

1 The use of pre-treatments in palynological processing

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11 ABSTRACT

12  
13 A sample of palynomorph-rich Upper Carboniferous mudstone from Scotland  
14 was separately pre-treated overnight with acetone, two detergent solutions, formic  
15 acid, household bleach (two methods), methylated spirits and white spirit prior to  
16 palynological preparation using sodium hexametaphosphate [(NaPO<sub>3</sub>)<sub>6</sub>]. The aim of  
17 this study was to identify effective methods of pre-treatment that would increase  
18 palynomorph yields using the (NaPO<sub>3</sub>)<sub>6</sub> method. Pre-treatment generally increased the  
19 mass of sample that was broken down by the (NaPO<sub>3</sub>)<sub>6</sub> technique. Detergent one  
20 (carpet cleaner), formic acid, household bleach and white spirit allowed the  
21 disaggregation of more rock than without any pre-treatment. However, formic acid  
22 produced a lower concentration of yield of Carboniferous miospores than with no pre-  
23 treatment. Pre-treatment with acetone, detergent two (industrial detergent) and  
24 methylated spirits actually decreased the weight of rock that was disaggregated with  
25 (NaPO<sub>3</sub>)<sub>6</sub>. Despite this, all these three pre-treatments improved the palynomorph yield  
26 as compared to with no pre-treatment. Moreover, all the pre-treatments except formic  
27 acid improved palynomorph productivity. The effectiveness of pre-treatments was  
28 demonstrated by the increased absolute numbers of indigenous palynomorphs  
29 extracted. However, the concentrations of miospores per gram of rock are more  
30 significant. Acetone, both detergent solutions, methylated spirit and white spirit  
31 significantly improved the amounts of palynomorph extracted. Household bleach was  
32 found to lighten and selectively destroy relatively delicate palynomorphs; this reagent  
33 should be used with caution, and only with robust material. In the subsample soaked  
34 overnight in 5% bleach solution, all the exotic *Lycopodium* spores added were  
35 destroyed. By contrast in the subsample treated with 2.5% bleach solution for six  
36 hours, a small proportion of the exotic *Lycopodium* spores survived. This study  
37 indicates that the (NaPO<sub>3</sub>)<sub>6</sub> method using either detergent or white spirit as a pre-  
38 treatment is highly effective at extracting palynomorphs from clay-rich lithotypes.  
39 However the concentration of palynomorphs obtained is generally lower than those  
40 from mineral acid digestions.

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42 *Keywords:* palynomorph preparation techniques; pre-treatment; sodium  
43 hexametaphosphate; Carboniferous; United Kingdom (Scotland).

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46 **1. Introduction**

47  
48 The pre-treatment of samples for palynology is not new. Raistrick (1934, p.  
49 143) for example reported that high rank coals macerate more effectively in  
50 Schultze's solution if the sample is pretreated by soaking in cold pyridine for 24

51 hours. Van Cleave and Ross (1947) subsequently noted that pre-treatment of  
52 palynomorph residues with a suitable detergent may help the penetration of stain.  
53 Samples of sedimentary rock or unconsolidated sediment for palynological analysis  
54 are sometimes soaked in water or surface-active substances such as detergent solution,  
55 ethanol or other reagents prior to the main (acid-based) processing procedure. This is  
56 to attempt to deflocculate or soften the sample material so that the subsequent  
57 processing proceeds quickly and effectively. Organic pre-treatment reagents such as  
58 acetone, methylated spirits and white spirit penetrate the interstices of the sample  
59 material and start to break it down by the pressure developed. A wetting agent may  
60 aid this penetration. Alternatives to pre-treatment are to use the power of  
61 crystallisation of, for example, sodium salts to physically break up the sample  
62 material or simply not to pre-treat (Faegri et al., 1989, p. 76).

63 In this study, the effects of seven reagents for the pre-treatment of an  
64 extremely palynomorph-rich Upper Carboniferous mudstone before processing using  
65  $(\text{NaPO}_3)_6$  were tested. The pre-treatment reagents used were acetone, two detergents  
66 (a domestic carpet cleaner and Decon 90), formic acid, household bleach (sodium  
67 hypochlorite solution – two methods), methylated spirits and white spirit. These were  
68 chosen because it was felt that they could all potentially soften and/or partially  
69 disaggregate the sample material and hence expedite clay deflocculation with  
70  $(\text{NaPO}_3)_6$ . Formic acid, methylated spirits, sodium hypochlorite and white spirit have  
71 been used to extract calcareous and phosphatic microfossils (Armstrong and Brasier,  
72 2005).

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## 75 **2. Background**

76

77 The present authors have recently developed methods of preparing  
78 palynomorphs from sedimentary rocks and sediments without using aggressive  
79 mineral acids such as hydrochloric acid (HCl) and hydrofluoric acid (HF). These acids  
80 dissolve carbonate and silicate minerals respectively, and acid digestion is the  
81 standard method of extracting palynomorphs (e.g. Gray, 1965a,b; Doherty, 1980;  
82 Phipps and Playford, 1984; Wood et al., 1996; Batten, 1999; Green, 2001; Brown,  
83 2008). The non-acid techniques involve the use of sodium hexametaphosphate  
84  $[(\text{NaPO}_3)_6]$ , and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Riding and Kyffin-Hughes, 2004, 2006;  
85 Riding et al., 2006; 2007).

86 Sodium hexametaphosphate, sometimes abbreviated to SHMP, is a hexamer  
87 which is prepared by melting monosodium orthophosphate followed by rapid cooling.  
88 Alternative names include Calgon, glassy sodium and Graham's Salt. It hydrolyzes in  
89 aqueous solution to sodium trimetaphosphate and sodium orthophosphate. The pH of  
90  $(\text{NaPO}_3)_6$  is neutral (7), and it is not an oxidising agent. This substance has a wide  
91 range of applications, and is used as a detergent, a powerful deflocculant or a  
92 dispersant for clay and soil and a water softener. It is also used as a food additive, and  
93 has the E-number E452i. Sodium hexametaphosphate is a relatively non-hazardous  
94 substance, however significant ingestion may cause an allergic reaction. It reduces the  
95 coherence of the clay fraction because phosphate ions are strongly adsorbed onto the  
96 particles of clay, which are broken up to sub-10  $\mu\text{m}$  particles due to the high ionic  
97 charges. This allows the dispersed clay to be separated from the organic fraction by  
98 sieving.

99 By contrast,  $\text{H}_2\text{O}_2$  is a strong oxidising agent, weakly acidic and slightly  
100 viscous. Pure  $\text{H}_2\text{O}_2$  is pale blue, but it becomes colourless when diluted. It is used in

101 the chemical industry, for bleaching, disinfecting and as a propellant. The major  
102 hazards pertaining to H<sub>2</sub>O<sub>2</sub> are its corrosive and oxidising properties, especially at  
103 high concentrations (i.e. >50%). Additionally, because it dissociates to form water  
104 and oxygen, it can form potentially explosive mixtures if allowed to mix with  
105 combustible materials. To minimise this phenomenon, a stabiliser is normally added  
106 to commercially-supplied H<sub>2</sub>O<sub>2</sub> to decrease the dissociation rate. Riding and Kyffin-  
107 Hughes (2007, p. 21, 22) described the health and safety issues surrounding H<sub>2</sub>O<sub>2</sub> in  
108 detail. By contrast with (NaPO<sub>3</sub>)<sub>6</sub>, H<sub>2</sub>O<sub>2</sub> disaggregates clay-rich materials physico-  
109 chemically. Because H<sub>2</sub>O<sub>2</sub> spontaneously dissociates into oxygen and water, it causes  
110 the physical disintegration of clays by ‘deposit swelling’. This is the action of the  
111 oxygen bubbles which are generated within the matrix of the sample material when  
112 H<sub>2</sub>O<sub>2</sub> dissociates. The expansion pressure of the dissociated H<sub>2</sub>O<sub>2</sub> which has soaked  
113 into the sample material breaks up the rock/sediment. Hydrogen peroxide is also a  
114 powerful oxidising agent, and this helps to simultaneously extract palynomorphs by  
115 breaking down amorphous organic material (Riding et al., 2007, pl. 2, 3). Naturally,  
116 this reagent must be used carefully because it can damage or destroy palynomorphs by  
117 oxidation (Hopkins and McCarthy, 2002).

