitored so that their effects on the crops phytolith ratios could be established.

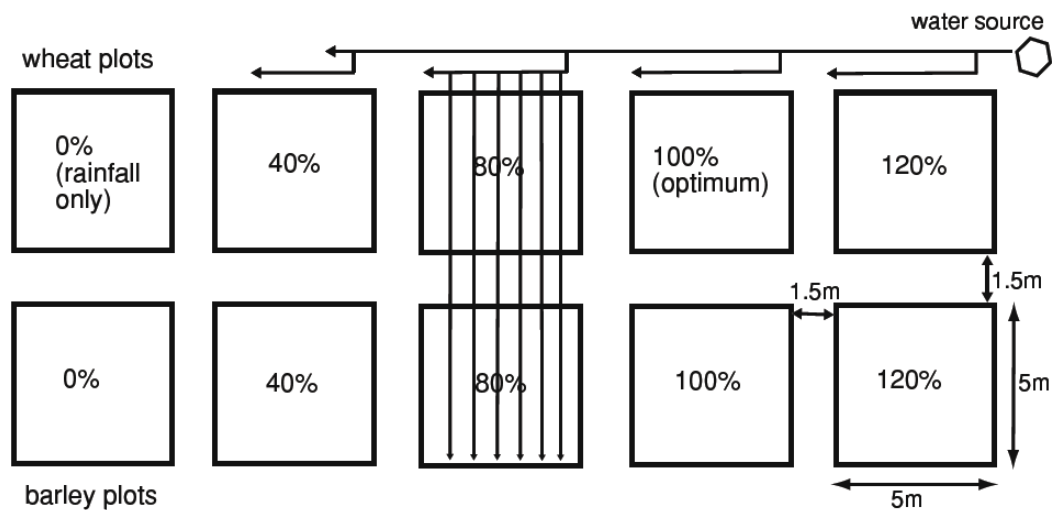


Figure 2: Schematic representation of the lay-out of the crop growing experiments, based on the lay-out of Khirbet as-Samra, but similar for all stations. (Flohr *et al.* 2011)

The sorghum was grown over two years from 2009 to 2010 and was sown in April and harvested in September/October. The crops were grown in 5 x 5 m plots, with 1.5 m in between each plot. Different irrigation regimes were employed: (1) no irrigation (0% of crop water requirements); (2) under-irrigated (80% of crop water requirements); (3) irrigated (100% of crop water requirements); (4) over-irrigated (120% of crop water requirements) (Jenkins 2011). The calculation for irrigation levels was based on knowledge of crop water requirements estimated by using Class A – Pan Evaporation readings (Jenkins 2011, Allen *et al.*, 1998).

Water was implemented by a drip irrigation system (Figure 2) with a 60 cm spacing between water pipes and a 40 cm spacing between the drippers on each pipe (Jenkins 2011). Each irrigation plot had eight lines (Jenkins 2011). The water that

was used for irrigation was treated wastewater at Ramtha and a mixture of treated wastewater and fresh water at Deir 'Alla (Jenkins *et al.* 2011b). The water used for irrigation at Salt was stored rain water and fresh water. No additional fertilizer was applied (Flohr, 2012). The crops were harvested in 50 cm intervals in a line diagonal over the plot, from the outside to the middle of the plot: 0-50 cm, 50-100 cm, 100-150 cm, 150-200 cm, 200-250 cm and 250-300 cm (Jenkins *et al.* 2011b). After harvesting, the crops were stored in paper bags.

Table 1: Sorghum data for Ramtha and Deir 'Alla.

Monthly relative humidity (av. RH%), average minimum temperature (av.min, in °C), average maximum temperature (av. Max T) and average temperature (av. T) for Deir 'Alla and Ramtha during the growing season (April to September) in 2009. September data were not available for Ramtha. Data not available for Salt. (After Flohr 2012)

Site	Year	Month	av. RH%	av. Min T	av. Max T	Av. T
Deir 'Alla	2009	April	45.0	16.5	30.1	23.3
		May	42.4	19.8	34.6	27.2
		June	38.6	23.4	39.9	31.6
		July	41.9	26.2	40.3	33.2
		August	44.2	26.4	40.2	33.3
		September	44.0	24.6	37.0	30.8
Ramtha	2009	April	47.7	9.4	23.7	16.5
		May	42.0	13.1	27.7	20.4
		June	31.2	17.9	32.3	25.1
		July	50.8	19.3	33.2	26.3
		August	55.2	18.4	33.1	25.8

3.2 Laboratory method

Due to the time limitations of this one year project only the sorghum samples from the 0% irrigated plots and 100% irrigated plots from 2009 and 2010 were analysed. Each year, site and irrigation had five samples taken from different grid locations. The husks, stem and leaves were taken from each sorghum sample for analysis. All of the 100% irrigated samples had husks but from the 0% samples only the plants grown at Deir 'Alla had husks present. The dry ashing method was used to conduct

the experiment because it has been demonstrated that dry ashing produces a higher weight percent of phytoliths to original plant matter (Jenkins 2011b). These dry samples were washed in distilled water and left to dry for at least 24 hours. The samples were cut up and placed in crucibles and weighed. The samples were then initially burnt in a muffle furnace for three hours at 500 °C. However, 500 °C was not high enough because the samples turned to charcoal and not ash. It transpired that the appropriate temperature for sorghum to fully ash is at 700 °C for three hours. Samples were weighed before and after heating to assess weight loss due to heating. After charring the samples were acid washed and centrifuged at 2000rpm for five minutes then the supernatant was discarded and the samples centrifuged twice more in distilled water. The samples were then put into glass beakers to dry overnight. When dried, 0.5 grams of the sample was mounted using the mounting medium *Entellan* and left for three days to dry. At first one gram of the dried sample was mounted and left to dry. However, when trying to count the slide, one gram was obviously far too much so it was reduced to 0.5 grams. In total 143 samples were mounted. The whole process was a learning curve and there were batches that were lost due to being new to this type of lab work. For example, when taking a batch out of the muffle furnace after it had been burnt the samples had a very noticeable blue colour. It must have been from when I was cutting up the plant pieces and parts of the blue rubber glove were going into the sample pots then mixing together and creating that colour. The batch was thrown away as I assumed they were ruined, however, when speaking with my supervisor about it I learnt that they would have been totally fine. Another batch in its final drying stage in the glass beakers was lost simply because it was dropped and everything smashed onto the floor. It was a time consuming process so losing batches was not ideal. Mounting the samples at times was very difficult. The scale that was used to measure the sample weight seemed to struggle when it would be below 1 gram, so to try and measure 0.5 grams sometimes took up to five minutes to try and get the correct amount and wait for the scale to adjust. This too added a lot more time to the whole process.

