A direct comparison of three palynological preparation techniques

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ABSTRACT

Two samples of palynomorph-rich Upper Jurassic (Lower Oxfordian) mudstone from western Scotland were quantitatively prepared using the traditional hydrochloric and hydrofluoric acid based palynological preparation technique and two non-acid procedures. The latter are protocols using sodium hexametaphosphate [$(NaPO_3)_6$] and hydrogen peroxide (H_2O_2). These non-acid techniques have previously been validated only in terms of the absolute numbers of palynomorphs extracted. By contrast, this study aimed to assess the numbers of palynomorphs extracted in terms of absolute numbers of the individual taxa present to test for any taxonomic biases. The $(NaPO_3)_6$ method proved around 50% as efficient as acid digestion in terms of absolute numbers of palynomorphs extracted. It produced clean residues, which are eminently suitable for most palynological studies. The majority of the taxa present were recovered in representative relative proportions, and no taxonomic biases were noted. The absolute numbers of most taxa decrease in a stepwise fashion from acid digestion via the $(NaPO_3)_6$ procedure to the H_2O_2 method. However, the concentrations of bisaccate pollen were apparently relatively unaffected by the three methods used. Similarly, the *Meiourogonyaulax catytonensis* group appears to be unusually resistant to oxidation damage by H_2O_2. It is considered that the $(NaPO_3)_6$ preparation method is an eminently viable alternative to acid digestion, especially in remote operational settings such as rigsites. The H_2O_2 technique proved to be significantly less effective, at approximately 10% of the extraction level of acid digestion which appears to be largely due to oxidation. Hydrogen peroxide is an aggressive oxidant. Therefore the

$(NaPO_3)_6$ technique is deemed to be both safer and more effective than the H_2O_2 method.

Keywords: palynomorphs; preparation techniques; Upper Jurassic (Oxfordian); United Kingdom (Scotland).

1. Introduction

This study aimed to test whether the sodium hexametaphosphate [$(NaPO_3)_6$] and hydrogen peroxide (H_2O_2) methods of extracting palynomorphs from sedimentary rocks and sediments are effective alternatives to the traditional technique of hydrochloric and hydrofluoric acid (HCl/HF) digestion and, in particular, do not introduce any taxonomic biases. It is acknowledged that some practitioners may be somewhat resistant to change in working practices. Consequently these 'proof of concept' studies should help to overcome any entirely understandable inertia to change and stimulate further research on, and testing of, non-acid methods of palynomorph preparation.

Two samples of palynomorph-rich Upper Jurassic mudstone from the Isle of Skye, western Scotland, were prepared quantitatively using the acid-based palynological preparation technique and two non-acid methods. The palynomorph assemblages extracted were thoroughly examined and counted. The two non-acid procedures use $(NaPO_3)_6$ and H_2O_2, and were first described by Riding and Kyffin-Hughes (2004) and Riding et al. (2007) respectively. This investigation aimed to assess the concentrations per gram of the individual palynomorph taxa across the entire taxonomic spectrum extracted using these three procedures in order to assess the relative effectiveness of the two non-acid techniques. Previous studies have concentrated wholly on the absolute numbers of palynomorphs extracted, and not on the concentrations of individual taxa. This approach should identify any biases, in terms of absolute numbers of palynomorphs and diversity, inherent with the $(NaPO_3)_6$ and H_2O_2 preparation techniques.

2. Background

The standard technique for the extraction of palynomorphs from sedimentary rocks and sediments uses acid digestion of the mineral matrix normally followed by oxidative maceration. Here HCl and HF separately remove the carbonate and silicate minerals respectively and nitric acid (HNO_3) is subsequently used for oxidation (Gray, 1965; Doher, 1980; Phipps and Playford, 1984; Wood et al., 1996; Green, 2001). Recently the present authors have developed techniques for palynomorph separation without the use of these highly hazardous acids. These non-acid techniques use $(NaPO_3)_6$, and H_2O_2 (Riding and Kyffin-Hughes, 2004; 2006; 2010; Riding et al., 2006; 2007).

Sodium hexametaphosphate, also known as Calgon and Graham's Salt, is a non-hazardous substance; it is neutral (pH 7) and non-oxidising. Sodium hexametaphosphate solution is used as a detergent, a deflocculant and a dispersent. The highly ionic solution breaks up the clay fraction due to the high charges of phosphate ions which are adsorbed onto the clay. This reduction of the coherence of the clay results in the production of sub-10 μm particles which can be separated from the palynomorphs and the other organic phytoclasts by sieving using a 20 μm mesh.