118 The (NaPO<sub>3</sub>)<sub>6</sub>, and H<sub>2</sub>O<sub>2</sub> procedures therefore differ from HCl and HF  
119 digestion in that the mineral fraction is broken up and sieved off, rather than being  
120 dissolved or etched away. Both (NaPO<sub>3</sub>)<sub>6</sub>, and H<sub>2</sub>O<sub>2</sub> appear to work well on most  
121 clay-rich materials. These procedures are however markedly less effective on  
122 carbonate lithotypes (Riding and Kyffin-Hughes, 2004, figs. 4E, 4F). Furthermore,  
123 H<sub>2</sub>O<sub>2</sub> appears to be superior to (NaPO<sub>3</sub>)<sub>6</sub> for preparing relatively indurated mudstones  
124 (Riding et al., 2007). The avoidance of using HCl and HF is important because these  
125 acids are hazardous to laboratory personnel and to the wider environment.  
126 Furthermore, the costs of installation and maintenance of acid-safe laboratory  
127 facilities are relatively high.

128 Riding and Kyffin-Hughes (2004, 2006) recommended the pre-treatment of  
129 samples with a strong detergent for several hours prior to preparation with (NaPO<sub>3</sub>)<sub>6</sub>.  
130 This pre-treatment appears to soften the sample material, and allow a greater surface  
131 area for deflocculation with the (NaPO<sub>3</sub>)<sub>6</sub>. The purpose of this study is to test seven  
132 different pre-treatment reagents prior to palynomorph preparation using (NaPO<sub>3</sub>)<sub>6</sub>.  
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### 135 3. Material and methods

137 In this study, a sample of Upper Carboniferous (Westphalian B) mudstone was  
138 prepared. The material is from British Geological Survey (BGS) offshore borehole  
139 number 74/13, which was rotary-drilled 17 km east of Fife Ness in the Forth  
140 Approaches, offshore southeast Scotland, United Kingdom (Owens and Marshall,  
141 1978, p. 19, figs. 1, 3). This borehole was fully cored, with excellent recovery  
142 achieved. The location of the borehole is 56° 18.10’N; 02° 19.30’W (Fig. 1). The  
143 material used is a composite sample of conventional core between 19.00 and 17.55 m,  
144 and was registered as BGS sample MPA 57940. This Upper Carboniferous mudstone  
145 is known to be extremely rich in well-preserved spores and pollen (Riding et al.,  
146 2007).

147 The composite sample was air-dried, crushed to approximately 1 mm  
148 fragments and thoroughly manually homogenised. Ten 5 g subsamples of this sample  
149 were measured, and eight of these were separately mixed with 50 ml of the pre-  
150 treatment reagents (acetone, two detergent solutions, formic acid, household bleach

151 [two methods], methylated spirits and white spirit), and left to stand overnight. It was  
152 anticipated that each of the pre-treatment regimes would soften the sample material  
153 and/or commence deflocculation of the clay. This would then enable the  $(\text{NaPO}_3)_6$  to  
154 more efficiently break down the clay fraction, thereby releasing significantly more  
155 palynomorphs. Two control subsamples were prepared. The first of these was  
156 prepared simply using the  $(\text{NaPO}_3)_6$  method of Riding and Kyffin-Hughes (2004;  
157 2006) with no pre-treatment. The second control subsample was prepared using the  
158 standard HCl/HF digestion method (e.g. Gray, 1965b; Doherty, 1980; Phipps and  
159 Playford, 1984; Wood et al., 1996; Green, 2001) without oxidation, and again with no  
160 pre-treatment. The hydrochloric and hydrofluoric acid treatments lasted until the  
161 respective reactions were complete. By contrast, the  $(\text{NaPO}_3)_6$  subsamples were  
162 treated for 20 minutes only.

163 To allow the relative effectiveness of each of the pre-treatment reagents, the  
164 concentrations of palynomorphs were calculated. The exotic marker method using  
165 *Lycopodium clavatum* tablets as a spike was used for this (Benninghoff, 1962;  
166 Stockmarr, 1971). Ten *Lycopodium* tablets were added to each of the nine subsamples  
167 prior to the preparation procedure; including the pre-treatment phase. At least 350  
168 Carboniferous pollen and spores were counted (Table 1). Damaged palynomorphs  
169 were counted. Fragments which are c. 50% were counted and aggregated into the  
170 count; however any small portions (<25%) were disregarded. The absolute  
171 abundances of Carboniferous miospores were calculated using the equation of  
172 Benninghoff (1962), i.e.:

173

$$174 \quad c = \frac{m_c \times L_t \times t}{L_c \times w}$$

175

176 This is where:

177  $c$  = the number of indigenous (i.e. Carboniferous) miospores per gram of dry rock (=

178 concentration)

179  $m_c$  = the number of indigenous (i.e. Carboniferous) miospores counted

180  $L_t$  = the number of *Lycopodium* spores in each tablet (i.e. 18,583)

181  $t$  = the number of tablets added to the sample (i.e. 10)

182  $L_c$  = the number of *Lycopodium* spores counted

183  $w$  = the weight of dry sediment processed in grams

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185 It should be noted that it has been demonstrated that exotic *Lycopodium* spores  
186 may be lost during preparation, largely during the decantation and sieving  
187 stages (Mertens et al., 2009). Selected low-magnification photomicrographs of the  
188 residues are presented in Figs. 2-11. The remaining sample material, organic residues,  
189 microscope slides, primary data and illustrated material are housed in the collections  
190 of the British Geological Survey (BGS), Keyworth, Nottingham NG12 5GG, United  
191 Kingdom.

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#### 194 **4. Results**

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196 The sample produced highly abundant and well-preserved spores and pollen  
197 which are mid/dark brown in colour (Figs. 2-11). Spores are more abundant than

198 pollen. This unit is a freshwater deposit, and no marine microplankton are present.  
199 The assemblage is of Middle Pennsylvanian–Duckmantian (Late Bashkirian–Early  
200 Moscovian or Atokan) age, and is dominated by *Lycospora pusilla* (Ibrahim 1932)  
201 Somers 1972 together with common *Crassispora* spp. and *Florinites* spp. The  
202 occurrences of *Endosporites globiformis* (Ibrahim 1932) Schopf et al. 1944 and  
203 *Florinites junior* Potonié & Kremp 1956 are indicative of the *Microreticulatisporites*  
204 *nobilis-Florinites junior* (NJ) Biozone of Clayton et al. (1977). Other miospores  
205 observed are entirely consistent with the NJ Biozone, and include *Cirratriradites*  
206 *saturni* (Ibrahim 1932) Schopf et al. 1944, *Cristatisporites indignabundus* (Loose  
207 1932) Staplin & Jansonius 1964, *Grumosisporites varioreticulatus* (Neves 1958)  
208 Smith & Butterworth 1967, *Raistrickia fulva* Artüz 1957, *Raistrickia saetosa* (Loose  
209 1932) Schopf et al. 1944, *Raistrickia superba* (Ibrahim 1933) Schopf et al. 1944,  
210 *Reinschospora triangularis* Kosanke 1950, *Simozonotriletes intortus* (Waltz 1938)  
211 Potonié & Kremp 1954, *Triquitrites bransonii* Wilson & Hoffmeister 1956,  
212 *Triquitrites sinani* Artüz 1957, *Vestispora cancellata* (Dybová & Jachowicz 1957)  
213 Wilson & Venkatachala 1963 and *Vestispora costata* (Balme 1952) Spode in Smith &  
214 Butterworth 1967.

