



COMPARATIVE STUDY OF THREE PALYNOLOGICAL PREPARATION TECHNIQUES

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ABSTRACT

Comparative study of three palynological preparation techniques was carried out on six samples obtained from the middle and lower Benue trough. The six samples of palynomorph-rich Cretaceous shales and clays from the middle and lower Benue trough of Nigeria were quantitatively prepared using the traditional hydrofluoric acid based palynological preparation and two non-acid techniques.

The palynomorph assemblages extracted were thoroughly counted. This investigation was able to assess the relative effectiveness of the two non-acid techniques. This approach identifies any biases, in terms of absolute numbers of palynomorphs and diversity, inherent with the $(\text{NaPO}_3)_6$ and H_2O_2 preparation techniques.

The H_2O_2 technique proved to be significantly less effective, at approximately 18% of the extraction level of salt digestion which appears to be largely due to oxidation, while the HF digestion proved to be approximately 69% of the extraction level of the salt digestion. Hydrogen peroxide is an aggressive oxidant. However, the H_2O_2 technique may be useful in breaking down more indurated lithotypes, and can be used in isolation or in combination with the $(\text{NaPO}_3)_6$ method. Therefore the $(\text{NaPO}_3)_6$ technique is deemed to be the safest and most effective of the three methods. It is evident that the $(\text{NaPO}_3)_6$ protocol is a viable alternative to acid digestion. Furthermore, it is safer, quicker to prepare and environmentally friendly.

INTRODUCTION

The palynological analysis was carried out on the six samples to using two different methods of palynological slides preparation so as to ascertain the most effective of the two method. The Samples were collected from the middle and lower Benue trough of Nigeria on latitudes 5°45'N and 8°45'N of the equator and longitudes 7°45'E and 9°00'E of the Greenwich meridian from Akunza Migili Lafia town to along the road near the Imo/Abia state boundary. The Benue Trough formed as a failed arm of the triple junction when the north and south Atlantic were created during the Jurassic (Burke *et al*, 1972). The accessibility is facilitated by the Enugu-Port Harcourt expressway and other roads linking to the area.

AIM AND OBJECTIVES OF THE STUDY

This study aimed to test whether the Sodium Hexametaphosphate [(NaPO₃)₆] and Hydrogen Peroxide (H₂O₂) methods of extracting palynomorphs from sedimentary rocks and sediments are effective alternatives to the traditional technique of Hydrofluoric Acid (HF) digestion. The objectives of the study are; to assess how safer, environmental friendly, quicker and efficient these non acid techniques are; identify any biases, in terms of absolute numbers of palynomorphs inherent with the HF, (NaPO₃)₆ and H₂O₂ preparation techniques; encourage researchers to develop other alternatives to microfossil extraction from sediment.

Location of Samples used for the Research: Sediments used for this research were gotten from the middle and lower Benue trough of Nigeria as shown in the map and lies within latitudes 5°45'N and 8°45'N of the equator and longitudes 7°45'E and 9°00'E of the Greenwich meridian from Akunza Migili Lafia town to along the road near the Imo/Abia state boundary. The accessibility is facilitated by the Enugu-Port Harcourt expressway and other roads linking to the area.

PREVIOUS WORKS ON PALYNOLOGICAL PREPARATION

Initially, the only chemical treatment used by researchers was treatment with the KOH to remove the humic substances, defloculation was accomplished through surface treatment or ultra-sonic treatment, although sonification may cause the pollen exine to rupture. The use of hydrofluoric acid (HF) to digest the silicate minerals was introduced by Assarson and Granlund in 1924, greatly reducing the amount of time required to scan slide for palynomorphs. Palynological studies using peats presented a particular challenge because of the presence of well preserved organic material including fine rootlets, moss leaflets and organic litter. This was the last major challenge in the chemical preparation of materials for palynological studies. Acetolysis was developed by Gunnar and Erdtman and his brother to remove these fine cellulose materials by dissolving them. In acetolysis, the specimen is treated with acetic anhydride and sulfuric acid, dissolving cellulosic materials and thus providing better visibility for palynomorphs. Some steps of the chemical treatment require special care for safety reasons, in particular the use of HF which diffuses very fast through

the skin and causes severe chemical burns and can be fatal. Chemical extraction of pollen samples was conducted at the Palynology Laboratory at Texas A&M University using a procedure designed for semi-arid southwestern sediments. The method specifically avoids use of such reagents as nitric acid and bleach, which have been experimentally demonstrated to be destructive to pollen grains. Most of the research works published on extraction techniques were applicable to western countries. Some of these work were published in journals, internet and text books.