3.3 Counting method

Slides were counted using a Meiji Infinity polarising microscope at x400 magnification with an attached camera. Phytoliths were grouped according to the mechanism of silification in their production:

- *Fixed forms* (short cells-in this study mainly bilobates): silification is under genetic control;
- *Sensitive forms* (long cells-in this study mainly elongate echinates and elongate smooth): silification is under environmental control.

Phytoliths were counted according to the number of long cells and short cells on the slide. Four hundred phytoliths were counted per slide equaling a total of 57,200 phytoliths counted in total for all slides. All the slides are labelled (see appendix), each number is one plant so the slides go up to 55 which means in total 55 sorghum plants were used. The letter used shows which part of the plant was used, A=leaf, B=stem and C=husk.

Table 2: Summary of samples analysed in this study

Sample numbers	Site name	Irrigation %	Year
1 - 5	Salt (SF)	0%	2009
6 - 10	Salt (SF)	100%	2009
11 - 15	Deir 'Alla (DA)	100%	2009
16 - 20	Ramtha (RA)	100%	2009
21 - 25	Ramtha (RA)	0%	2009
26 - 30	Deir 'Alla (DA)	0%	2010
31 - 35	Ramtha (RA)	0%	2010
36 - 40	Ramtha (RA)	100%	2010
41 - 45	Deir 'Alla (DA)	100%	2010
46 - 50	Salt (SF)	100%	2010
51 - 55	Salt (SF)	0%	2010

3.4 Statistical analyses

Statistical analysis was conducted using R with assistance from Prof Rick Stafford, Bournemouth University. Short to long phytolith ratio was calculated and a log₁₀ transformation was used as a dependent variable in the analysis. The log₁₀

transformation allowed the data to meet the assumptions of parametric statistic. A three-way ANOVA design with all interactions was conducted using 'Year', 'Site' and 'Irrigation' as fixed factors in the analysis. After the tests were completed a confidence interval was applied to the mean of the results.

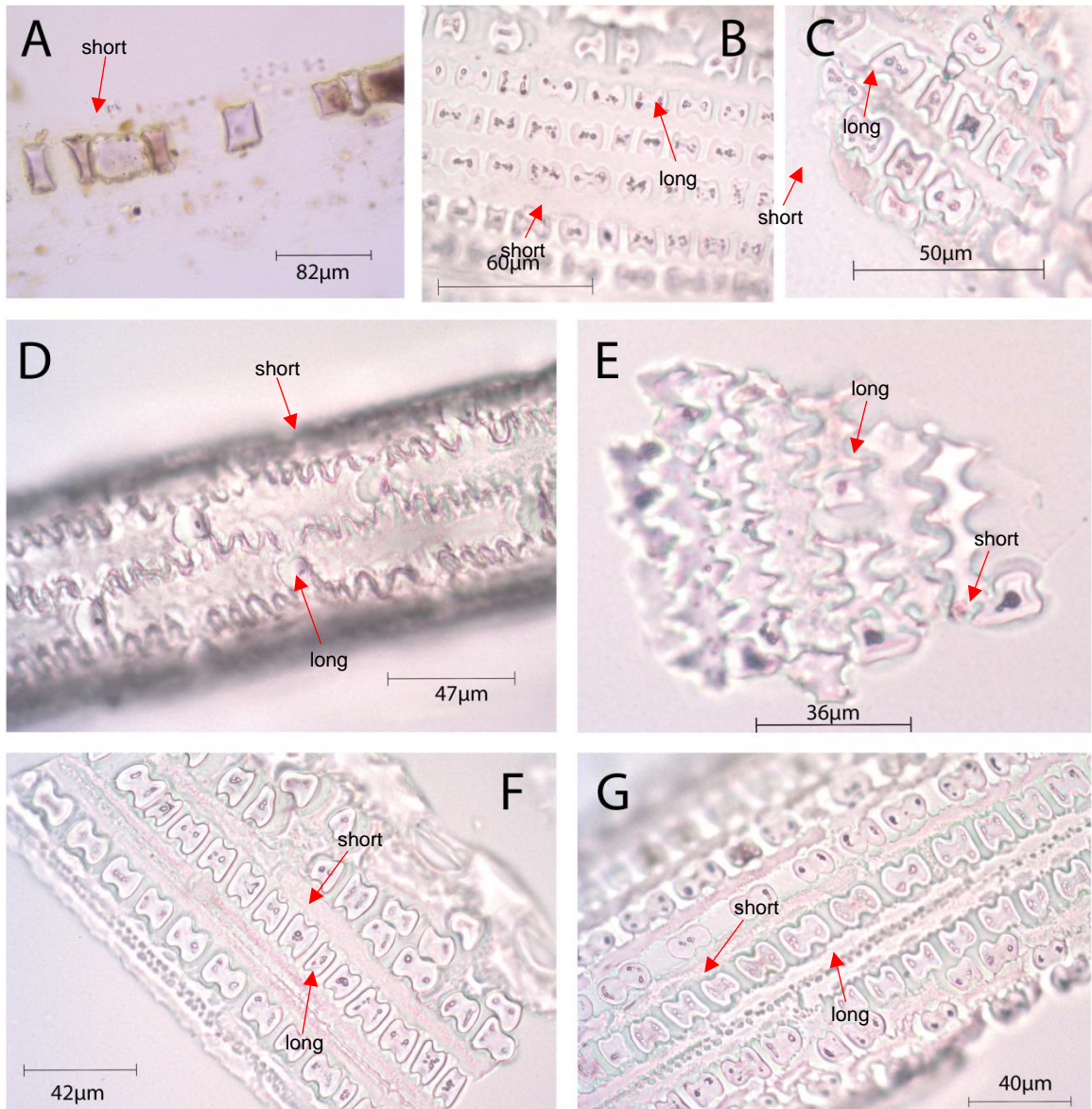


Figure 3: Photomicrographs of phytoliths from the modern sorghum: A) short cells from Ramtha 100% irrigated 2009 husk; B) Bilobes (short) from Sameeh's Farm 100% irrigated stem; C) Bilobes interspersed with elongate echinates (long) from Deir Alla 100% irrigated stem D) Elongate echinates interspersed with short cells from Ramtha 2009 100% stem; E) Elongate echinates interspersed with short cells from Deir Alla 2009 100% husks; F) Bilobes interspersed with elongate echinates from Ramtha 2009 100% stem; G) Bilobes interspersed with elongate echinates Ramtha 2009 100% stem