215 As previously mentioned, the acid preparation was allowed to proceed until  
216 the reactions were complete, but the  $(\text{NaPO}_3)_6$  treatments were given 20 minutes. The  
217 prepared residues from the ten subsamples studied were examined and the indigenous  
218 Carboniferous pollen and spores and the exotic *Lycopodium* spores were counted.  
219 These data, together with the dry weight of sample macerated, the concentration of  
220 indigenous palynomorphs (based on the actual weight of rock broken down and on 5.0  
221 g) and the calculated number of indigenous palynomorphs are presented as Table 1.  
222 The actual weights of the subsamples prepared are considered to be highly significant  
223 (see below).

224 Following both the acid and  $(\text{NaPO}_3)_6$  preparations, the residues were sieved  
225 to remove the  $>500 \mu\text{m}$  fraction. This largely comprises undigested or  
226 undeflocculated rock as appropriate. Unsurprisingly, the acid digestion gave the  
227 lowest amount (0.9 g) of undigested rock residue. The remaining  $(\text{NaPO}_3)_6$   
228 preparations deflocculated between 1.5 and 4.0 g of the initial 5.0 g used (Table 1);  
229 hence the undeflocculated residues using  $(\text{NaPO}_3)_6$  were between 1.0 and 3.5 g. The  
230 concentrations of indigenous palynomorphs based on the actual weight of rock broken  
231 down, and on the full 5.0 g of each subsample are presented in Table 1. This strategy  
232 was adopted to emphasise the difference in palynomorph concentrations if the actual  
233 weight of rock disaggregated or dissolved is taken into account. Many quantitative  
234 studies do not allow for any unprocessed raw sample material which potentially can  
235 liberate palynomorphs. Moreover, this methodology clearly demonstrate that the  
236  $(\text{NaPO}_3)_6$  method normally does not fully break down relatively indurated lithotypes.

237 The results of this study are discussed in the remainder of this section,  
238 subsample by subsample. Generally, the eight overnight pre-treatments did not cause  
239 any discernible physical changes to the sample material. However, it was notable that,  
240 except for formic acid, when the material was mixed with  $(\text{NaPO}_3)_6$ , it generally  
241 disaggregated significantly faster than material which had no pre-treatment.  
242 Prolonged soaking in pre-treatment reagents however can cause physical changes. For  
243 example, in another experiment which is not described in detail here, a subsample of  
244 this Carboniferous mudstone was completely disaggregated after soaking for one  
245 week in white spirit.

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248 4.1. *The control subsample prepared by hydrochloric/hydrofluoric acid digestion*  
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250 In order to make comparisons with the seven (NaPO<sub>3</sub>)<sub>6</sub> preparations, a  
251 subsample was prepared using the standard mineral acid digestion technique. This  
252 subsample received no pre-treatment, and the residue was not oxidised following  
253 hydrofluoric acid treatment. The sample was crushed to pea-sized fragments and  
254 treated separately with hydrochloric acid and hydrofluoric acid to remove the  
255 carbonate and silicate minerals respectively. The acid digestion proved highly  
256 effective; 4.1 g of the initial 5.0 g of rock was eliminated. Following the acid  
257 treatment, the organic concentrate was sieved using a 10 µm mesh to remove the fine  
258 material which tends to obscure the palynomorphs. The sample prepared in this way  
259 produced 341,746 palynomorphs per gram and 1,401,158 grains in total (Table 1).  
260 The concentration is significantly higher than that obtained by Riding et al. (2007) for  
261 similar material using the volume aliquot method described by Dale (1976) and  
262 Harland (1989). A sample of this Carboniferous unit was prepared from borehole  
263 74/13 at 18.07 m, and a palynomorph concentration of 54,600 grains per gram was  
264 determined (Riding et al. (2007, table 1). The reasons behind this apparent  
265 underestimation are not clear. The volume aliquot method requires accurate  
266 measurements, but the disparity noted here is well beyond confidence limits and  
267 experimental error. Another reason may be that this mudstone unit exhibits  
268 significantly variable palynomorph concentrations because the sample material in this  
269 study is from between 19.00 and 17.55 m in BGS borehole 74/13.

270 It seems most likely that this anomaly is largely due to significant losses of  
271 palynomorphs during the various laboratory procedures. This will affect aliquot  
272 methods more than the exotic *Lycopodium* spore method, which uses a ratio  
273 (Stockmarr, 1971). De Vernal et al. (1987) noted that concentrations of palynomorphs  
274 determined using the weight aliquot method are 33% lower than those worked out  
275 with the marker-grain method. However, in a similar test, Mertens et al. (2009) found  
276 that exotic *Lycopodium* spores are prone to losses during preparation.

277 The preparation is of a reasonable standard, however moderate levels of  
278 amorphous organic material (AOM) are present (Fig. 2). This AOM could be removed  
279 by oxidising the residue with nitric acid or Schultze's solution. However, the  
280 (NaPO<sub>3</sub>)<sub>6</sub> preparations were not separately oxidised, hence it was decided to maintain  
281 consistency and not to oxidise the HCl/HF preparation.

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284 4.2. *The control subsample prepared with sodium hexametaphosphate*  
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286 So that the subsamples prepared using (NaPO<sub>3</sub>)<sub>6</sub> with pre-treatments can be  
287 objectively assessed, a control subsample was processed. This was using the (NaPO<sub>3</sub>)<sub>6</sub>  
288 method without any pre-treatment prior to the addition of flakes of (NaPO<sub>3</sub>)<sub>6</sub> (Riding  
289 and Kyffin-Hughes (2004, appendix 2; 2006, appendix 3). The treatment proved  
290 moderately effective, but 3.0 g of the initial 5.0 g of sample was not broken down  
291 after soaking overnight (Table 1). The 2.0 g sample prepared in this way produced  
292 104,427 palynomorphs per gram; this represents 208,853 grains in the subsample  
293 prepared (Table 1). This concentration compares with 341,746 pollen/spores per gram  
294 using HCl/HF. In this highly productive lithotype, the fact that the preparation is  
295 somewhat less efficient in terms of absolute extraction has no bearing in terms of  
296 normal palynological analysis. The effectiveness disparity does not bias the relative  
297 proportions of the taxa in the sample. In fact, because palynologists routinely study

298 only a miniscule proportion of the grains extracted from any one sample, this is hardly  
299 ever likely to be a serious problem. The ‘efficiency gap’ using  $(\text{NaPO}_3)_6$  would only  
300 be a problem with extremely organic-lean samples such as the Neoproterozoic  
301 material from Australia studied by Grey (1999). The fact that  $(\text{NaPO}_3)_6$  cannot  
302 entirely disaggregate relatively indurated lithotypes such as the Carboniferous  
303 mudstone tested here emphasises the need for an effective pre-treatment regime. In a  
304 previous study, Riding et al. (2007) used the volume aliquot method for quantitative  
305 assessments. However these authors did not undertake a quantitative study of the  
306 mudstone used in this work using  $(\text{NaPO}_3)_6$ , so a meaningful comparison between the  
307 volume aliquot method and the *Lycopodium* spore spiking method for this sample  
308 cannot be made in this case.