A comparison of palynological and paleontological extraction techniques using samples from the Silurian Bainbridge Formation, Missouri, U.S.A carried out by G.Kent Colbath in 2005 have been documented. Well preserved assemblages of organic-walled micro-phytoplanktons and spores of land type were recovered using cold, concentrated HNO_3 . Further research by Riding and Kyffin (2011) carried out a direct comparison of palynological techniques with sediments from the Upper Jurassic mudstone from western Scotland. An effective palynological preparation procedure using hydrogen peroxide by Riding et al (2007) have been documented by the British Geological Survey.

METHODOLOGY

SAMPLE COLLECTION: The field work was carried out with the aid of some basic instruments such as road map, Global Positioning System (G.P.S), digital camera, marker, field notebook, pencil, eraser, masking tape, sample bags (used to collect samples from different locations. The samples collected from different locations were well-labelled with sample and location number and then kept in a sample bag. It is also necessary to note that samples were collected from fresh and undisturbed vegetations that has not experienced bush burning, farming or contains construction materials. Six samples of Cretaceous shales and clays were collected from bed outcrops. The samples are informally termed **Sp.1- Sp.6 (Table 1)**

Apparatus:

- a) Weighing balance, Hand gloves, Nose mask, Plastic cups, Litmus paper, Nylon sieve mesh (10μ), SPT sieve holder, Watch glasses (24cm diameter), Centrifuge tubes with racks, Pasteur pipettes, Hot plate, Slide mounting plate, Rectangular cover slips
- b) Glass slides (usually 76 x 26mm), Palynological microscope

Reagents:

- a) Hydrofluoric acid (HF), Distilled water or mains tap water filtered at 1micron, Sodium Hexametaphosphate (NaPO_3)₆, White gum, Hydrogen Peroxide (H_2O_2), Orland glue

The laboratory work involved washing, sieving, processing, mounting, viewing and identification of palynormorphs and micro-fauna. Photomicrographs were taken with palynological microscope and shown on

plate 1. Palynomorphs were counted. Damaged palynomorphs were also considered and aggregated into the count. The six samples were prepared each using 5g of sediments. Six control subsamples were each prepared using the $(\text{NaPO}_3)_6$ technique, H_2O_2 method and the standard HF digestion method without pre-treatment or oxidation.

SAMPLE NO.	LITHOLOGY	CONDITION
1	Clay	Grey, very fine grained, wet.
2	Clay	Grey, very fine grained, wet.
3	Shale	Light grey, fissile, carbonaceous, dry.
4	Shale	Dark grey, fissile, carbonaceous, dry.
5	Shale	Dark, sub-fissile to fissile, dry with rootlets.
6	Shale	Bluish grey, sub-fissile to fissile, dry with rootlets.

Table 1: Showing sample lithology and condition

DESCRIPTION OF PALYNOLOGICAL PREPARATION TECHNIQUES:

Hydroflouric acid (HF):

Hydroflouric acid is a solution of hydrogen fluoride in water. It is also a valued source of fluorine and is a precursor to numerous pharmaceuticals such as fluoxetine and diverse materials such as PTFE (Teflon). Because of its ability to dissolve most oxides and silicates, hydrofluoric acid is useful for dissolving rock samples (usually powdered) prior to analysis. In similar manner, this acid is used in acid macerations to extract organic fossils from silicate rocks

Sodium hexametaphosphate $(\text{NaPO}_3)_6$:

This is a hexamer of composition $(\text{NaPO}_3)_6$. Sodium hexametaphosphate of commerce is typically a mixture of polymeric metaphosphates, of which the hexamer is one, and is usually the compound referred to by this name. It is more correctly termed sodium polymetaphosphate. It is used as a deflocculant or dispersant for the ASTM D422-63 (2007) Standard Test Method for Particle-Size Analysis of Soils.