Hydrogen peroxide is used as a bleach and a disinfectant. It is a powerful oxidant and is weakly acidic, hence is significantly hazardous (Riding et al., 2007, p. 21, 22). Furthermore, it readily dissociates and the resultant hydrogen and oxygen can potentially form explosive mixtures with combustible substances. Hence H_2O_2 should always be used in a fume cupboard. Hydrogen peroxide disaggregates clay-bearing sediments and sedimentary rocks both physically and chemically. It causes ‘deposit swelling’ in fine-grained siliciclastic lithotypes. As H_2O_2 soaks into the sample material, hydrogen and oxygen bubbles are produced by dissociation. The resultant expansion pressure caused by these gases physically breaks up the rock matrix. Because H_2O_2 is an oxidising agent, it breaks down amorphous organic material (AOM). This destruction of AOM occurs simultaneously with deposit swelling, and hence can liberate palynomorphs from suitable material. Therefore organic-rich lithotypes which are rich in AOM are especially suitable for the H_2O_2 method. However, H_2O_2 should be used very carefully because oxidation can damage or entirely destroy palynomorphs (Hopkins and McCarthy, 2002).

The palynomorph extraction techniques using $(NaPO_3)_6$ and H_2O_2 fundamentally differ from acid digestion in that the mineral matrix is disaggregated

and sieved away, rather than simply being dissolved. Both non-acid protocols work well on most relatively soft clay-rich materials. However, they are significantly less effective on indurated mudstones and carbonates (Riding and Kyffin-Hughes, 2004; 2006).

All the palynology laboratory operations in this study were undertaken with strict reference to the appropriate and up-to-date health and safety guidelines (see http://www.hse.gov.uk/coshh), the manufacturer's safety data sheets and internal British Geological Survey risk assessments. In particular, protective clothing and footwear should be worn at all times, great care should be taken with all mineral acids, and hydrogen peroxide should be heated with great caution in a fully operational fume cupboard (see Riding et al., 2007, p. 21-22).

3. Material and methods

Two samples of Upper Jurassic mudstone were prepared using the three techniques. The material is from coastal (foreshore) outcrops at Dunans, Isle of Skye, western Scotland, United Kingdom (NG 472 708) (Fig. 1). The samples were collected from Bed 8 of Sykes and Callomon (1979) from the Lower Oxfordian part of the Dunans Clay Member of the Staffin Shale Formation; they are referable to the Cardioceras scarburgense Subzone of the Quenstedtoceras mariae Zone (Riding and Thomas, 1997, fig. 2). The samples are informally termed DUN 42 and DUN 43; they are from 32.12 m and 33.31 m from the base of the section at Dunans respectively. The Dunans Clay Member is known to be extremely rich in well-preserved dinoflagellate cysts, pollen and spores (Riding and Thomas, 1997).

The six subsamples prepared each used 5 g of rock. Two control subsamples were prepared using the standard HCl/HF digestion method (e.g. Wood et al., 1996) without pre-treatment or oxidation. The durations of the hydrochloric and hydrofluoric acid treatments were until the respective reactions were complete. The four non-acid subsamples were prepared using the methods described by Riding and Kyffin-Hughes (2004; 2006) and Riding et al. (2007) (Appendix 1). It was ensured that all the sample material was disaggregated.

To allow the relative effectiveness of each of the three protocols, the concentrations of palynomorphs were calculated using the exotic marker technique

using a spike of *Lycopodium clavatum* tablets (Benninghoff, 1962; Stockmarr, 1971). Five *Lycopodium* tablets were added to each of the six subsamples prior to the preparation procedures. At least 250 Jurassic palynomorphs were counted (Table 1). Damaged palynomorphs were considered; fragments which represented ca. 50% of the grain were counted as such and aggregated into the count. However any portions below 25% were disregarded. The absolute abundances of palynomorphs were calculated using the equation of Benninghoff (1962), i.e.:

$$c = \frac{m_c \times L_t \times t}{L_c \times w}$$

This is where:

c = the number of indigenous palynomorphs per gram of dry rock (= concentration)

m_c = the number of indigenous palynomorphs counted

L_t = the number of *Lycopodium* spores in each tablet (the mean for the batch of tablets used is 18,583)

t = the number of tablets added to the sample (i.e. 5)

L_c = the number of *Lycopodium* spores counted

w = the weight of dry sediment processed in grams (i.e. 5)

It should be remembered that exotic *Lycopodium* spores may be lost during the preparation procedure, largely during the decantation and sieving stages (Mertens et al., 2009). Selected photomicrographs of the organic residues are presented in Plates I and II. The palynomorphs identified in this study at and below species level are listed in Appendix 2. The sample material, organic residues, microscope slides, primary data and illustrated materials are all housed in the collections of the British Geological Survey, Keyworth, Nottingham NG12 5GG, United Kingdom.