309 The  $(\text{NaPO}_3)_6$  preparation proved very clean, and was largely devoid of AOM  
310 (Fig. 3). This phenomenon was also noted by Riding and Kyffin-Hughes (2004; 2006,  
311 pl. 4) and it appears that  $(\text{NaPO}_3)_6$  can disaggregate AOM, in addition to clay  
312 minerals. This reagent is not an oxidising agent and it seems likely that  $(\text{NaPO}_3)_6$   
313 breaks up AOM using ionic charges, i.e. in a similar way to how it disaggregates  
314 clays. This phenomenon is extremely useful in that it potentially negates the need to  
315 use hydrochloric, hydrofluoric and nitric acids in palynological preparation.  
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#### 318 4.3. *The subsample pre-prepared with acetone*

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320 Acetone ( $\text{CH}_3\text{COCH}_3$ ) is a colourless, flammable liquid ketone. It is miscible  
321 with most liquids, and is used as nail polish remover and in paint thinners. Acetone is  
322 a solvent for most plastics, and should always be used in glass containers. It auto-  
323 ignites at  $465^\circ\text{C}$ , and acetone pre-treatments should be done in a fume cupboard. This  
324 substance is not highly toxic but it can be harmful by inhalation, ingestion or  
325 absorption.

326 It was thought that acetone may help to soften the sample material. However,  
327 because acetone is a volatile substance, the vessel should be monitored and topped-up  
328 if the pre-treatment is done over several days. In another experiment, during the  
329 course of several days of pre-treatment, all the acetone evaporated despite the vessel  
330 being partially covered. An overnight treatment however does not encounter this  
331 problem.

332 The acetone pre-treatment appears to have been significantly beneficial.  
333 However, acetone pre-treatment did not increase the mass of sample which was  
334 disaggregated by the  $(\text{NaPO}_3)_6$ . Following overnight pre-treatment with acetone, 3.3 g  
335 of the 5.0 g subsample remained following the 20 minute treatment with  $(\text{NaPO}_3)_6$   
336 (Table 1). This means that acetone pre-treatment enabled 0.3 g less rock to be  
337 disaggregated compared with no pre-treatment (Table 1). Despite this, the  
338 palynomorph yield was increased by approximately 50% by the acetone pre-  
339 treatment. The pre-treated sample yielded 151,929 grains per gram, as opposed to  
340 104,427 palynomorphs per gram with no pre-treatment (Table 1). The numbers of  
341 Carboniferous spores in the acetone pre-treated and control subsamples are 258,280  
342 and 208,853 respectively (Table 1). Hence it appears that this pre-treatment softened  
343 the material, and began to deflocculate the clay fraction.

344 The acetone pre-treatment had no adverse effect on the  $(\text{NaPO}_3)_6$  preparation.  
345 The residue was clean, the palynomorphs were abundant, and had not been bleached  
346 or damaged in any way (Fig. 4). Furthermore, no differential degradation or  
347 destruction of the pollen and spores was noted.

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#### 350 4.4. The subsample pre-prepared with detergent solutions

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352 Two types of detergent were used in this study; these are a household carpet  
353 cleaner and an industrial grade detergent.

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##### 355 4.4.1 Domestic carpet cleaner solution

356 The first detergent is a domestic carpet cleaner especially formulated for use  
357 on heavily-used carpets. It is claimed to be an effective pre-treatment for soiled  
358 carpets, specifically breaking down oil and soil. The cleaner is a clear liquid which  
359 smells of the active ingredient, diethylene glycol monobutyl ether. It is not hazardous,  
360 but accidental spillage may cause eye and skin irritation, and it is moderately toxic if  
361 ingested.

362 It was thought that a 3% solution of this domestic carpet cleaner would  
363 partially disaggregate the sample prior to the main preparation procedure. According  
364 to Riding and Kyffin-Hughes (2004; 2006), samples to be prepared with  $(\text{NaPO}_3)_6$   
365 should be soaked overnight in a detergent solution. The overnight pre-treatment with  
366 the carpet cleaner solution increased the weight of sample which was then treated by  
367 the  $(\text{NaPO}_3)_6$ . Following the overnight pre-treatment with carpet cleaner solution, 1.7  
368 g of the 5.0 g subsample remained undisaggregated following treatment with  
369  $(\text{NaPO}_3)_6$  (Table 1). This represents a significant improvement compared to no pre-  
370 treatment. The yield of palynomorphs, however, was enhanced by approximately  
371 150%; this is assumed to be largely due to the pre-treatment with the carpet cleaner  
372 solution. The pre-treated subsample yielded 262,790 *in situ* palynomorphs per gram  
373 compared with 104,427 palynomorphs per gram with no pre-treatment (Table 1). This  
374 increase is also reflected in the absolute numbers of indigenous palynomorphs  
375 extracted; the numbers of Carboniferous spores in the control subsample and  
376 subsample pre-treated with carpet cleaner solution are 208,853 and 867,207  
377 respectively (Table 1). Hence the pre-treatment with carpet cleaner solution  
378 apparently appears to be extremely effective. The pre-treatment apparently started the  
379 clay disaggregation process, thus allowing the  $(\text{NaPO}_3)_6$  to act on partially softened  
380 clay and thereby extracting a higher proportion of Carboniferous palynomorphs. The  
381 pre-treatment with carpet cleaner solution does not appear to cause damage to either  
382 the *in situ* or the exotic palynomorphs (Fig. 5).

383

##### 384 4.4.2 Industrial detergent solution

385 The second detergent used was Decon 90, an industrial grade concentrated  
386 liquid detergent suitable for use in ultrasonic baths. It is a biodegradable emulsion  
387 including anionic and non-ionic surface-active agents. Decon 90 is used for cleaning  
388 and decontaminating a wide variety of media, however, it is unsuitable for use on  
389 non-ferrous metals such as aluminium and zinc. A 2-5% solution is normally  
390 adequate. The toxicity level is low, but the high alkalinity (the concentrate has a pH of  
391 >13) means that it is potentially hazardous, especially at high concentrations.

392 It was anticipated that a 3% solution of Decon 90 may significantly soften the  
393 sample. Riding and Kyffin-Hughes (2004; 2006) recommended that samples to be  
394 prepared with  $(\text{NaPO}_3)_6$  are soaked overnight in a dilute solution of a strong detergent.  
395 The overnight pre-treatment with Decon 90 did not increase the weight of sample  
396 which was then treated by the  $(\text{NaPO}_3)_6$ . Like with acetone, after the overnight pre-  
397 treatment with Decon 90, 3.3 g of the 5.0 g subsample remained undisaggregated



398 following the  $(\text{NaPO}_3)_6$  treatment (Table 1). This therefore does not represent an  
399 improvement on no pre-treatment. However, the palynomorph yield was more than  
400 doubled, presumably by the Decon 90 pre-treatment. The Decon 90 pre-treated  
401 subsample yielded 216,599 grains per gram compared with 104,427 palynomorphs  
402 per gram with no pre-treatment (Table 1). The numbers of Carboniferous spores in the  
403 Decon 90 pre-treated and control subsamples are 368,219 and 208,853 respectively  
404 (Table 1). Hence the pre-treatment with Decon 90 also appears to have been highly  
405 effective. The pre-treatment initiated the deflocculation of the clay fraction, allowing  
406 the  $(\text{NaPO}_3)_6$  to work on partially broken down clay and thus extracting a higher  
407 proportion of palynomorphs. The pre-treatment with Decon 90 did not apparently  
408 selectively degrade or destroy the palynomorphs (Fig. 6).

409  
410

#### 411 4.5. *The subsample pre-prepared with formic acid*

412

413 Formic acid ( $\text{CH}_2\text{O}_2$ ) is a simple carboxylic acid, and occurs in the venom of  
414 ant and bee stings. It is miscible in water and most organic solvents, is partially  
415 soluble in hydrocarbons and may be dissociated by heat. Formic acid is not an  
416 oxidising agent, and has some reducing properties. This substance is used as an  
417 antibacterial agent and as a preservative. The principal hazards associated with formic  
418 acid are eye and respiratory tract damage, and skin burns. Thus full personal  
419 protective equipment should be worn when working with >10% formic acid. All use  
420 of this reagent should be done in a fume hood as carbon monoxide (CO) may be  
421 present in the vapours produced.