Hydrogen peroxide:

Hydrogen Peroxide is the simplest peroxide (a compound with an oxygen-oxygen single bond). It is also a strong oxidizer. Hydrogen Peroxide is a colourless liquid, slightly more viscous than water. In dilute solution, it appears colourless. Due to its oxidizing properties, it is often used as a bleach or cleaning agent.

The oxidizing capacity of hydrogen peroxide is so strong that it is considered a highly reactive oxygen species. Because of its ability to dissolve most oxides and silicates, hydrogen peroxide is useful for dissolving rock samples

DESCRIPTION OF THREE PALYNOMORPH PREPARATION TECHNIQUES USED IN THIS STUDY:

Sodium Hexametaphosphate(NaPO_3)₆ digestion:

- a) Air-dry the sample material so moisture can be taken off.
- b) Add ca. 400 ml of water to the sample material and bring to the boil.
- c) Add around 40 g of $(\text{NaPO}_3)_6$ to the mixture, stir very thoroughly and simmer and leave for approximately 60 minutes.
- d) Sieve the mixture using a $10\mu\text{m}$ mesh to remove the $<10\mu\text{m}$ fraction of deflocculated clay particles, then wash out the $(\text{NaPO}_3)_6$ from the retained $>10\mu\text{m}$ fraction.
- e) If any of the sample material remains unsegregated, treat with H_2O_2 as necessary until it has all broken down.
- f) Centrifuge and/or swirl the final residue to remove any resistant mineral grains.
- g) Concentrate the palynomorph concentrate and mount on microscope slides.

Hydrogen Peroxide technique:

- a) Air-dry the sample material to keep moisture off.
- b) Place the sample material into a ceramic dish and place this on a pre-heated (to ca. $100\text{ }^\circ\text{C}$) hot plate in a fume hood for around one minute.
- c) Cover the sample material with 30% H_2O_2 , and heat extremely gently with great care until the rock/sediment begins to segregate.
- d) Cover the sample material with 30% H_2O_2 , and heat extremely gently with great care until the rock/sediment begins to disaggregate.
- e) Decant off any floating segregated sample material into a beaker of cold water to stop the reaction.
- f) Repeat steps three and four as necessary until all the sample material has been disaggregated.
- g) Centrifuge and/or swirl the final residue to remove any resistant mineral grains.
- h) Place the palynomorphs concentrate and mount on microscope slides.

RESULT AND DISCUSSION

PERCENTAGE DISTRIBUTION OF PALYNOMORPHS:

Comparative study of three palynological preparation techniques was carried out on shale and clay samples from the middle and lower Benue trough. The samples both produced abundant and well-preserved dinoflagellate cysts, pollen and spores. Dinoflagellate cysts and Acritarch are significantly less abundant than pollen and spores and are entirely typical of the Early – late cretaceous of middle-lower Benue trough. The associations are overwhelmingly dominated by *Echitricolpites spinosus*, *Fenetrites spinosus*, *Nympheapollis clarus*, *Multiarolites formosus*, *Fromea tornatilis*, *Echimonocolpites rarispinosus*, *Cingulatisporites ornatus*, *Maerotyloma brevicaules*, *Tubistephanocolpites cylindricus*, *Hexaporotricolpites emelianova*, *Psilaperiporites minimus*, *Retistephanocolpites gracillis*, *Elaeis guineensis*, *Ambonosphaera staffinernsis*.