4. Results

Table 3: Results of three-factor ANOVA investigating differences in phytolith ratio between sites, irrigation regimes and years

	Df	Sum Sq	Mean Sq	F Value	P
Site	2	3.050	1.525	6.366	0.00229
Irrigation	1	20.429	20.429	85.280	56.67<0.0016
Year	1	1.594	1.594	6.656	0.1098
Site:Irrigation	2	1.116	0.558	2.330	0.10132
Site:Year	2	0.091	0.045	0.190	0.82731
Irrigation:Year	1	0.243	0.423	1.015	0.31560
Site:Irrigation:Year	1	0.315	0.1315	1.314	0.25368
Residuals	132	31.622	0.240		

Results show that there were significant differences in the mean ratio of short to long forms between sites Salt (SF), Deir 'Alla (DA) and Ramtha (RA). (Table 1, Figure 4). Tukey tests were employed to find out which site means (SF, DA and RA) (compared with each other) are significantly different. Tukey tests indicated that SF is significantly different to DA ($p<.05$), that RA was borderline significantly different to DA ($p=0.072$) and that RA is not necessarily different to SF.

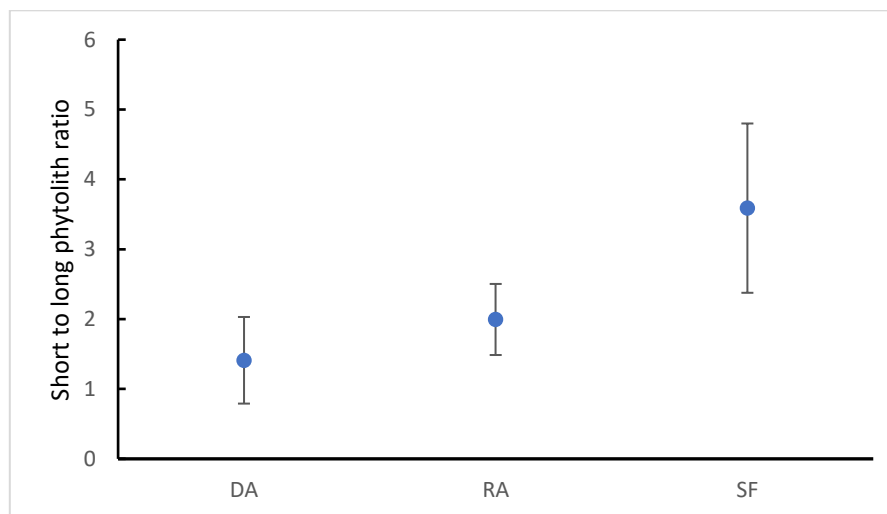


Figure 4: Fixed to sensitive cell ratio at different sites Salt (SF), Deir 'Alla (DA) and Ramtha (RA)

Figure 5 shows the short to long phytolith ratio between non-irrigated and irrigated samples for all plant parts. The results show that there is a difference between the irrigated and non-irrigated samples in the fixed to sensitive cell ratio. There is no overlap between the samples and the confidence interval is much smaller for the irrigated samples. Samples above 3.5 are likely to have been unirrigated while samples with a fixed to sensitive cell ratio of less than 1.5 are likely to have been irrigated. Figure 6 shows that there is little difference between the ratio of short to long forms for all plant parts between the two growing years and that while the mean ratio of short to long cells for 2010 is lower the confidence intervals overlap.

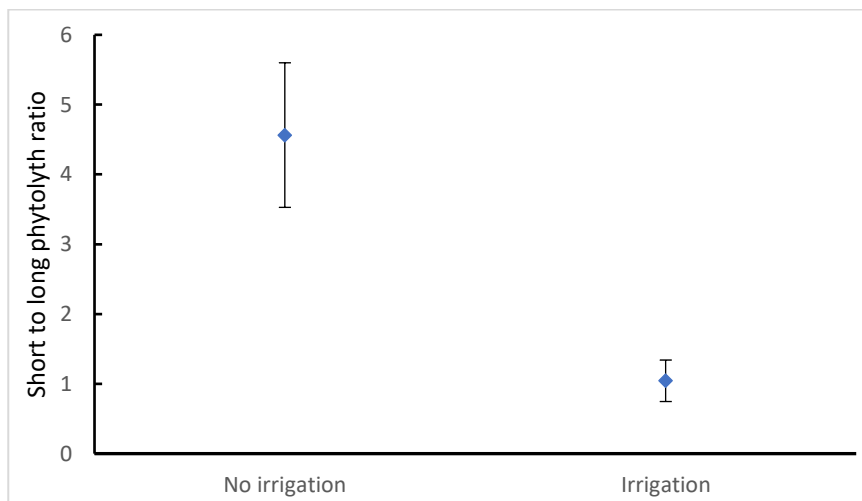


Figure 5: Fixed to sensitive cell ratio at different irrigation levels

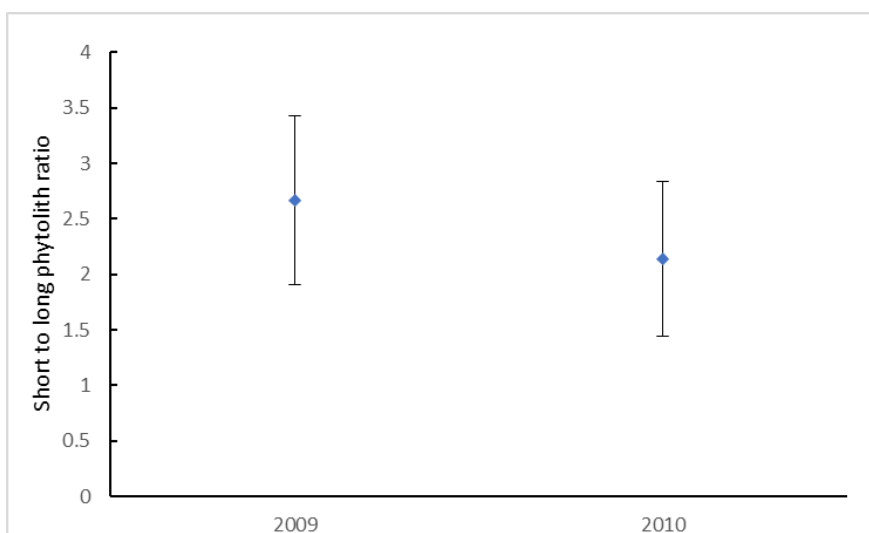


Figure 6: Fixed to sensitive cell ratio at different years

Table 4: Meant percent with standard deviation of long cells to short cells by sites Salt (SF), Deir 'Alla (DA) and Ramtha (RA), regime and year from the sorghum samples