4. Results

The samples both produced abundant and well-preserved dinoflagellate cysts, pollen and spores (Tables 1, 2; Plates I, II). Dinoflagellate cysts are significantly more abundant than pollen and spores. The dinoflagellate cyst assemblages are moderately

diverse and are entirely typical of the Early Oxfordian of Europe. The associations are overwhelmingly dominated by *Mendicodinium groenlandicum*, with common *Gonyaulacysta jurassica* subsp. *adecta*, the *Meiourogonyaulax caytonensis* group, *Rigaudella aemula*, the *Sentusidinium rioultii* group and *Wanaea* spp. (Tables 1, 2). The presence of taxa such as *Evansia deflandrei*, *Gonyaulacysta centriconnata*, *Gonyaulacysta dentata, Gonyaulacysta jurassica* subsp. *jurassica, Rigaudella aemula* and *Trichodinium scarburghense* is characteristic of the Late Callovian-Early Oxfordian interval. Comparable palynofloras have been described by Woollam (1980), Riding (1982; 1987; 2005), Smelror (1988a,b), Kunz (1990) and Riding and Thomas (1997). The association is assigned to the earliest Oxfordian DSJ20 Zone of Poulsen and Riding (2003).

The prepared organic residues from the six subsamples were each examined and the indigenous Jurassic palynomorphs and the exotic *Lycopodium* spores counted. All numerical data and the concentrations of the indigenous palynomorphs are presented as Tables 1 and 2 respectively. The results of this study are discussed below, both sample-by-sample and in general terms. In summary, the two non-acid procedures extracted significantly fewer palynomorphs than acid digestion, with the $(NaPO_3)_6$ method proving far more effective than the H_2O_2 technique.

4.1. Sample DUN 42

Sample DUN 42 produced an abundant, moderately diverse palynoflora (Tables 1, 2). The acid digestion method produced a concentration of 159,960 palynomorphs per gram (Table 2). This concentration is by far the highest of the three techniques, and is comparable to other palynomorph-rich material (Riding and Kyffin-Hughes, 2010, table 1). The $(NaPO_3)_6$ method yielded a significantly lower concentration of in situ palynomorphs, i.e. 71,781 grains per gram. This figure is 44.9% of the concentration of the acid digestion subsample. This marked reduction in productivity is virtually identical (45.4%) in terms of the indigenous marine palynomorphs. By contrast, the concentration of in situ terrestrial palynomorphs with the $(NaPO_3)_6$ method is 37.5% of that produced by the acid digestion procedure. The H_2O_2 technique proved to be the least effective of the three techniques, yielding 15,356 palynomorphs per gram (Table 2). This represents 9.6% and 21.4% of the concentrations achieved with acid digestion and the $(NaPO_3)_6$ method respectively.

This reduction in palynomorph productivity from the acid digestion method using H_2O_2 is far more for the indigenous marine palynomorphs, than the reduction in the level of in situ terrestrial palynomorphs. This strongly suggests that dinosporin is generally more susceptible to oxidation than the sporopollenin in pollen and spores. It appears that the diminution in the concentration of in situ pollen and spores is less using the H_2O_2 technique than with the $(NaPO_3)_6$ method (Table 2).

For the most common dinoflagellate cysts, i.e. *Gonyaulacysta jurassica* subsp. *adecta,* the *Meiourogonyaulax caytonensis* group, *Mendicodinium groenlandicum*, the *Sentusidinium rioultii* group and *Wanaea* spp., the diminutions in concentration between acid digestion and the $(NaPO_3)_6$ method are similar. Generally, this reduction in concentration is around 50%. The further reductions with the H_2O_2 technique are normally very high, but are significantly more variable. For example the *Meiourogonyaulax caytonensis* group is 54.1% of the concentration derived using the $(NaPO_3)_6$ method, and no specimens of *Wanaea* spp. were recorded in the H_2O_2 preparation (Table 2). Generally, however, the numbers of dinoflagellate cyst taxa per gram decrease in a stepwise fashion from acid digestion via the $(NaPO_3)_6$ procedure to the H_2O_2 method. An exception to this is *Batiacasphaera* spp., which proved most concentrated in the H_2O_2 preparation. Similarly, *Evansia deflandrei* was more relatively abundant in the H_2O_2 preparation than in the $(NaPO_3)_6$ method subsample. Species which are more concentrated using the $(NaPO_3)_6$ method are *Gonyaulacysta dentata* and *Pareodinia ceratophora*. Trends such as this based on low counts are less reliable than for the abundant species, for example the concentrations of *Pareodinia ceratophora* are relatively similar in both samples for all three methods (Table 2).