SAMPLE 1 (SP.1):

Sample 1 produced an abundant diverse palynoflora. The salt digestion method produced a concentration of diverse palynomorphs . This concentration is by far the highest of the three techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is about 27% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ terrestrial palynomorphs with the acid method is 60% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding six absolute palynomorph. This represents about 27% and 17% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly, *Echitricolpites spinosus* and *Nympheapollis clarus* were more abundant than the rest forms. Forms obtained include *Downiesphaeridium polytrichum*, *Cerebropollenites macroverrucosus*, *Ischyosporites variegates*, *Traquitrite crassisus*, *Convintricyst circulus*, *Vitreisporites pallidus*, *Chytroeisphaeridia chytroeides* ,*Chytroeisphaeridia Chytroeides*, *Atopodinium prostaticum*, *Perinopollenites elatoides*, *Classopollis classoides*, *Endoscrinium galeritum* ,*Evansia deflandrei*, *Nympheapollis clarus*, *Laevigatosporites discordatus*.

Sample 1 (Table 2) is deficient of dinoflagellate but rich in pollen and spores and is composed of 51% pollen, 37% of spore, 6% fungal spore and 6% dinoflagellate. The dominance of pollen and spore indicate terrestrial depositional environment.

Sample 1 (Table 3) is deficient in fungal spore but rich in pollen and spores and is composed of 76% pollen, 19% of spore, and 5% dinoflagellate. This indicate terrestrial depositional environment.

Sample 1 (Table 4) is devoid of dinoflagellate cyst but 100% pollen. The account for a few forms can be as a result of oxidation of majority of the forms by the peroxide. This indicates a terrestrial depositional environment.

Sodium Hexametaphosphate extraction technique yielded 35 absolute number of palynomorphs which represents 56% of the total forms. Hydroflouric acid digestion yielded 21 palynomorphs which

represent 34% of the total forms while the hydrogen peroxide technique was obtained 6 forms which represent only 10% of the extraction levels of the three methods. In all, 62 forms were extracted. Dominance of pollen and spore indicate terrestrial environment.

SAMPLE 2 (SP.2):

Sample 2 produced an abundant diverse palynoflora . The salt digestion method produced a concentration of diverse palynomorphs. This concentration is the highest of the three techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is 46% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ palynomorphs with the acid method is 62% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding six absolute palynomorph. This represents about 46% and 29% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly, *Fenetrites rarispinosus*, *Multiarolites formosus*, were more abundant than the rest forms. Forms obtained include *Densosporites sp*, *Convintricyst circulus*, *Impagidinium sp*, *Netmatospaeroopsisn grandis*, *Vitreisporites pallidus*, *Hystrichokolpoma granulatum*, *Cyclopsiella eliptica*, *Ascostomocystis potane*, *Peripollenites elatrides*, *Concavissimisporites verucosus*, *Glyphyricysta exuberans*.

Sample 2 is deficient of dinoflagellate but rich in pollen and spores and is composed of 76% pollen, 14% of spore, 5% fungal spore and 5% dinoflagellate. The dominance of pollen and spore indicate terrestrial depositional environment.

Plate 1:

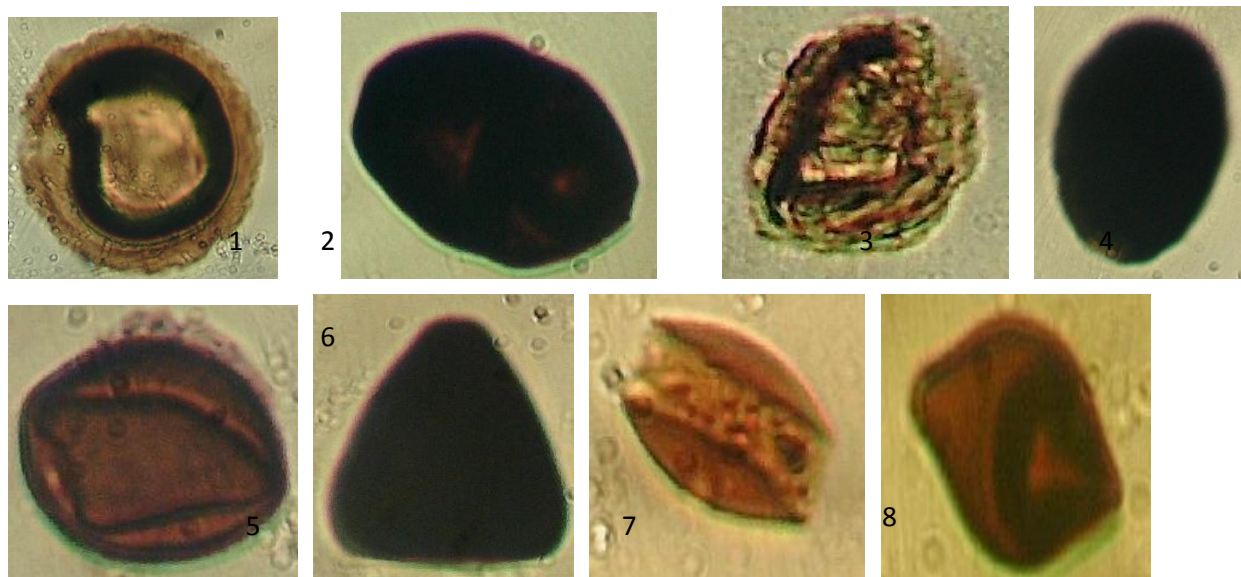


Plate 1: Showing some of the palynomorphs encountered in the study

1.*Psilatricolporites sp*, **2.** *Striadiporites reticulates*, **3***Verrucatosporites sp*

4. *Monoporisporites* sp. 5 *Zygnemasporites* sp, 6. *Indeterminate fungal sporen* 7. *Cycadosporites* sp. 8 *Polyporisporites* sp.

Sample 2 is deficient of dinoflagellate but rich in pollen and spores and is composed of 77% pollen, 15% of spore, 0% fungal spore and 8% dinoflagellate. This is indicative of a terrestrial depositional environment.

Sample 2 here is deficient of dinoflagellate but rich in pollen composed of 100% pollen. This is indicative of a terrestrial depositional environment.

Sodium Hexametaphosphate extraction technique yielded 21 absolute number of palynomorphs which represents 52% of the total forms. Hydroflouric acid digestion yielded 13 palynomorphs which represent 33% of the total forms while the hydrogen peroxide technique was obtained 6 forms which represent only 15% of the extraction levels of the three methods. In all, 40 forms were extracted. This is indicative of a terrestrial depositional environment.

SAMPLE 3 (SP.3):

Sample 3 produced an abundant diverse palynoflora . The salt digestion method produced a concentration of diverse palynomorphs (Plate 7). This concentration is the highest of the three techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is 16% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ palynomorphs with the acid method is 61% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding seven absolute palynomorph.. This represents about 16% and 10% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly, the presence of *Echimonocolpites rarispinosus*, *Cingulatisporites ornatus* were more abundant. Forms obtained include *Pentadinium* sp, *Perinopollenites elatoides*, *Cerebropollenites macroverrucosus*, *Chytroeisporites chytrooides*, *Verrusporites microporous*, *Arecipites*, *Magnastraitites howadi*, *Nematosphaeopsis*, *Shagnamsporites* sp.

Sample 3 is deficient of dinoflagellate but rich in pollen and spores and is composed of 52% pollen, 39% of spore, 6% fungal spore and 3% Acritarch. The presence of Acritarch indicates a gradual move from terrestrial to marine environment.

Sodium Hexametaphosphate extraction technique yielded 31 absolute number of palynomorphs which represents 55% of the total forms. Hydroflouric acid digestion yielded 19 palynomorphs which represent 33% of the total forms while the hydrogen peroxide technique was obtained 7 forms which represent only 12% of the extraction levels of the three methods. In all, 57 forms were extracted. This is indicative of a gradual change from terrestrial to marine environment due to the presence of few dinoflagellate cyst, probably presence of fungal spores and acritarch.