Site/regime/year	Sorghum						
	n slides	Long cells	Mean	SD	Short cells	Mean	SD
Year: 2009							
SF 0%	10	694	69.4	29.12	3306	330.6	29.12
SF 100%	15	2985	199	121.9	3020	201.3	120.89
RA 0%	9	703	78.11	25.22	2987	321.88	25.22
RA 100%	15	3708	247.2	79.27	2297	153.13	78.71
DA 100%	15	3566	237.73	109.61	2468	164.53	109.57
Year: 2010							
SF 0%	10	647	64.7	31.48	3353	335.3	31.27
SF 100%	15	4034	268.93	70.96	1966	131.06	70.96
RA 0%	10	958	95.8	13.62	3042	304.2	13.62
RA 100%	15	3853	256.88	85.56	2147	256.86	85.56
DA 0%	15	2732	182.13	101.34	3268	217.86	101.34
DA 100%	14	3882	277.28	70.84	1718	122.71	70.84

The data was further analysed by splitting up the results into plant part. The most striking observation that can be made from the results is that the husks have a smaller ratio of fixed to sensitive forms and hence have more sensitive phytolith forms than the leaves and stems. Results show that there is a difference between irrigated and non-irrigated leaf samples (Figure 7) but that the ratio of short to long forms is much higher than the ratio seen with the husk phytoliths (Figure 8). Figure 7 shows that in the leaf anything above 3.2 is likely to have been irrigated and anything below 1.1 is likely to have been unirrigated. Figure 8 illustrates that husks have a clear difference in the fixed to sensitive cell ratio between irrigated and non-irrigated samples. The confidence interval is small for irrigated husks and demonstrates that anything under 0.2 is likely to have been irrigated whereas anything above 0.2 is likely to have been unirrigated. Figure 9 demonstrates that there is some difference between irrigated and non-irrigated phytoliths for the stems. Samples with a short to long forms ratio of under three indicates that irrigation has occurred and anything above three indicates that no irrigation has occurred.

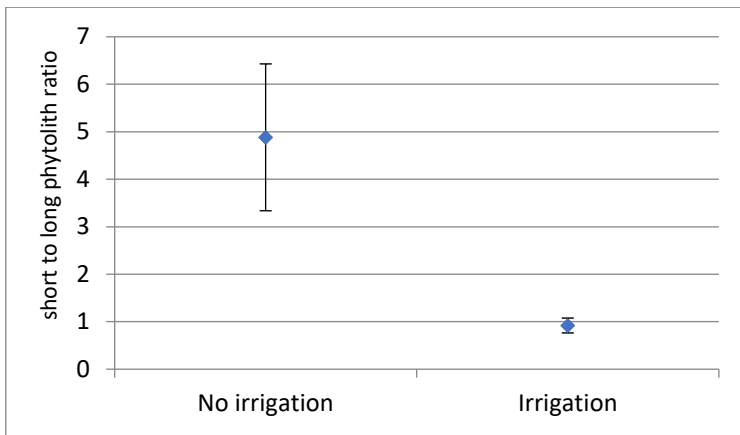


Figure 7: Fixed to sensitive cell ratio in the leaf at different irrigation levels. The non-irrigated samples were at 0% and the irrigated at 100%

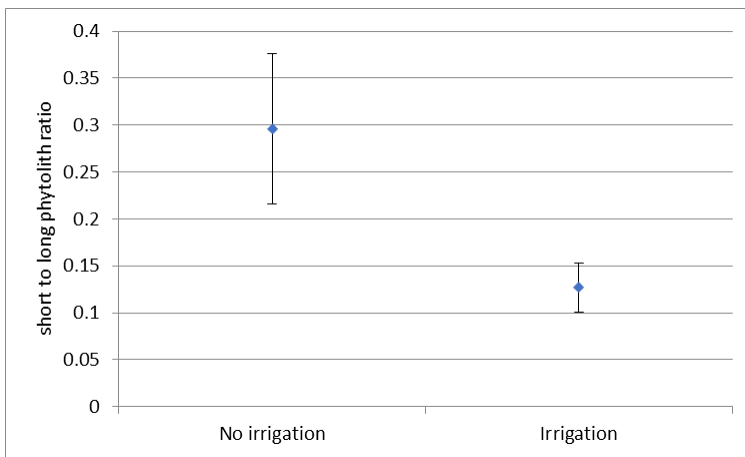


Figure 8: Fixed to sensitive cell ratio in the husks at different irrigation levels. The non-irrigated samples were at 0% and the irrigated at 100%

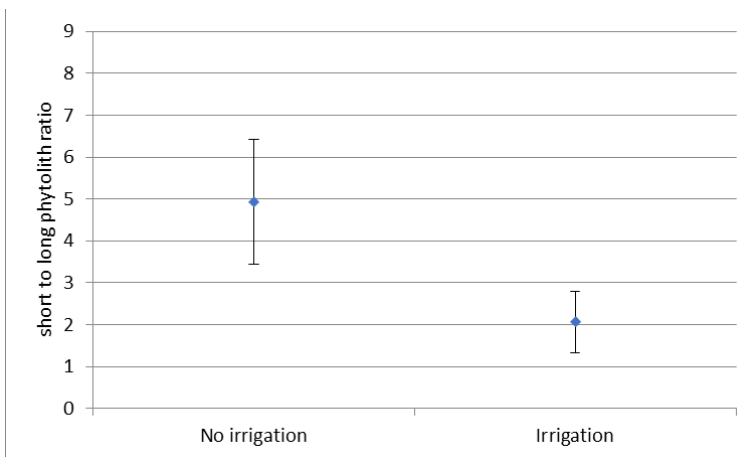


Figure 9: Fixed to sensitive cell ratio in the stems at different irrigation levels. The non-irrigated samples were at 0% and the irrigated at 100%

Figure 10 shows the overall percentage of fixed to sensitive forms per plant part in the irrigated samples. Sensitive forms in the husk dominate taking up 90% for the plant part. Sensitive forms in the leaf are just under 50%, sensitive forms in the stem are similar to this taking up just over 50%.