The trend of decreasing concentrations from acid digestion through $(NaPO_3)_6$ and H_2O_2 in the miospores is only followed by *Cerebropollenites macroverrucosus*. The concentrations of undifferentiated bisaccate pollen and *Classopollis classoides* appear to be relatively unaffected by the preparation method used. The concentrations of *Callialasporites* spp. per gram were markedly less using the two non-acid techniques, but this diminution is relatively similar. Furthermore, the concentration of *Perinopollenites elatoides* appears to have increased using the H_2O_2 method (Table 2).

Similarly, the diversity of palynomorphs diminishes using the two non-acid techniques. This trend is most marked in the marine forms, reducing from 30 to 20

taxa between acid digestion and the H_2O_2 method. The diversity of terrestrially-derived forms is reduced, but not as significantly (Table 1).

In terms of the percentages of the main kerogen macerals, the most marked trends are the increase in black/brown wood and the decrease in AOM and non-woody plant fragments from acid digestion through the $(NaPO_3)_6$ and the H_2O_2 methods. The percentages of palynomorphs are relatively similar in the acid and $(NaPO_3)_6$ preparations, but these are significantly sparser in the H_2O_2 slides (Table 1; Plates I, II).

4.2. Sample DUN 43

DUN 43 also produced an abundant and moderately diverse palynomorph assemblage (Tables 1, 2). The acid digestion method yielded 126,413 palynomorphs per gram; this concentration is by far the highest for all the three protocols employed (Table 2). The $(NaPO_3)_6$ method yielded 73,494 in situ grains per gram, which represents 58.1% of the density produced by the acid digestion method. This reduction in concentration is virtually identical (57.0%) for the indigenous marine palynomorphs. However, the concentration of in situ terrestrial palynomorphs produced using the $(NaPO_3)_6$ method is 73.5% of that produced by the acid digestion procedure. The H_2O_2 protocol was the least effective method, yielding 17,532 palynomorphs per gram; this represents 13.9% and 23.9% of the densities produced using the acid and $(NaPO_3)_6$ techniques respectively. This reduction in productivity using the H_2O_2 technique rather than the acid digestion method is far more for the in situ marine palynomorphs by comparison with the indigenous terrestrial palynomorphs. In marked contrast with DUN 42, the reduction in the concentrations of in situ miospores reduces stepwise from acid digestion through the $(NaPO_3)_6$ and H_2O_2 methods (Table 2).

For the majority of the most common dinoflagellate cysts, i.e. the *Meiourogonyaulax caytonensis* group, *Mendicodinium groenlandicum*, *Rigaudella aemula*, the *Sentusidinium rioultii* group and *Wanaea* spp., the concentrations produced reduced markedly between the acid and the $(NaPO_3)_6$ methods. These diminutions varied somewhat in magnitude, i.e. 38.2% for *Wanaea* spp. and 69.4% for *Rigaudella aemula*. However the concentration of *Gonyaulacysta jurassica* subsp.

adecta proved similar (Table 2). The concentrations of the majority of these abundant dinoflagellate cysts also reduce significantly with the H_2O_2 technique, and most of these reductions are relatively large. The reductions between the H_2O_2 and $(NaPO_3)_6$ methods are normally between 78.7% (for the *Sentusidinium rioultii* group) and 96.9% (for *Rigaudella aemula*). However, the reduction in the concentration of the *Meiourogonyaulax caytonensis* group between the H_2O_2 and $(NaPO_3)_6$ protocols is only 28.7% (Table 2). Hence *Meiourogonyaulax caytonensis* appears to be relatively resistant to oxidation with H_2O_2 (see above). This may be due to the relatively thick, robust autophragm. Typically, the concentrations of dinoflagellate cysts decrease in a stepwise fashion from acid digestion through the $(NaPO_3)_6$ method to the H_2O_2 technique. Examples of these forms include *Endoscrinium galeritum, Pareodinia ceratophora* and *Rhynchodiniopsis cladophora*. Exceptions to this trend are *Evansia deflandrei* and *Surculosphaeridium*? *vestitum*, which proved slightly more concentrated in the $(NaPO_3)_6$ preparation (Table 2). However, phenomena such as these that are based on low counts are less significant than analagous trends for the more abundant taxa.

The trend of decreasing concentrations from the acid technique through the $(NaPO_3)_6$ and H_2O_2 methods for the miospores is followed by *Cerebropollenites macroverrucosus, Cyathidites* spp. and *Perinopollenites elatoides*. The concentration of undifferentiated bisaccate pollen however, seems to be unaffected by the preparation method used; this is a similar trend seen in sample DUN 42 (see above). Of the two non-acid methods, the concentration of *Classopollis classoides* appears to be greater using the H_2O_2 technique (Table 1).