SAMPLE 4 (SP.4):

Sample 4 produced an abundant diverse palynoflora (Plate 10-12). The salt digestion method produced a concentration of diverse palynomorphs (Plate 10). This concentration is the highest of the three

techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is 14% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ palynomorphs with the acid method is 82% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding five absolute palynomorph. This represents about 14% and 11% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly, *Macrotyloma breviacules* and *Elaeis guineensis*, were more abundant. Forms obtained include *Cyclopsiella elliptica*, *Magnaperiporite ornatus*, *Aquatraradites sp*, *Meliacenes sp*, *Laevigatosprites sp*, *Anthoceras sp*.

Sodium Hexametaphosphate extraction technique yielded 45 absolute number of palynomorphs which represents 52% of the total forms. Hydroflouric acid digestion yielded 37 palynomorphs which represent 42% of the total forms while the hydrogen peroxide technique was obtained 5 forms which represent only 6% of the extraction levels of the three methods. In all, 87 forms were extracted. This is indicative of infiltration of marine water, swamp condition, a gradual change from terrestrial to marine environment, due to the presence of few dinoflagellate cyst, probably presence of fungal spores and acritarch.

SAMPLE 5 (SP.5):

Sample 5 produced an abundant diverse palynoflora. The salt digestion method produced a concentration of diverse palynomorphs. This concentration is the highest of the three techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is 19% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ palynomorphs with the acid method is 60% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding three absolute palynomorphs. This represents about 19% and 11% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly Hexaporocolpites emelianova and *psilaperiporites minimus* were abundant. Forms obtained include *Cyperepollis sp*, *Amaranthaceae pollen*, *Concavissimisorites sp.*, *Scabraporities ibadaensis*, *Polyperopollenites reticulate*, *Neilsonela aceras*, *Acostomocystis potane*, *Perigrinipollis nigericus*, *Monocolpites proxepertites*.

Sodium Hexametaphosphate extraction technique yielded 27 absolute number of palynomorphs which represents 59% of the total forms. Hydroflouric acid digestion yielded 16 palynomorphs which represent 35% of the total forms while the hydrogen peroxide technique was obtained 3 forms which represent only 6% of the extraction levels of the three methods. In all, 46 forms were extracted. This is indicative of a terrestrial to marine environment and also the presence of plant debris further confirms swampy condition.

SAMPLE 6 (SP.6):

Sample 6 produced an abundant diverse palynoflora. The salt digestion method produced a concentration of diverse palynomorphs. This concentration is the highest of the three techniques. The peroxide method yielded a significantly lower concentration of in situ palynomorphs. This figure is 32% of the concentration of the acid digestion subsamples. By contrast, the concentration of in situ palynomorphs

with the acid method is 51% of that produced by the salt digestion procedure. The H₂O₂ technique proved to be the least effective of the three techniques, yielding three absolute palynomorph. This represents about 32% and 27% of the concentrations achieved with acid digestion and the (NaPO₃)₆ method respectively. Similarly, Tubestephanocolpites gracillis and Retistephanocolpites gracillis were abundant. Forms obtained Monocolpites clavatus, Monocolpites anulatus, Cannigia sp., Venezuelites sp, Perigrinipollis nigericus, Polygonum sp., Sapotaceoidaepollenites sp., Acostomocystis potane, Anacolosidites sp, Bombacacidites noromi.

Sodium Hexametaphosphate extraction technique yielded 23 absolute number of palynomorphs which represents 48% of the total forms. Hydroflouric acid digestion yielded 19 palynomorphs which represent 40% of the total forms while the hydrogen peroxide technique was obtained 6 forms which represent only 12% of the extraction levels of the three methods. In all, 48 forms were extracted.

Pollen and spore indicate a terrestrial depositional environment. The presence of fungal probably further confirms a swamp depositional environment. Acritarch indicate a marine environment.