422 It was thought that 80% formic acid may be a potentially effective pre-  
423 treatment reagent and could effect some disaggregation before the  $(\text{NaPO}_3)_6$  treatment  
424 is begun. The pre-treatment with formic acid initially appeared to have been effective  
425 because it increased the weight of sample which was available for treatment by  
426  $(\text{NaPO}_3)_6$ . Following the overnight pre-treatment with formic acid, 1.8 g of the 5.0 g  
427 subsample remained following treatment for 20 minutes with  $(\text{NaPO}_3)_6$  (Table 1). The  
428 subsample prepared simply using  $(\text{NaPO}_3)_6$  with no pre-treatment left 3.0 g of rock  
429 undisaggregated (Table 1). However, this improved disaggregation did not translate to  
430 a higher palynomorph yield per gram. The yield was slightly reduced in comparison  
431 to the  $(\text{NaPO}_3)_6$  control subsample. The sample pre-treated with formic acid yielded  
432 93,889 grains per gram compared with 104,427 palynomorphs per gram for the  
433 control subsample (Table 1). The numbers of Carboniferous spores in the formic acid  
434 pre-treated and control subsamples are 300,444 and 208,853 respectively (Table 1).  
435 Therefore the pre-treatment with formic acid does not apparently give any advantage.  
436 However, the formic acid pre-treatment did not cause any discernible damage to the  
437 palynomorphs (Fig. 7).

438

439

#### 440 4.6. *The subsample pre-prepared with household bleach*

441

442 Household bleach is a 3–6% aqueous solution of sodium hypochlorite  
443 ( $\text{NaClO}$ ). The concentration gradually decreases during storage. A weak solution (i.e.  
444 ca. 1%) will sanitise kitchen surfaces; stronger solutions (12–15%) are used to  
445 chlorinate and disinfect water supplies. Sodium hypochlorite solution (0.5–5.25%) is  
446 also used in endodontics during root canal treatment to remove necrotic nerve tissue.  
447 Sodium hypochlorite is corrosive due to its alkaline nature, and concentrated solutions

448 can cause eye damage and burn skin. It is a strong oxidising agent, and it may release  
449 chlorine if mixed with acids.

450 Sodium hypochlorite solution is an oxidant, and is used in the processing of  
451 siliciclastic rocks for palynomorphs after the acid digestion stage (e.g. Lee, 1964;  
452 Batten, 1999; Green, 2001; Traverse, 2007). It is one of the gentlest oxidising agents  
453 used in palynological processing, being significantly less aggressive than any of the  
454 nitric acid-based reagents such as Schultze's solution (Evitt, 1984; Eshet and Hoek,  
455 1996). Sodium hypochlorite has been used to macerate coals (Hoffmeister, 1960;  
456 Smith and Butterworth, 1967), to remove pyrite (Merrill, 1980), as a bleach for  
457 darkened palynomorphs such as chitinozoa and megaspores (Jenkins, 1967) and to  
458 remove organic matter from soils to allow clay mineral analysis (Siregar et al., 2005).  
459 Because of its bleaching and oxidising properties, this reagent should be used  
460 carefully to avoid the degradation or destruction of palynomorphs. Doher (1980, p.  
461 21) stated that sodium hypochlorite solution corrodes pollen and spores over  
462 prolonged periods, and can cause grain size changes. This was confirmed by Traverse  
463 (1990), who warned how bleaching dark palynomorphs by oxidation may have  
464 adverse effects. Traverse (1990) demonstrated that modern *Althea rosea* (hollyhock)  
465 pollen is significantly altered by brief treatment with sodium hypochlorite bleach.  
466 *Althea rosea* pollen grains that have simply been acetolysed are dark, spherical and  
467 have numerous spines. However, if the pollen is acetolysed then bleached with a  
468 dilute sodium hypochlorite solution for two minutes, the pollen morphology is  
469 changed beyond recognition. The grains are lighter, the outermost layer including the  
470 spines is destroyed, and the remaining exine shrinks producing a rounded square  
471 outline. If these pre-and post-bleached forms were fossil pollen, they would be placed  
472 in entirely different taxa.

473 It was anticipated that sodium hypochlorite solution will partially disaggregate  
474 the sample material prior to the  $(\text{NaPO}_3)_6$  treatment. The overnight pre-treatment with  
475 5% sodium hypochlorite solution appears to have been highly effective. It greatly  
476 increased the mass of sample which was broken down by the  $(\text{NaPO}_3)_6$ . Following the  
477 overnight 5% sodium hypochlorite solution pre-treatment, only 1.0 g of the 5.0 g  
478 subsample remained following 20 minutes treatment with  $(\text{NaPO}_3)_6$  (Table 1). This  
479 means that this sodium hypochlorite solution pre-treatment has enabled 2.0 g more  
480 rock to be disaggregated, compared with no pre-treatment (Table 1). However, the  
481 pre-treatment destroyed all the *Lycopodium* spores and noticeably bleached the  
482 Carboniferous spores. The palynomorph residue comprises relatively light coloured  
483 Carboniferous spores only (Fig. 8); no *Lycopodium* spores could be found, despite  
484 scanning entire slides. This confirms the findings of Traverse (1990) that sodium  
485 hypochlorite solution is highly destructive to modern pollen and spores. The  
486 Carboniferous palynomorphs are markedly lighter in colour than with all the other  
487 preparation strategies in this study (Figs. 2-7 and 10-11). However, the residue is still  
488 extremely rich in palynomorphs and is devoid of AOM (Fig. 8). There does not  
489 appear to have been any selective destruction of the Carboniferous palynomorphs.

490 A second test using sodium hypochlorite solution was undertaken to attempt to  
491 establish if a gentler treatment would be less destructive to the *Lycopodium* spores. A  
492 5.0 g subsample was pre-treated with 2.5% sodium hypochlorite solution for 6 hours.  
493 This second sodium hypochlorite solution pre-treatment enabled 0.8 g more rock to be  
494 disaggregated compared to the control with no pre-treatment (Table 1). The organic  
495 material produced by this subsample was also noticeably lightened, and the  
496 preservation of the *Lycopodium* spores was poor (Fig. 9). Only 29 of these poorly-  
497 preserved *Lycopodium* spores were counted in an overall population of 617 grains

498 (Table 1). This ratio, as compared to the others in Table 1, means that significant  
499 numbers of *Lycopodium* spores were destroyed by this gentler treatment. Hence this  
500 count cannot be used to assess the concentration of the Carboniferous spores, which  
501 do not appear to have been destroyed by the bleach. This means that the calculation of  
502 3,767,863 palynomorphs in the preparation and the two concentrations depicted in  
503 Table 1 are spurious due to the destruction of significant proportion of the  
504 *Lycopodium* spores.

505 It is therefore clear that sodium hypochlorite solution is extremely corrosive to  
506 modern and relatively young palynomorphs, and should be used with great care. This  
507 reagent can apparently be used with caution on material which contains old (i.e.  
508 Palaeozoic) and/or robust palynomorphs. By contrast, it should not be used to pre-  
509 treat Neogene and younger material because of its highly corrosive nature.

510  
511

#### 512 4.7. *The subsample pre-prepared with methylated spirits ('meths')*

513

514 Methylated spirits is ethanol (C<sub>2</sub>H<sub>5</sub>OH), which has been mixed with aniline  
515 dye in order to render it toxic and unpalatable. Methanol (CH<sub>3</sub>OH) is also added to  
516 make the separation of pure ethanol via distillation difficult. Ethanol is a versatile fuel  
517 and solvent; it is miscible with light aliphatic hydrocarbons, other organic solvents  
518 and water. Methylated spirits is slightly basic (pH 7.33), and is volatile. This means  
519 that the level of the liquid should be monitored, if the pre-treatment with methylated  
520 spirits is prolonged (i.e. several days).