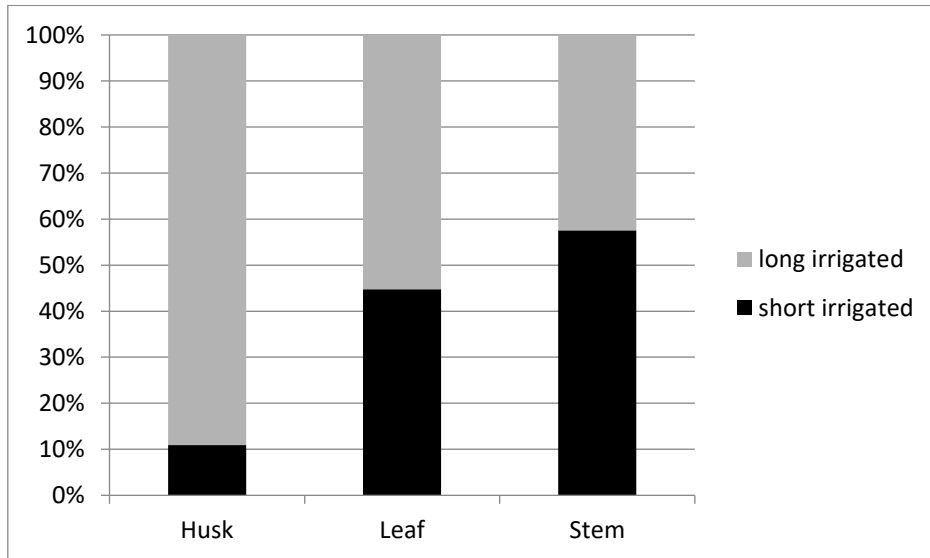


Figure 10: Percentage of fixed and sensitive cells per plant part in irrigated samples. The irrigated samples were 100% irrigated.

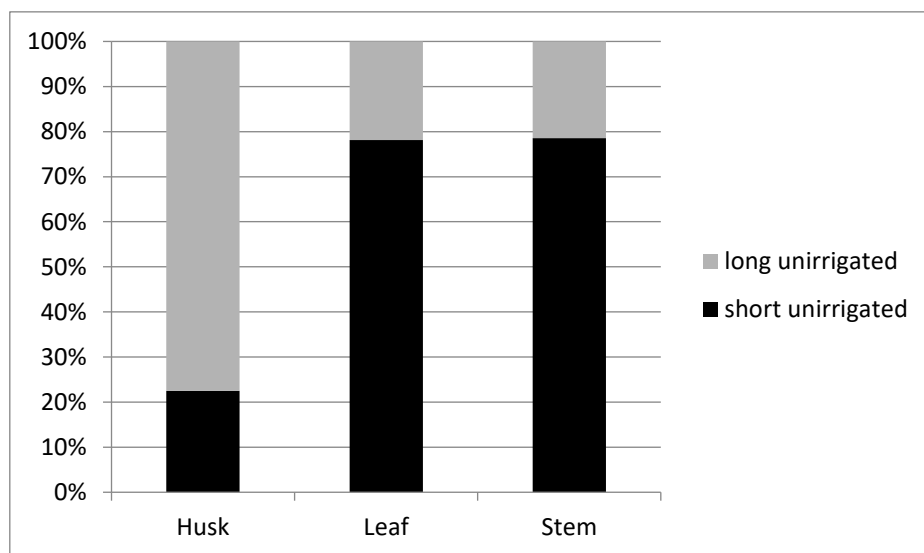


Figure 11: Percentage of fixed and sensitive cells in non-irrigated samples. The unirrigated samples were 0%.

5. Discussion

5.1 Inter-site and Inter-annual variation

The results demonstrate that irrigation has an effect on the ratio of short to long phytolith forms in sorghum. They also indicate that irrigation had different effects on the phytolith samples from the different sites. As shown, the effect of irrigation to the crops in Salt was significantly different to Deir 'Alla but not necessarily different to Ramtha. Salt also had a higher mean ratio when compared with the other two sites. However, there is only a slight difference between the 2009 and 2010 assemblages.

It is not clear what causes these inter-site and inter-annual differences but it is likely to be due to an interaction of different environmental factors. All three sites have different micro climates and vary in environmental factors. Other than water availability the following could also have an impact on water uptake and phytolith production: salinity, nutrient availability, humidity, temperature, evaporation rates, altitude and differences in light or irradiance. Temperature is also important as it primarily affects plant development and probably nutrient availability. The effects of these variables are not straightforward, but act in complex ways (Flohr 2012).

There is a possibility that the slight inter-annual variation is caused by a combination of genetic and environmental factors. Two different varieties of sorghum were sown in the two years, with the one in 2010 belonging to a taller variety than the one in 2009. Genetic differences can possibly affect the reaction of the plants to environmental differences (Flohr 2012). The 2009 variety could have been more easily affected by differences in temperature between the sites. Another possibility is that the 2010 variety could have grown larger roots, thereby accessing water from neighbouring plots, so that it would be less water stressed than the 2009 variety (Flohr, 2012). However, these genetic differences seem not have had a great effect on the sorghum, because there is little inter-annual variation. It seems that environmental variation has a stronger effect than plant genotype on the study of water availability. There are other factors that were not measured in this experiment that could have had an effect on the sorghum for example soil chemistry.

Rainfall was absent for all the sites in both years because the crops were grown over summer though environmental differences are apparent between Deir 'Alla and Ramtha, with Deir 'Alla being warmer and less humid than Ramtha. Evaporation also differed between sites and years with Ramtha having the highest evaporation rate in 2009 (Figure 10) (Flohr 2012). This could explain why there were slightly more long celled phytoliths at Deir 'Alla than Ramtha.