Palynomorph diversity diminishes using the two non-acid techniques. This phenomenon is greatest in the marine forms, reducing from 26, to 15 and 16 taxa between acid digestion and the $(NaPO_3)_6$ and H_2O_2 methods respectively. The diversity of terrestrially-derived forms is similar for all three preparation methods (Table 2).

The percentages of black/brown wood increased and the relative proportions of AOM and non-woody plant fragments decreased through the acid digestion, $(NaPO_3)_6$ and H_2O_2 techniques. By contrast the percentages of palynomorphs are comparable in the acid and $(NaPO_3)_6$ slides, but are significantly reduced in the H_2O_2 preparations (Table 1; Plates I, II).

4.3. Overview of samples DUN 42 and 43

This study has shown that, for these palynomorph-rich Jurassic marine claystones, acid digestion produces organic microfossil concentrations which are reduced by between 41.9% and 55.1% using the $(NaPO_3)_6$ technique. This reduction is reflected in the marine palynomorphs more than in the pollen and spores. The H_2O_2 preparations yield sparser palynomorph assemblages than the acid digestion and the $(NaPO_3)_6$ techniques. This reduction occurs in a stepwise fashion, and the H_2O_2 preparations produced between 9.6% and 13.9% of the concentrations produced by acid digestion. The palynomorph diminution in the H_2O_2 preparations, by comparison to the acid-based preparations, are most profound for the marine palynomorph associations (largely dinoflagellate cysts). However, the *Meiourogonyaulax caytonensis* group appears to be more resistant to oxidation damage by H_2O_2. Most miospores are also reduced in concentration stepwise from acid digestion through the $(NaPO_3)_6$ technique to the H_2O_2 protocol. However, the concentrations of undifferentiated bisaccate pollen and *Classopollis classoides* seem to be relatively unaffected by the preparation method used. The diversity of palynomorphs was diminished with the two non-acid techniques. This reduction was consistently higher in the marine palynomorphs. By contrast, the diversity of terrestrially-derived forms is substantially similar for all three preparation methods. This phenomenon may be a function of total diversity, which is significantly greater in the marine forms. Consistent trends were perceived in the kerogen macerals. The percentages of black/brown wood increased, and the relative proportions of AOM and non-woody plant fragments decreased through the acid digestion, $(NaPO_3)_6$ and H_2O_2 protocols. However, the percentages of palynomorphs are broadly comparable in the acid and $(NaPO_3)_6$ preparations, but these are reduced in the H_2O_2 preparations.

5. Discussion

This study has confirmed the conclusions of Riding et al. (2007) that the traditional acid digestion procedure is normally the most effective for extracting all

palynomorph groups. Clearly, the chemical removal of the calcareous and silicate mineral matrix maximises the concentration of the organic materials.

5.1. The sodium hexametaphosphate technique

The reduction in palynomorph productivity of the acid digestion method of approximately 50% with the $(NaPO_3)_6$ technique is most likely to be due to the incomplete dissagregation of the clay fraction, coupled with losses during the extensive sieving away of the small (<10 μm) clay particles. Notwithstanding the reduced palynomorph concentrations using the $(NaPO_3)_6$ technique, it unequivocally produces eminently workable palynofloras which are only slightly reduced in diversity compared to those produced by acid digestion. The marine palynomorphs are more reduced in diversity than the miospores, however this may be due to the greater diversity of dinoflagellate cysts in the material studied herein. It is highly likely that the full diversity of palynomorphs would be revealed by studying more slides of the $(NaPO_3)_6$ residue. This means that, at least for routine analyses, the $(NaPO_3)_6$ technique is an eminently viable alternative to acid digestion. Only a tiny proportion of the palynomorphs extracted from a sample are ever studied, so this factor is unlikely to be a serious problem with palynologically-productive material. It seems clear that the $(NaPO_3)_6$ method does not cause any significant oxidation of the palynomorphs. However, the AOM content of the organic residue was markedly reduced, so the $(NaPO_3)_6$ treatment does somehow affect the AOM. This is probably by physico-chemical means, and appears not to be through oxidation. This confirms the findings of Riding and Kyffin-Hughes (2004; 2006; 2010), who also noted this phenomenon. This means that any reduction in effectiveness is due to physical factors. This will hence allow future development of the technique to increase efficacy. This study has analysed the concentrations of individual taxa, and this has proved that the $(NaPO_3)_6$ method does not cause any significant taxonomic biases. However, the concentrations of bisaccate pollen and *Classopollis classoides* seem to be similar in both the mineral acid and the $(NaPO_3)_6$ preparations. The reason for this slight disparity to the majority of the remaining palynomorph taxa is not clear.