521 It was considered that methylated spirits may possibly help to render the  
522 sample material more susceptible to disaggregation using (NaPO<sub>3</sub>)<sub>6</sub>. Treatment with  
523 methylated spirits did not increase the weight of sample which was broken down by  
524 the (NaPO<sub>3</sub>)<sub>6</sub>. Following pre-treatment with methylated spirits, 3.5 g of the 5.0 g  
525 subsample remained after 20 minutes (NaPO<sub>3</sub>)<sub>6</sub> treatment (Table 1). The methylated  
526 spirits pre-treatment thus enabled 0.5 g less rock to be disaggregated compared with  
527 no pre-treatment (Table 1). However, in terms of the palynomorph yield, the pre-  
528 treatment with methylated spirits appears to have been marginally beneficial. This  
529 pre-treatment yielded 142,449 grains per gram, compared with 104,427 palynomorphs  
530 per gram for the control subsample (Table 1). The absolute numbers of Carboniferous  
531 spores in the methylated spirit pre-treated subsample also show a marginal increase on  
532 the control subsample; these figures are 213,674 and 208,853 respectively (Table 1).  
533 The pre-treatment with methylated spirits apparently had no adverse effects on the  
534 palynomorphs. The organic concentrate proved generally free of extraneous materials,  
535 and the palynomorphs were abundant and undamaged (Fig. 10).

536  
537

#### 538 4.8. *The subsample pre-prepared with white spirit*

539

540 White spirit (also known as mineral spirits, Stoddard solvent and Varsol) is a  
541 petroleum-based distillate; it is a mixture of alicyclic, aliphatic and aromatic  
542 hydrocarbons. This clear liquid is used as an extraction solvent in degreasing and dry  
543 cleaning, a fuel additive, a viscosity-reducer and a general-purpose organic solvent  
544 (e.g. paint thinners). It is flammable, with a flash point of 39°C. Despite having a low  
545 acute toxicity, white spirit is an irritant and may cause contact dermatitis, various  
546 other skin complaints and lung damage. White spirit is a potential freshwater or  
547 marine pollutant, and hence should be disposed of responsibly.

548 In this study, technical grade white spirit was used. Its grade is determined by  
549 the nature of the crude oil used, and the conditions of distillation. It is highly volatile,  
550 and the level in the vessel should be checked if the pre-treatment lasts for a few days.  
551 Brown (1960; 2008, p. 76, 88, 89) described using white spirit (as Varsol) to  
552 disaggregate shale and to dissolve asphalt and other heavy hydrocarbons.

553 It was anticipated that white spirit will help to soften the sample material. It is  
554 well known as a disaggregating agent that can liberate microfossils *sensu lato* from  
555 partially indurated clay-rich lithotypes (Armstrong and Brasier, 2005, p. 275). This  
556 pre-treatment appears to have been markedly beneficial. It slightly increased the  
557 amount of sample material which was eventually disaggregated by the  $(\text{NaPO}_3)_6$ .  
558 Following pre-treatment with white spirit, 2.7 g of the 5.0 g subsample remained  
559 following the 20 minute treatment with  $(\text{NaPO}_3)_6$  (Table 1). This means that the pre-  
560 treatment with white spirit enabled 0.3 g more rock to be disaggregated than with no  
561 pre-treatment (Table 1). Regarding palynomorph yield, the pre-treatment with white  
562 spirit proved highly effective. The pre-treatment yielded 203,199 grains per gram of  
563 rock prepared, compared with 104,427 palynomorphs per gram for the control  
564 subsample (Table 1). The numbers of Carboniferous spores in the white spirit pre-  
565 treated and control subsamples are 467,357 and 208,853 respectively (Table 1). This  
566 marked enhancement of the palynomorph extraction process is comparable to that  
567 given by pre-treatment with Decon 90 (Table 1). The white spirit apparently started to  
568 deflocculate the clay, hence allowing the  $(\text{NaPO}_3)_6$  to break down the partially  
569 disaggregated clay and explaining the higher palynomorph yield. The white spirit pre-  
570 treatment does not cause adverse preservational effects on the palynomorphs. The  
571 organic residue was extremely clean and pollen and spores were abundant and well-  
572 preserved (Fig. 11).

## 573 574 575 **5. Summary** 576

577 This study aimed to objectively assess the relative effectiveness of several pre-  
578 treatment regimes on a single sample of highly palynologically productive  
579 sedimentary rock. Another objective was to improve the effectiveness of the  
580 preparation method using  $(\text{NaPO}_3)_6$  developed by Riding and Kyffin-Hughes (2004;  
581 2006). Generally, pre-treatment increased the mass of sample that was eventually  
582 broken down by the  $(\text{NaPO}_3)_6$  treatment. This is unsurprising because more soaking  
583 should soften lithified rocks. The pre-treatment aims at softening the sample material,  
584 thereby allowing the  $(\text{NaPO}_3)_6$  to act on an increased surface area, and hence releasing  
585 more palynomorphs. Specifically, detergent one (carpet cleaner), formic acid,  
586 household bleach and white spirit allowed the disaggregation of more raw rock  
587 sample than without any pre-treatment. This also clearly demonstrates that the  
588  $(\text{NaPO}_3)_6$  preparation method is made more effective by pre-treatment. However,  
589 acetone, detergent two (Decon 90) and methylated spirits actually reduced the amount  
590 of rock broken down by  $(\text{NaPO}_3)_6$ . Unsurprisingly, the largest weight of rock prepared  
591 was with the control subsample prepared using HCl and HF digestion (Table 1).

592 The efficacy of pre-treatments is clear based on the absolute numbers of  
593 indigenous palynomorphs extracted from the subsamples. Only the pre-treatment  
594 using methylated spirits gave fewer specimens than the control subsample with no  
595 pre-treatment (Table 1). However, this comparison is somewhat misleading because  
596 the amounts of rock broken down were different.

597 The most significant data are the Carboniferous miospores per gram of rock  
598 which was disaggregated. Based on this, the  $(\text{NaPO}_3)_6$  treatment with no pre-treatment  
599 produced 104,427 Carboniferous miospores per gram. Of the seven pre-treatment  
600 reagents tested, only formic acid was relatively ineffective; this reagent produced a  
601 yield of 93,889 Carboniferous miospores per gram. This represents a lower  
602 concentration of Carboniferous miospores than with no pre-treatment. Five of the  
603 others (i.e. acetone, both detergents, methylated spirits and white spirit) produced  
604 significantly higher concentrations of Carboniferous miospores from the sample  
605 studied than with no pre-treatment. Of these, the detergents and white spirit  
606 essentially doubled the palynomorph yield. Again, the subsample digested with HCl  
607 and HF produced the largest concentration of Carboniferous miospores (Table 1).

608 It is abundantly clear that household bleach is unsuitable for the pre-treatment  
609 of post-Neogene palynomorphs. It should be used with extreme caution as a pre-  
610 treatment reagent because it lightens (bleaches) and selectively destroys relatively  
611 young palynomorphs. All the *Lycopodium* spores were destroyed in the subsample  
612 which was soaked overnight in 5% sodium hypochlorite solution (Fig. 8). In the  
613 subsample treated with 2.5% sodium hypochlorite solution for six hours, a small  
614 proportion of the *Lycopodium* spores survived, however these are poorly-preserved.  
615 To summarise, bleach/sodium hypochlorite solution should be used only with extreme  
616 care on relatively old and robust palynomorphs.