If there is no other severe stress from other factors then temperature is the most important factor in plant development and growth (Fitter and Hay 2002). Plants require a specific amount of heat to develop from one point to another (Miller 2018). Only once a certain number of growing degree days (GDD) is reached then it can move on to the next stage of development (Miller 2018). GDD is the sum of the average daily temperature above a certain base level (Flohr 2012). Deir 'Alla has a higher temperature than the other two sites, and more days above the base temperature (Flohr 2012). It is possible that humidity, which is the amount of water vapor in the air, played a role for the three sites. Though Ramtha was the most humid of all three sites it also had the highest rate of evaporation. The crops grown at Ramtha in 2009 gave a more water stressed signal. This could explain why it did not develop as fast as the crops grown at Deir 'Alla.

5.2 Effect of irrigation on sorghum

The results of the effect that irrigation had on the sorghum demonstrate that the ratio of fixed (short) to sensitive (long) forms could be used to identify water availability. Analysis found that 'irrigation' was the variable with the strongest explanatory power which indicates that water availability is the most significant factor in determining the ratio of fixed to sensitive forms. Figures 5, 7, 8 and 9 compare the mean of the sensitive forms for the unirrigated and irrigated samples in all plant parts. It shows that the irrigated samples have a higher mean ratio than the unirrigated samples. Results show that phytolith assemblages with a mean fixed to sensitive cell ratio of <1.7 indicate that irrigation has occurred while assemblages with a fixed to sensitive form ratio of >3.5 suggests that irrigation has not occurred.

This result establishes that there was increased Si uptake with irrigation. Irrigation had different effects on the separate sites but what is most important is that irrigation has the greatest effect overall. Jenkins et al. (2016) looked at phytolith ratios in husks from, six-row barley (*Hordeum vulgare*) and durum wheat (*Triticum durum*) both C3 plants which were grown under different irrigation regimes in Jordan but from two of the same sites as the current study. The results indicated that while variation in the ratio of fixed to sensitive forms could be explained by differences in species, both durum wheat and six-row barley produce more sensitive forms when irrigation is increased (Jenkins et al. 2016). Logistical regression showed that across all sites, the ratio of fixed to sensitive cells explained around 15.7% of the variability in levels of irrigation (Jenkins et al. 2016). However, where the fixed to sensitive ratio was >1 it could be assumed with 80% confidence that irrigation had occurred. At the site of Kherbet as-Samra, the explanatory was much greater with a ratio of >0.5 meaning irrigation could be predicted with $>99\%$ confidence (Jenkins et al. 2016). Results from the current study compare favourably with Jenkins et al. (2016) with husks having a mean fixed to sensitive form ratio of >0.2 . Both these studies demonstrate that at the higher ratios phytoliths can be used to effectively determine past water availability.

Madella et al's. (2009) results also show that in bread and emmer wheat, the production of sensitive form phytoliths are greatly enhanced when there is a higher availability of water to the plant. In their study fixed forms predominate but all taxa (other than six-row barley) show some increase in the sensitive form counts in the wet samples.

Weisskopf (2015) showed that there are higher proportions of sensitive cell forms when the plants are in a wetter setting. Their study analysed phytoliths from Neolithic China. Riverside samples from Tianluoshan, consistent with a wetland setting, from their study illustrated this point with sensitive cells taking up 70% of the assemblage. The results from the current study also show that wetter samples have a higher percentage of sensitive forms in the assemblage (See Figure 10). In Weisskopf's (2015) study, phytoliths from a small field in Caoxieshan had much more fixed morphotypes that are consistent with drier signatures in decrue fields showing that these fields were kept drier. This matches results from the current

study, figure 11 shows that when samples are drier there are much more fixed forms. The results from the current study suggest that phytoliths from sorghum are able to show if they are from wet or dry environments.

The most interesting observation in the results is the variability of sensitive forms per plant part. The results highlight that husks have a greater proportion of long forms than the leaves and stems which suggests that they uptake water to a greater degree than the leaves and stems. Stem appears to be the least responsive to water variability in the plant with fixed form phytoliths being more prominent in this data set. This could be because of the anatomical function of the stem. In the stem, water moves through vessels primarily as a consequence of evapo-transpiration in leaves and inflorescence (Madella *et al.* 2009). Most of the water and silica solute are moved away to other organs, therefore loss of water in the stem is minimal and deposition of phytoliths is not influenced by high rates of evapo-transpiration (Shahack Gross *et al.* 1996). Most Si is deposited up cell walls of roots, leaves, stems and husks, where it may form a thin layer consisting of silica gel ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Shahack Gross *et al.* 1996).

The effect of irrigation on plant parts has been more widely explored on C_3 plants. In Madella *et al.* (2009) study, morphological analysis showed that the production of environmentally sensitive forms in the leaf is influenced by the water regime. The results in the current study also indicate this, figures 9 and 10 show that the amount of sensitive forms varies per plant part. In Madella's *et al.* (2009) study it shows that the stem in emmer and spelt wheat appear to be less responsive to water variability in the plant, fixed form phytoliths are constantly the dominant morphotypes and dry and wet crops do not differ much in sensitive form production. In Madella *et al.* 2009 study, bread and emmer wheat and two-row barley showed that sensitive forms in the leaf differed significantly between the wet and dry crops. Spelt wheat and six-row barley did not show any difference in this respect. In the results presented by Madella *et al.* (2009) results are in agreement with the study presented here because with sorghum the number of fixed form phytoliths in the stem was always larger. However, there are some differences, in irrigated samples sensitive phytolith forms in the stem did increase which was not seen in Madella *et al.* (2009). The results of

sensitive phytolith forms in the leaf match Madella *et al.* (2009) study. In the current study sensitive phytolith forms in the leaf of the sorghum also differed significantly between wet and dry crops with sensitive forms increasing when the sorghum is irrigated.

Hutton and Norrish (1974) suggest that the percentage of silica in the husks is closely related to the amount of water transpired in wheat and hence more accurately reflects water availability during growth. Silica is concentrated in the husks, containing about one-third of the total silica in the plant (Hutton and Norrish, 1974). In their studies, Handreck and Jones (1968) show that, of all the parts of the oat plant they analysed, the husks contained the highest concentration of silica, and accounted for about 40% of the total weight of silica. The same was found for wheat with husks representing 10% of the total weight of the plant which contained 40% of the total weight of silica (Handreck 1968). It is probable that this is also the case with sorghum. This suggests that the most informative data would most likely be obtained by analysing the husks.