5.2. The hydrogen peroxide technique

The H_2O_2 protocol used here produced reasonable palynomorph associations, but these were relatively sparse compared with the other methods tested. This significantly reduced effectiveness is consistent with the findings of Riding and Kyffin-Hughes (2006) and Riding et al. (2007). This profoundly reduced palynomorph recovery, coupled with the reduced levels of AOM, means that the H_2O_2 technique unequivocally destroys significant proportions of the organic residue by oxidation. This is a significant problem with the use of H_2O_2, which is known to be an aggressive oxidising agent (Hopkins and McCarthy, 2002). These results strongly suggest that the $(NaPO_3)_6$ method is significantly superior to the H_2O_2 technique. However the H_2O_2 method will clearly produce workable palynomorph assemblages which can, for example, produce satisfactory biostratigraphical data and interpretations. The reason for this is that the palynomorph diversities in this study were comparable to those produced by the $(NaPO_3)_6$ technique (Table 1). Analysis of the concentrations of individual taxa has proved that the H_2O_2 method does not cause any significant taxonomic biases. This is probably largely due to the fact, in this study, the dinoflagellate cysts are overwhelmingly gonyaulacacean and hence not especially susceptible to destruction by oxidation. Peridiniacean dinoflagellate cysts are known to be more prone to damage by oxidation in comparison to gonyaulacalean forms (Dale, 1976; Schrank, 1988; Harland, 1989; Head, 1996; Williams et al., 2005; Riding et al., 2007). However, the concentration of bisaccate pollen is similar in both the mineral acid and the H_2O_2 preparations (Table 2). The reason for this slight disparity is not known.

6. Summary

Acid digestion using HCl and HF generally is the most efficient method of extracting palynomorphs from sediments and sedimentary rocks in terms of absolute numbers and diversities, however this protocol is potentially hazardous. Alternative methods using $(NaPO_3)_6$ and H_2O_2 have been developed recently, and these techniques are relatively safe. However, in most cases, these techniques are not as effective as acid digestion in terms of absolute palynomorph extraction. For example,

406 in this experiment on two samples of palynomorph-rich Upper Jurassic claystone, the
407 $(NaPO_3)_6$ method proved approximately 50% as effective as acid digestion. It appears
408 that the effectiveness of the $(NaPO_3)_6$ method is indirectly proportional to the levels of
409 lithification/induration of the material studied. Despite the disparity in effectiveness,
410 the $(NaPO_3)_6$ method produces clean (i.e. largely AOM-free) residues, and is suitable
411 for most projects using palynology. However, the apparent reduction in AOM may
412 make this technique unsuitable for palynofacies work. This is because the majority of
413 the taxa present were recovered in representative relative proportions using the
414 $(NaPO_3)_6$ method. This means that the key marker taxa will be recovered, and the
415 final interpretations will not be adversely affected by the reduced palynomorph
416 concentrations. No taxonomic biases were introduced by the $(NaPO_3)_6$ method.
417 The H_2O_2 technique was significantly less efficient, at around 10% of the acid
418 digestion level probably because it destroys palynomorphs by oxidation. Hydrogen
419 peroxide is hazardous as it is an oxidant and gives off a highly combustible mixture of
420 hydrogen and oxygen, hence a fume hood is required for this technique. Therefore the
421 $(NaPO_3)_6$ technique is safer and more effective. The latter thus appears to have
422 significant advantages over the H_2O_2 method. However, the H_2O_2 technique may be
423 useful in breaking down more indurated lithotypes, and can be used in isolation or in
424 combination with the $(NaPO_3)_6$ method (Riding and Kyffin-Hughes, 2004, appendix
425 2).
426 It is evident that the $(NaPO_3)_6$ protocol is a viable alternative to acid digestion.
427 This method clearly does not introduce any taxonomic biases. Furthermore, it is safe,
428 quick and inexpensive. It does not require particularly sophisticated laboratory
429 equipment and significant infrastructure such as a fume hood, and it is thus ideal for
430 work on drilling rigs and in remote fieldwork operations. Future research should be
431 undertaken to attempt to increase the effectiveness of this procedure in terms of the
432 absolute numbers of palynomorphs extracted using the acid digestion as a benchmark.
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434
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APPENDIX 1 – BRIEF DESCRIPTIONS OF THE THREE PALYNOMORPH PREPARATION TECHNIQUES USED IN THIS STUDY

This Appendix provides brief descriptions of the three preparation methods used herein.