617 This study confirms that the  $(\text{NaPO}_3)_6$  method of Riding and Kyffin-Hughes  
618 (2004; 2006) is a highly effective technique for the extraction of palynomorphs from  
619 siliciclastic/clay-rich lithotypes, although the concentration of palynomorphs is  
620 generally lower than those obtained by HCl/HF digestions. Furthermore, the  $(\text{NaPO}_3)_6$   
621 treatment can help remove AOM from organic residues. Overnight pre-treatment with  
622 acetone, detergent, methylated spirits and white spirit makes the  $(\text{NaPO}_3)_6$  preparation  
623 significantly more effective. These reagents all increase the concentration of the  
624 indigenous palynomorphs extracted. One detergent (the carpet cleaner) and white  
625 spirit increase the amount of rock that is disaggregated by the  $(\text{NaPO}_3)_6$ . Of the seven  
626 substances tested, detergent and white spirit are the most effective pre-treatment  
627 reagents. Consequently, these reagents are recommended as the best pre-treatment  
628 reagents in palynological preparation. It is interesting that one is essentially liquid  
629 soap and the other is an organic substance, hence they work in softening claystones in  
630 different ways.

631 It should be borne in mind that this study was only based on a single sample so  
632 these results should not be considered as being definitive; more research is needed.  
633 There is clearly scope for further investigations on non-acid palynological  
634 preparation. Tests for example using other reagents, different timings and different  
635 sample materials would enhance capability in this important area. It is also possible  
636 that pre-treating samples would make HCl-HF digestions faster, and enhance the final  
637 residue.

638

639

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641

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648

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## 650 **References**

651

652 Armstrong, H.A., Brasier, M.D., 2005. *Microfossils*. Second Edition. Blackwell  
653 Publishing, Oxford, 296 p.

654

655 Batten, D.J., 1999. 4. Small palynomorphs. In: Jones, T. P., Rowe, N. P. (Eds.), *Fossil*  
656 *plants and spores: modern techniques*. Geological Society, London, pp. 15-19.

657

658 Benninghoff, W.S., 1962. Calculation of pollen and spores density in sediments by  
659 addition of exotic pollen in known quantities. *Pollen et Spores* 4, 332-333.

660

661 Brown, C.A., 1960. *Palynological Techniques*. Privately published, 1180 Stanford  
662 Avenue, Baton Rouge, Louisiana, U.S.A., 188 p.

663

664 Brown, C.A., 2008. *Palynological Techniques*. Second Edition. Riding, J.B. and  
665 Warny, S. (Eds). American Association of Stratigraphic Palynologists Foundation,  
666 Dallas, Texas, U.S.A., 137 p.

667

668 Clayton, G., Coquel, R., Doubinger, J., Gueinn, K.J., Loboziak, S., Owens, B., Streef,  
669 M., 1977. Carboniferous miospores of western Europe: illustration and zonation.  
670 *Mededelingen Rijks Geologische Dienst* 29, 71 p.

671

672 Dale, B., 1976. Cyst formation, sedimentation, and preservation: factors affecting  
673 dinoflagellate assemblages in Recent sediments from Trondheimsfjord, Norway.  
674 *Review of Palaeobotany and Palynology* 22, 39-60.

675

676 de Vernal, A., Larouche, A., Richard, P.J.H., 1987. Evaluation of palynomorph  
677 concentrations: do the aliquot and the marker-grain methods yield comparable results?  
678 *Pollen et Spores* 29, 291-303.

679

680 Doherty, L.I., 1980. Palynomorph preparation procedures currently used in the  
681 paleontology and stratigraphy laboratories, U.S. Geological Survey. United States  
682 Geological Survey Circular 830, 29 p.

683

684 Eshet, Y., Hoek, R., 1996. Palynological processing of organic-rich rocks, or: How  
685 many times have you called a palyniferous sample "barren"? *Review of Palaeobotany*  
686 *and Palynology* 94, 101-109.

687

688 Evitt, W.R., 1984. Some techniques for preparing, manipulating and mounting  
689 dinoflagellates. *Journal of Micropalaeontology* 3(2), 11-18.

690

691 Faegri, K., Kaland, P.E., Krzywinski, K., 1989. *Textbook of Pollen Analysis*, by Knut  
692 Faegri and Johs. Iversen. Fourth edition. John Wiley and Sons, Chichester, U.K., 328  
693 p.

694

695 Gray, J., 1965a. Palynological techniques. In: Kummel, B., Raup, D. (Eds.),  
696 Handbook of Paleontological Techniques. W.H. Freeman and Company, San  
697 Francisco, U.S.A., pp. 471-481.  
698

699 Gray, J., 1965b. Extraction techniques. In: Kummel, B., Raup, D. (Eds.), Handbook of  
700 Paleontological Techniques. W.H. Freeman and Company, San Francisco, U.S.A., pp.  
701 530-587.  
702

703 Green, O.R., 2001. Chapter 25. Extraction techniques for palaeobotanical and  
704 palynological material. In: A manual of practical laboratory and field techniques in  
705 palaeobiology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 256-  
706 287.  
707

708 Grey, K., 1999. A modified palynological preparation technique for the extraction of  
709 large Neoproterozoic acanthomorph acritarchs and other acid-insoluble microfossils.  
710 Geological Survey of Western Australia Record 1999/10, 23 p.  
711

712 Harland, R., 1989. A dinoflagellate cyst record for the last 0.7 Ma from the Rockall  
713 Plateau, northeast Atlantic Ocean. Journal of the Geological Society 25, 113-117.  
714

715 Hoffmeister, W.S., 1960. Sodium hypochlorite, a new oxidising agent for the  
716 preparation of microfossils. Oklahoma Geology Notes 20, 34-35.  
717

718 Hopkins, J.A., McCarthy, F.M.G., 2002. Post-depositional palynomorph degradation  
719 in Quaternary shelf sediments: a laboratory experiment studying the effects of  
720 progressive oxidation. Palynology 26, 167-184.  
721

722 Jenkins, W.A.M., 1967. Ordovician chitinozoa from Shropshire. Palaeontology 10,  
723 436-488.  
724

725 Lee, H.W., 1964. A modified method of coal maceration and a simple technique for  
726 slide preparation. Micropaleontology 10, 486-490.  
727

728 Merrill, G.K., 1980. Removal of pyrite from microfossil samples by means of sodium  
729 hypochlorite. Journal of Paleontology 54, 633-634.  
730

731 Mertens, K.N., Verhoeven, K., Verleye, T., Louwye, S., Amorim, A., Ribeiro, S.,  
732 Deaf, A.S., Harding, I., de Schepper, S., Kodrans-Nsiah, M., de Vernal, A., Henry,  
733 M., Radi, T., Dybkjaer, K., Poulsen, N.E., Feist-Burkhardt, S., Chitolie, J., González  
734 Arango, C., Heilmann-Clausen, C., Londeix, L., Turon, J.-L., Marret, F., Matthiessen,  
735 J., McCarthy, F., Prasad, V., Pospelova, V., Kyffin Hughes, J.E., Riding, J.B.,  
736 Rochon, A., Sangiorgi, F., Welters, N., Sinclair, N., Thun, C., Soliman, A., van  
737 Nieuwenhove, N., Vink, A., Young, M., 2009. Determining the absolute abundance of  
738 dinoflagellate cysts in recent marine sediments: the *Lycopodium* marker-grain method  
739 put to the test. Review of Palaeobotany and Palynology in press.  
740

741 Owens, B., Marshall, J., 1978. Micropalaeontological biostratigraphy of samples from  
742 around the coasts of Scotland. Report of the Institute of Geological Sciences 78/20, 35  
743 p.  
744

745 Phipps, D., Playford, G., 1984. Laboratory techniques for extraction of palynomorphs  
746 from sediments. Papers of the Department of Geology, University of Queensland  
747 11(1), 23 p.  
748

749 Raistrick, A., 1934. The correlation of coal seams by microspore content. Part I. – The  
750 seams of Northumberland. Transactions of the Institution of Mining Engineers 88,  
751 142-153 and 259-264.  
752