5.3 Application in Archaeology and Suggestions for Further Work

In this study only one C4 plant species was analysed *Sorghum bicolor*. Further work is needed to establish if this method is applicable to other C4 plant species such as millet. Though the most effective method for identifying water availability is the ratio of fixed cells to sensitive cells it does not remain unproblematic, factors such as taphonomy, bioturbation and possibility of a mixed assemblage may have an impact on the method used. Phytoliths can be transported by wind or water which may result in pitting and corrosion of single celled forms and breakdown of conjoined or multi-celled forms. Phytoliths recovered from archaeological sites may have been consumed either by animals or humans. If consumed by humans then the plants are likely to have undergone extensive processing and cooking prior to consumption. Phytoliths are subjected to translocation, bioturbation and, stratigraphic mixing processes after being incorporated into the soil. They can suffer from vertical

movement, with the smallest phytoliths removed (eluviation) and accumulated (illuviation) in deeper strata (Zurro *et al.* 2016). This process is known as translocation or percolation and it can damage the single phytolith particles (Zurro *et al.* 2016). Heavy translocation and extreme bioturbation may produce a phytolith pool that differs in chronology and composition even given the same soil profiles (Zurro *et al.* 2016). Post burial phytoliths are also affected by soil conditions, it has been documented that phytoliths are harmed by alkaline conditions (Jenkins 2009). Though this study is an example that shows there are more phytoliths with increased water availability, taphonomic processes in the archaeological record, especially over long periods of time, can cause these cells to disaggregate which can make this method problematic unless the phytoliths assemblage has remained undisturbed through time. Phytoliths also go through more taphonomic processes during laboratory processing. In this study the process involved immersion in 10% hydrochloric acid, burning in a muffle furnace at temperatures of 700 °C and centrifugation. In a previous study using emmer wheat it was shown that both dry ashing and the acid extraction method causes a change in the number of conjoined forms in emmer wheat (Jenkins 2009). The conjoined phytoliths method was not used because single celled forms are not subject to break up. This study and Jenkins *et al.* (2016) shows that the method can provide a strong indication of past water availability, and of irrigation if it is known that the study region was arid or semi-arid during the period of occupation, when the ratios of fixed to sensitive forms are high. Further work such as the full disclosure of taphonomy indicators (diagnosis, pH, etc.) could be added to strengthen interpretation and level of comparability between different datasets.

Irrigation and rainfall also have the potential to inform on agricultural methods in the past. An example of this is the in the record of very early agriculture in South Asia where regional cropping patterns established thousands of years ago are still important for understanding forms of production (Morrison 2019). In this region there is an especially sharp distinction between “wet” and “dry” farming, the former closely associated with rice and with artificial irrigation and the latter with hardy millets and rainfed production (Morrison 2019). A large number of Southern Neolithic sites are

located in the lower rainfall parts of the peninsula (Morrison 2009). In one well-studied region in northern Karnataka (India), most Neolithic settlements of any size are located near the bases of the high granite outcrops that dot the landscape which suggests that the control of seasonal monsoon runoff was an important factor in site location (Morrison 2019). Irrigation is an artificial application of water to the soil it is usually used to assist in growing crops in dry areas and during periods of inadequate rainfall. Archaeological investigation has identified evidence of irrigation in Mesopotamia and Egypt as far back as the 6th millennium BCE, where barley was grown in areas where the natural rainfall was insufficient to support such a crop (Zaghloul et al. 2013). It was found that the ancient inhabitants of the land used a system comprised of a network of irrigation canals. These canals are the earliest record of irrigation probably in the period of the Roman time (Zaghloul et al. 2013).

Multiplicity, when different phytolith morphotypes are produced within a taxon, and redundancy, when the same phytolith morphotypes are produced by different taxa, are persistent challenges in phytolith analysis. Micromorphology and phytolith analysis of soil thin sections can be integrated to address issues redundancy and multiplicity, as well as depositional history when studying archaeological soils and sediments (Vrydaghs 2016). It can also indicate a degree of bioturbation. This is seen in a study of an early modern burial complex which combined micromorphology and phytolith analysis. The combination of these two techniques suggested that there was considerable bioturbation but that the effects were localized (Grave and Kealhofer 1999).

Furthermore, while the data demonstrates that there is a difference in the ratio of short to long forms depending on plant part, archaeological assemblages are likely to reflect an assemblage comprised of forms from all parts of the plant. As such, this study takes a conservative approach and suggests that, based on the data generated here, an archaeological assemblage comprised of sorghum with a short to long cell ratio of <3 suggests that the plants received an optimum amount of water for successful growth. This water could either be coming from irrigation or increased rainfall. Sometimes on archaeological sites specific features or contexts are found

that do not represent mixed assemblages for example grain storage features which contain a phytolith assemblage comprised largely of husk phytoliths. However, such features tend to be the exception rather than the norm. This study showed that the method is applicable to the leaves and stems as well as the husk. It is important because the types of long cells found in leaves and stems have smoother edges and are very distinct from the wavy edged sensitive forms found in husks, the corresponding fixed forms formed in leaves are morphologically very similar too cork-silica cells. This means that in an archaeological assemblage derived from a mixture of plant parts, it would be difficult to isolate short cells formed in husks from those formed in leaves. Further work is now needed to create a probabilistic model in order to determine the expected fixed to sensitive form ratio mean for a mixed plant part/species assemblage based on the results presented here and those previously published in Jenkins et al. 2016 for wheat and barley. This would make the method more useful to archaeologists who are likely to be dealing with assemblages from mixed plant parts and, in some regions, mixed C3/C4 assemblages.

6. Conclusion

The aim of this study was to validate whether or not phytoliths in sorghum, a C₄ plant, can be indicators of past water availability. The reason behind this was to create the beginning of a new data set on phytoliths from C₄ plants that are able to provide an indication of past water availability. This study reports that phytoliths in sorghum were affected by water availability. The results of this study show that water availability is clearly important in determining the ratio of fixed to sensitive phytolith forms in sorghum though other environmental variables also play a role in sensitive form production. Results show that there were unknown factors that influenced phytolith production between site and years however irrigation showed to be the biggest influence in determining the ratio of fixed (short) to sensitive (long) forms. Samples above 3.5 are likely to have been unirrigated while samples with a short to long form ratio of less than 1.5 are likely to have been irrigated. When comparing the results to C₃ plants the method proves that the C₄ sorghum is also able to give information about the past water availability. The percentages per plant part show that husks have a smaller ratio of fixed to sensitive forms and hence have more sensitive phytolith forms than the leaves and stems. The percentages also show that wetter samples have more sensitive cells and drier samples have more fixed cells. This demonstrates that sorghum is able to tell us if they were grown in a wet or dry setting which in turn could help understand the environment they grew in and whether socio-economic changes have occurred.