i) Acid digestion

1 Air-dry the sample material and crush ca. 25-40 g to approximately five mm fragments.

2 Decalcify the crushed sample using 36% HCl until there is no more reaction with fresh HCl.

3 Decant-wash the decalcified sample residue until the supernatant liquor is neutral.

4 Treat the neutral residue with 40% HF to remove the silicate fraction until the reaction is complete.

5 Decant-wash the sample residue until the supernatant liquor is neutral.

6 Remove any resistant minerals and dense woody material by centrifugation and/or swirling in a large watch glass.

7 Concentrate the palynomorph concentrate and mount on microscope slides.

ii The sodium hexametaphosphate technique (adapted from Riding and Kyffin-Hughes, 2004, p. 42; 2006, p. 86)

1 Air-dry the sample material and crush ca. 100 g to approximately five mm fragments.

2 Add ca. 400 ml of water to the sample material and bring to the boil.

3 Add around 40 g of $(NaPO_3)_6$ to the mixture, stir very thoroughly and simmer for approximately 20 minutes.

4 Sieve the mixture using a 10 μm mesh to remove the <10 μm fraction of deflocculated clay particles, then wash out the $(NaPO_3)_6$ from the retained >10 μm fraction.

5 If any of the sample material remains undisaggregated, treat with H_2O_2 (see below) as necessary until it has all broken down.

6 Centrifuge and/or swirl the final residue to remove any resistant mineral grains.

7 Concentrate the palynomorph concentrate and mount on microscope slides.

606

607 **iii The hydrogen peroxide technique (adapted from Riding et al., 2007, p. 34)**

608 1 Air-dry the sample material and crush ca. 50-100 g to approximately one mm
609 fragments.

610 2 Place the sample material into a ceramic dish and place this on a pre-heated (to
611 ca. 100 °C) hot plate in a fume hood for around one minute.

612 3 Cover the sample material with 15%-30% H_2O_2, and heat extremely gently with
613 great care until the rock/sediment begins to disaggregate.

614 4 Decant off any floating disaggregated sample material into a beaker of cold
615 water to stop the reaction.

616 5 Repeat steps three and four as necessary until all the sample material has been
617 disaggregated.

618 6 Centrifuge and/or swirl the final residue to remove any resistant mineral grains.

619 7 Concentrate the palynomorph concentrate and mount on microscope slides.

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621

622 **APPENDIX 2 – LIST OF PALYNOMORPH SPECIES AND SUBSPECIES**

623

624 This Appendix lists all valid, formally defined palynomorph taxa below generic level
625 which were identified in samples DUN 42 and DUN 43 with full author citations. The
626 palynomorphs are listed alphabetically within their constituent groups. References to
627 the dinoflagellate cyst author citations can be found in Fensome and Williams (2004).

628

629 **Dinoflagellate cysts:**

630 *Ambonosphaera*? *staffinensis* (Gitmez 1970) Poulsen & Riding 1992

631 *Atopodinium prostatum* Drugg 1978

632 *Chytroeisphaeridia chytroeides* (Sarjeant 1962) Downie & Sarjeant 1965

633 *Ctenidodinium ornatum* (Eisenack 1935) Deflandre 1939

634 *Downiesphaeridium polytrichum* (Valensi 1947) Masure in Fauconnier & Masure
635 2004

636 *Endoscrinium galeritum* (Deflandre 1939) Vozzhennikova 1967

637 *Evansia deflandrei* (Wolfard & Van Erve 1981) Below 1990

638 *Fromea tornatilis* (Drugg 1978) Lentin & Williams 1981

639 *Gonyaulacysta centriconnata* Riding 1983

Gonyaulacysta dentata (Raynaud 1978) Lentin & Vozzhennikova 1990
Gonyaulacysta eisenackii (Deflandre 1939) Górka 1965
Gonyaulacysta jurassica (Deflandre 1939) Norris & Sarjeant 1965
subsp. *adecta* Sarjeant 1982
subsp. *jurassica* (autonym)
Meiourogonyaulax caytonensis (Sarjeant 1959) Sarjeant 1969
Mendicodinium groenlandicum (Pocock & Sarjeant 1972) Davey 1979
Nannoceratopsis pellucida Deflandre 1939
Pareodinia ceratophora Deflandre 1947
Pareodinia halosa (Filatoff 1975) Prauss 1989
Rhynchodiniopsis cladophora (Deflandre 1939) Below 1981
Rigaudella aemula (Deflandre 1939) Below 1982
Rigaudella filamentosa (Cookson & Eisenack 1958) Below 1982
Scriniodinium dictyotum Cookson & Eisenack 1960
Sentusidinium rioultii (Sarjeant 1968) Sarjeant & Stover 1978
Sirmiodinium grossii Alberti 1961
Surculosphaeridium? *vestitum* (Deflandre 1939) Davey et al. 1966
Trichodinium scarburghense (Sarjeant 1964) Williams et al. 1993
Tubotuberella dangeardii (Sarjeant 1968) Stover & Evitt 1978