753 Riding, J.B., Kyffin-Hughes, J.E., 2004. A review of the laboratory preparation of  
754 palynomorphs with a description of an effective non-acid technique. Revista  
755 Brasileira de Paleontologia 7(1), 13-44.  
756

757 Riding, J.B., Kyffin-Hughes, J.E., 2006. Further testing of a non-acid palynological  
758 preparation procedure. Palynology 30, 69-87.  
759

760 Riding, J.B., Wilkinson, I.P., Jones, L.D., Freeborough, K., 2006. The occurrence of  
761 dinoflagellate cysts in calcareous/siliceous microfossil preparations from the Eocene  
762 of southeast England. Journal of Micropalaeontology 25, 35-36.  
763

764 Riding, J.B., Kyffin-Hughes, J.E., Owens, B., 2007. An effective palynological  
765 preparation procedure using hydrogen peroxide. Palynology 31, 19-36.  
766

767 Siregar, A., Kleber, M., Mikutta, R., Jahn, R., 2005. Sodium hypochlorite oxidation  
768 reduces soil organic matter concentrations without affecting inorganic soil  
769 constituents. European Journal of Soil Science 56, 481-490.  
770

771 Smith, A.V.H., Butterworth, M.A., 1967. Miospores in the coal seams of the  
772 Carboniferous of Great Britain. Special Papers in Palaeontology 1, 324 p.  
773

774 Stockmarr, J., 1971. Tablets with spores used in absolute pollen analysis. Pollen et  
775 Spores 13, 615-621.  
776

777 Traverse, A., 1990. The ravages of oxidation on pollen of *Althaea rosea*  
778 (“hollyhock”). Stuifmail 8.1, 12.  
779

780 Traverse, A., 2007. Paleopalynology. Second Edition. Springer, Dordrecht, The  
781 Netherlands, 813 p.  
782

783 van Cleave, H.J., Ross, J.A., 1947. Use of trisodium phosphate in microscopical  
784 technic. Science 106(2748), 194.  
785

786 Wood, G.D., Gabriel, A.M., Lawson, J.C., 1996. Chapter 3. Palynological techniques  
787 – processing and microscopy. In: Jansonius, J., McGregor, D.C. (Eds.), Palynology:  
788 principles and applications. American Association of Stratigraphic Palynologists  
789 Foundation, Dallas 1, 29-50.  
790

791

792 **Fig. 1.** The location of BGS offshore borehole 74/13, offshore southeast Scotland,  
793 United Kingdom.  
794



795 **Fig. 2.** A representative low-magnification photomicrograph of the organic residue  
796 from the subsample of sample MPA 57940 which was prepared as a control with  
797 hydrochloric and hydrofluoric acids. Slide ‘HF, 1 count, test B, #1’, England Finder  
798 coordinate S65/1. The specimen of *Lycospora pusilla* in the centre-left is 24 µm in  
799 diameter. Note the presence of amorphous organic material at the top of the frame; for  
800 consistency with the non-acid preparations, this was not removed by oxidation.

801

802 **Fig. 3.** A representative low-magnification photomicrograph of the organic residue  
803 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> and no  
804 pre-treatment. Slide ‘control, test B’, England Finder coordinate O56. The specimen  
805 of *Lycospora pusilla* in the centre is 24 µm in diameter. Note the relatively clean  
806 nature of the residue, i.e the relative rarity of amorphous organic material.

807

808 **Fig. 4.** A representative low-magnification photomicrograph of the organic residue  
809 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
810 an overnight pre-treatment with acetone. Slide ‘acetone, test B’, England Finder  
811 coordinate M50/4. The saccate pollen grain in the centre-left is 84 µm long. Note the  
812 abundance of both Carboniferous miospores and *Lycopodium* spores.

813

814 **Fig. 5.** A representative low-magnification photomicrograph of the organic residue  
815 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
816 an overnight pre-treatment with 3% solution of domestic carpet cleaner. Slide ‘R.D.,  
817 overnight, 3 count’, England Finder coordinate J51/2. The cracked specimen of  
818 *Lycospora pusilla* in the centre-left is 38 µm in diameter. Note the well-preserved  
819 miospores and the absence of amorphous organic material.

820

821 **Fig. 6.** A representative low-magnification photomicrograph of the organic residue  
822 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
823 an overnight pre-treatment with 3% Decon 90 detergent solution. Slide ‘Decon 90,  
824 test B’, England Finder coordinate M56. The specimen of *Densosporites* sp. near the  
825 bottom of the frame in the centre-left is 36 µm in maximum diameter. Note the ‘clean’  
826 nature of the residue, i.e. the absence of amorphous organic material.

827

828 **Fig. 7.** A representative low-magnification photomicrograph of the organic residue  
829 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
830 an overnight pre-treatment with formic acid. Slide ‘formic acid, test B’, England  
831 Finder coordinate N50/4. The specimen of *Lycospora pusilla* in the centre is 33 µm in  
832 maximum diameter. Note the abundance of *Lycopodium* spores.

833

834 **Fig. 8.** A representative low-magnification photomicrograph of the organic residue  
835 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
836 an overnight pre-treatment with household bleach (a 5% solution of sodium  
837 hypochlorite). Slide ‘NaOCl, test B’, England Finder coordinate O40/2. The monolet  
838 spore in the upper-right is 45 µm in diameter. Note the light (bleached) Carboniferous  
839 miospores, and the complete absence of *Lycopodium* spores which have been  
840 destroyed by the bleach.

841

842 **Fig. 9.** A representative low-magnification photomicrograph of the organic residue  
843 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using a  
844 pre-treatment with household bleach (a 2.5% solution of sodium hypochlorite) for six

845 hours. Slide '2.5% NaOCl, 6 hours', England Finder coordinate O48. The prominent  
846 specimen of *Lycospora pusilla* in the centre-left is 31 µm in maximum diameter. Note  
847 the apparent absence of *Lycopodium* spores; these are present but in relatively low  
848 numbers (Table 1). This reflects partial destruction of the *Lycopodium* spores by the  
849 bleach. Note also the fact that the Carboniferous spores are only slightly bleached, as  
850 compared to the significantly lightened forms in Fig. 8.

851

852 **Fig. 10.** A representative low-magnification photomicrograph of the organic residue  
853 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
854 an overnight pre-treatment with methylated spirits. Slide 'meths, test B', England  
855 Finder coordinate N66/1. The prominent specimen of *Lycospora pusilla* in the upper-  
856 centre is 33 µm in maximum diameter. Note the well-preserved Carboniferous  
857 miospores and the absence of amorphous organic material.

858

859 **Fig. 11.** A representative low-magnification photomicrograph of the organic residue  
860 from the subsample of sample MPA 57940 which was prepared with (NaPO<sub>3</sub>)<sub>6</sub> using  
861 an overnight pre-treatment with white spirit. Slide 'white spirit, test B', England  
862 Finder coordinate O69/3. The specimen of *Lycospora pusilla* in the upper-right is 31  
863 µm in maximum diameter. Note the abundant, well-preserved Carboniferous  
864 miospores and the absence of amorphous organic material.

865

#### 866 **Table 1**

867 The key data in this study. The numbers of Carboniferous miospores and marker  
868 *Lycopodium* spores which were counted, the dry weight of the rock sample that was  
869 broken down, the indigenous palynomorph concentrations (based on the actual weight  
870 prepared and 5.0 g) and the absolute numbers of indigenous palynomorphs based on  
871 5.0 g are given for each of the subsamples prepared. It should be noted that the  
872 numbers and concentrations of palynomorphs in the row pertaining to the pre-  
873 treatment with 2.5% household bleach for six hours (*italicised*) are entirely spurious  
874 due to the destruction of large numbers of the exotic *Lycopodium* spores. The  
875 *italicised* abbreviations (e.g. *mc*) refer to equation of Benninghoff (1962) where  
876 appropriate.