Forms	Numerical count	% count
Pollen	18	51
Spore	13	37
Fungal spore	2	6
Dinoflagellate	2	6
TOTAL COUNT	35	100%

Table 2: Showing the percentage distribution of palynomorphs with (NaPO₃)₆ digestion for SP.1

Forms	Numerical count	% count
Pollen	16	76
Spore	4	19
Fungal spore	0	0
Dinoflagellate	1	5
TOTAL COUNT	21	100%

Table 3: Showing the percentage distribution of palynomorphs with (HF) digestion for SP.1

Forms	Numerical count	% count
Pollen	6	100
Spore	0	0
Fungal spore	0	0
Dinoflagellate	0	0
TOTAL COUNT	6	100%

Table 4: Showing the percentage distribution of palynomorphs with H₂O₂ digestion for SP.1

DISCUSSION CONCLUSION

Comparative study of three palynological preparation techniques were carried out on six samples obtained from the middle and lower Benue trough. Two samples made up of clays were obtained from the middle Benue trough (Lafia Formation) while four samples made up of shale were obtained from the lower Benue trough (Nsukka Formation and Imo Shale). In the middle Benue trough, the Lafia Formation is the youngest formation in the area and was deposited under continental condition (fluvatile) in the Maastrichtian and characterized by ferruginized sandstones, red, loose sands, flaggy mudstones, clay and clay stones. Sedimentologic and micro-fossil data from Maastrichtian outcrops indicate a regressive phase and fossiliferous associations which are characterized by Foraminifera and palynomorphs like *Echitricolpites spinosus*, *Fenetrites spinosus*, *Nympheapollis clarus*, *Multiarolites formosus* as also recognized by Oloto and Ihunda (2013) in relation to forms found in the middle Benue trough.

Palynomorphs recorded in this basin are *Echimonocolpites rarispinosus*, *Cingulatisporites ornatus*, *Macrotyloma brevicaulis*, *Tubistephanocolpites cylindricus*, *Hexaporotricolpites emelianova*, *Psilaperiporites minimus*, *Tubistephanocolpites cylindricus*, *Retistephanocolpites gracillis*.

Acid digestion using HF generally is a method of extracting palynomorphs from sediments and sedimentary rocks however this protocol is potentially hazardous. Alternative methods using (NaPO₃)₆ and H₂O₂ have been developed and these techniques are relatively safe. However, in most cases, the H₂O₂ techniques are not as effective as acid digestion in terms of absolute palynomorph extraction. The (NaPO₃)₆ method proved more effective than the acid digestion. It appears that the effectiveness of the (NaPO₃)₆ method is indirectly proportional to the levels of lithification/induration of the material studied. Despite the disparity in effectiveness, the (NaPO₃)₆ method produces clean residues, and is suitable for most projects using palynology. The H₂O₂ technique was significantly less efficient than the acid digestion level probably because it destroys palynomorphs by oxidation. Hydrogen peroxide is hazardous as it is an oxidant and gives off a highly combustible mixture of hydrogen and oxygen; hence a fume hood is required for this technique. Therefore the (NaPO₃)₆ technique is safer and more effective. The latter thus appears to have significant

advantages over the H₂O₂ method. In total, 182 palynomorphs were derived through the use of the sodium hexametaphosphate technique; 125 forms from the hydrofluoric acid method and 33 forms through the hydrogen peroxide preparation technique respectively.

The H₂O₂ technique proved to be significantly less effective, at approximately 18% of the extraction level of salt digestion which appears to be largely due to oxidation, while the HF digestion proved to be approximately 69% of the extraction level of the salt digestion. Hydrogen peroxide is an aggressive oxidant. However, the H₂O₂ technique may be useful in breaking down more indurated lithotypes, and can be used in isolation or in combination with the (NaPO₃)₆ method. Therefore the (NaPO₃)₆ technique is deemed to be the safest and most effective of the three methods. It is evident that the (NaPO₃)₆ protocol is a viable alternative to acid digestion. Furthermore, it is safer, quicker to prepare and environmentally friendly. It does not require particularly sophisticated laboratory equipment and significant infrastructure such as a fume hood, and it is thus ideal for work on drilling rigs and in remote fieldwork operations.

RECOMMENDATION:

The use of Sodium Hexametaphosphate salt in palynological analysis should be encouraged as it has proven to be a better alternative to Hydrofluoric acid

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