Gymnospermous pollen:
Cerebropollenites macroverrucosus (Thiergart 1949) Schulz 1967
Classopollis classoides (Pflug 1953) Pocock & Jansonius 1961
Perinopollenites elatoides Couper 1958
Vitreisporites pallidus (Reissinger 1950) Nilsson 1958

Pteridophyte spores:
Concavissimisporites verrucosus Delcourt & Sprumont 1955
Ischyosporites variegatus (Couper 1958) Schulz 1967

Display material (Figure/Plate/Table) captions:

Fig. 1. The location of Dunans at Staffin Bay, northwest Skye, western Scotland. A – a sketch map of the Staffin Bay area illustrating the foreshore at Dunans where samples DUN 42 and DUN 43 were collected. B, C – the broader geographical context of the Staffin Bay area. Adapted from Riding (1992) and Riding and Thomas (1997).

Plate I. Photomicrographs of the different preparations of sample DUN 42. Figures 1-3 are representative low-magnification photomicrographs of the organic residues from each of the three preparation methods used.

1 Acid digestion preparation. Slide MPA 14067Acid/1, England Finder coordinate U61/1. The specimen of *Medicodinium groenlandicum* in the top left hand corner is 69 μm wide. Note the presence of AOM; for consistency with the non-acid preparations, this was not removed by oxidation.

2. Hydrogen peroxide preparation. Slide MPA 14067H_2O_2/1, England Finder coordinate H59. The specimen of *Rhynchodiniopsis cladophora* in the centre left is 91 μm long. Note the abundant dark wood fragments and the lack of AOM.

3. Sodium hexametaphosphate preparation. Slide MPA 14067$(NaPO_3)_6$/2, England Finder coordinate N43. The specimen of *Gonyaulacysta dentata* in the top left hand corner is 111 μm long. Note the lack of AOM.

4. *Rhynchodiniopsis cladophora.* Sodium hexametaphosphate preparation. Slide MPA 14067$(NaPO_3)_6$/2, BGS figured specimen MPK 14208, England Finder coordinate N39/4. The specimen is 87 μm in length.

Plate II. Photomicrographs of the different preparations of sample DUN 43. Figures 1-3 are representative low-magnification photomicrographs of the organic residues from each of the three preparation methods used.

1 Acid digestion preparation. Slide MPA 14068Acid/2, England Finder coordinate N64. The specimen of *Wanaea* sp. in the centre left is 84 μm wide. Note the presence of AOM; for consistency with the non-acid preparations, this was not removed by oxidation.

2. Hydrogen peroxide preparation. Slide MPA 14068H_2O_2/2, England Finder coordinate J52/1. The specimen of *Meiourogonyaulax* sp. in the top right hand corner is 67 μm long. Note the abundant dark wood fragments and the lack of AOM.

3. Sodium hexametaphosphate preparation. Slide MPA 14068$(NaPO_3)_6$/2, England Finder coordinate T54. The specimen of *Medicodinium groenlandicum* in the centre left is 69 μm wide. Note the sparsity of AOM.

4. *Sirmiodinium grossii.* Sodium hexametaphosphate preparation. Slide MPA 14068$(NaPO_3)_6$/1, BGS figured specimen MPK 14209, England Finder coordinate R63. The specimen is 60 μm in length.

Table 1. Key data on palynomorph counts and kerogen maceral distributions. This table illustrates the numbers of indigenous Jurassic palynomorphs and the spiked *Lycopodium* spores counted for the three subsamples each for samples DUN 42 and DUN 43 prepared using the three different protocols. Occurrences recorded outside the main count are indicated with an 'X'. Three dots (…) indicate that the respective palynomorphs are absent. The palynomorphs are listed alphabetically in five groups. The totals of in situ marine palynomorphs, in situ terrestrial palynomorphs, all in situ palynomorphs and exotic *Lycopodium* spores are given. The diversities of indigenous marine palynomorphs, terrestrial palynomorphs and all palynomorphs are listed. The information on the distribution of the four main kerogen macerals are given as percentages.

Table 2. Key data on palynomorph concentrations. This table illustrates the concentrations per gram of indigenous Jurassic palynomorphs for the three subsamples each of samples DUN 42 and DUN 43 prepared using the three different protocols. The palynomorphs are listed alphabetically in four groups. The total concentrations of in situ marine palynomorphs, in situ terrestrial palynomorphs and all in situ palynomorphs are listed.