To make the method more useful for archaeologists further work is needed to create a probabilistic model to determine the expected fixed to sensitive form ratio mean for a mixed plant part/species assemblage based on the results presented here and those previously published in Jenkins et al. 2016 for wheat and barley, since most features on archaeological sites represent mixed assemblages.

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Appendix 1**Raw data counts**

Slide	Site	Irrigation	Year	Short	Long
1A	SF	0	2009	319	81
1B	SF	0	2009	325	75
2A	SF	0	2009	305	95
2B	SF	0	2009	362	38
3A	SF	0	2009	338	62
3B	SF	0	2009	299	101
4A	SF	0	2009	378	22
4B	SF	0	2009	302	98
5A	SF	0	2009	367	33
5B	SF	0	2009	311	89
6A	SF	100	2009	274	126
6B	SF	100	2009	347	53
6C	SF	100	2009	54	346
7A	SF	100	2009	207	193
7B	SF	100	2009	359	41
7C	SF	100	2009	43	357
8A	SF	100	2009	256	144
8B	SF	100	2009	315	85
8C	SF	100	2009	35	365
9A	SF	100	2009	184	216
9B	SF	100	2009	300	100
9C	SF	100	2009	102	298
10A	SF	100	2009	218	182
10B	SF	100	2009	304	96
10C	SF	100	2009	22	378
11A	DA	100	2009	83	323
11B	DA	100	2009	312	88
11C	DA	100	2009	30	370
12A	DA	100	2009	214	186

12B	DA	100	2009	294	106
12C	DA	100	2009	45	355
13A	DA	100	2009	186	214
13B	DA	100	2009	205	195
13C	DA	100	2009	67	333
14A	DA	100	2009	164	236
14B	DA	100	2009	345	55
14C	DA	100	2009	46	354
15A	DA	100	2009	201	199
15B	DA	100	2009	250	150
15C	DA	100	2009	27	373
16A	RA	100	2009	266	134
16B	RA	100	2009	288	112
16C	RA	100	2009	30	370
17A	RA	100	2009	205	195
17B	RA	100	2009	200	200
17C	RA	100	2009	85	315
18A	RA	100	2009	170	230
18B	RA	100	2009	143	257
18C	RA	100	2009	82	318
19A	RA	100	2009	177	223
19B	RA	100	2009	203	197
19C	RA	100	2009	56	344
20A	RA	100	2009	188	212
20B	RA	100	2009	160	240
20C	RA	100	2009	44	356
21A	RA	0	2009	348	52
21B	RA	0	2009	303	97
22A	RA	0	2009	346	54
22B	RA	0	2009	299	101
23A	RA	0	2009	339	61

23B	RA	0	2009	343	57
24A	RA	0	2009	275	125
24B	RA	0	2009	N/A	N/A
25A	RA	0	2009	320	80
25B	RA	0	2009	324	76
26A	DA	0	2010	309	91
26B	DA	0	2010	318	82
26C	DA	0	2010	99	301
27A	DA	0	2010	296	104
27B	DA	0	2010	280	120
27C	DA	0	2010	95	305
28A	DA	0	2010	237	163
28B	DA	0	2010	209	191
28C	DA	0	2010	48	352
29A	DA	0	2010	277	123
29B	DA	0	2010	370	30
29C	DA	0	2010	101	299
30A	DA	0	2010	243	157
30B	DA	0	2010	279	121
30C	DA	0	2010	107	293
31A	RA	0	2010	327	73
31B	RA	0	2010	298	102
32A	RA	0	2010	291	109
32B	RA	0	2010	300	100
33A	RA	0	2010	309	91
33B	RA	0	2010	313	87
34A	RA	0	2010	303	97
34B	RA	0	2010	290	110
35A	RA	0	2010	288	112
35B	RA	0	2010	323	77
36A	RA	100	2010	177	223

36B	RA	100	2010	198	202
36C	RA	100	2010	60	340
37A	RA	100	2010	190	210
37B	RA	100	2010	312	88
37C	RA	100	2010	49	351
38A	RA	100	2010	159	241
38B	RA	100	2010	208	192
38C	RA	100	2010	36	364
39A	RA	100	2010	177	223
39B	RA	100	2010	186	214
39C	RA	100	2010	18	382
40A	RA	100	2010	189	211
40B	RA	100	2010	176	224
40C	RA	100	2010	22	378
41A	DA	100	2010	169	231
41B	DA	100	2010	202	198
41C	DA	100	2010	45	355
42A	DA	100	2010	166	234
42B	DA	100	2010	197	203
42C	DA	100	2010	51	349
43A	DA	100	2010	170	230
43B	DA	100	2010	193	207
43C	DA	100	2010	13	387
44A	DA	100	2010	133	267
44B	DA	100	2010	154	246
44C	DA	100	2010	35	365
45A	DA	100	2010	N/A	N/A
45B	DA	100	2010	161	239
45C	DA	100	2010	29	371
46A	SF	100	2010	188	212
46B	SF	100	2010	180	220

46C	SF	100	2010	67	333
47A	SF	100	2010	196	204
47B	SF	100	2010	218	182
47C	SF	100	2010	55	345
48A	SF	100	2010	196	204
48B	SF	100	2010	185	215
48C	SF	100	2010	47	353
49A	SF	100	2010	157	243
49B	SF	100	2010	169	231
49C	SF	100	2010	10	390
50A	SF	100	2010	135	265
50B	SF	100	2010	142	258
50C	SF	100	2010	21	379
51A	SF	0	2010	327	73
51B	SF	0	2010	369	31
52A	SF	0	2010	375	25
52B	SF	0	2010	346	54
53A	SF	0	2010	312	88
53B	SF	0	2010	330	70
54A	SF	0	2010	297	103
54B	SF	0	2010	329	71
55A	SF	0	2010	289	111
55B	SF	0	2010	378	22