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Outcrop Sediments

Alcohol-Based Hand Sanitizers

Highlights

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Discovering Thoughts, Inventing Future

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Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates

By Enwa Felix O, Anie Clement O, Oghenejobo Micheal & Ilaya Sonia A
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Abstract- This study is aimed at comparing the activity of alcohol based hand sanitizers (Dettol® and Lovillea®) and toilet soaps (Lux® and Premier®) against bacterial isolates. The activity of Dettol® and Lovillea® was compared with the activity of Lux® and Premier® toilet soaps against the bacterial isolates (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus specie and Shigella specie*) obtained from the palms of some individuals in Abraka, Delta state. Susceptibility of the bacterial isolates was evaluated using the agar well diffusion method by measuring the zones of inhibition observed after 24hours of incubation. Their minimum inhibitory concentrations (MIC) were also determined using the agar dilution technique. Dettol® hand sanitizer had the highest zones of inhibition (5mm, 5mm and 3mm) against *Staph aureus*, *Staph epidermis* and *Shigella specie* respectively.

Keywords: sanitizers, toilet soaps, hand washing, bacterial.

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Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates

Enwa Felix O [α], Anie Clement O [σ], Oghenejobo Micheal [ρ] & Ilaya Sonia A [ω]

Abstract- This study is aimed at comparing the activity of alcohol based hand sanitizers (Dettol® and Lovillea®) and toilet soaps (Lux® and Premier®) against bacterial isolates. The activity of Dettol® and Lovillea® was compared with the activity of Lux® and Premier® toilet soaps against the bacterial isolates (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus specie and Shigella specie*) obtained from the palms of some individuals in Abraka, Delta state. Susceptibility of the bacterial isolates was evaluated using the agar well diffusion method by measuring the zones of inhibition observed after 24hours of incubation. Their minimum inhibitory concentrations (MIC) were also determined using the agar dilution technique. Dettol® hand sanitizer had the highest zones of inhibition (5mm, 5mm and 3mm) against *Staph aureus*, *Staph epidermis* and *Shigella specie* respectively. Both soaps had no activity against Gram negative *Shigella specie.* Dettol® antiseptic was used as control and it gave zones of inhibition (36mm, 45mm, 35mm and 50mm) against *Shigella specie, Streptococcus specie, Staph aureus, and Staph epidermidis* respectively. For the MIC; the hand sanitizers inhibited all four organisms at 2ml, Premier® soap had MIC of 5mg/ml against *Staph epidermidis* while Lux® soap had MIC of 5mg/ml against *Staph epidermidis and Streptococcus specie*. The result revealed the efficacy of the antiseptic (control), hand sanitizers and soaps in descending order as follows; Antiseptic > Hand sanitizers > soaps. The use of soaps for hand hygiene appeared to be less efficacious against Gram negative organisms. However, both hand sanitizers and soaps can be used as effective measures to control the spread of diseases.

Keywords: sanitizers, toilet soaps, hand washing, bacterial.

I. Introduction

Hand washing is one of the most important steps to avoid spreading germs. Germs are microorganisms such as bacteria and viruses that may lead to harmful diseases. They can live on the skin, mouth, intestines and breathing passages. They can enter the body through openings such as the nose, mouth and also through breaks in the skin. Today, hygiene is associated with disease prevention and health promotion, and the importance of hygiene is universally recognized and evidence based. Physical contact between people and between people and objects is a key vehicle for the transmission of pathogens. Therefore, effective hand hygiene is a key intervention in disease prevention (Aiello *et al*, 2008). It is an integral procedure in the health care environment with healthcare workers receiving regular training about hand hygiene procedures (Hilburn *et al*; 2008, Johnson *et al* 2005, Harrington *et al* 2007). In the community outside of the healthcare environment, studies have reported association between improvements in hand hygiene and reduction in rates of infectious disease (Mello *et al*, 2007). It is estimated that simple hand washing could save one million lives a year (Curtis and Cairncross; 2003, WHO; 2000), many public health campaigns worldwide have addressed "hand hygiene "with varying success (Erasmus *et al* 2010, Pittet *et al*, 2009).

Bacteria are a tiny group of unicellular microorganisms. They can be classified into two groups, namely Gram Negative and Gram Positive organisms. An example of a Gram negative organism is *Escherichia Coli;* this type of bacteria is shaped like tiny pink rods and is found in raw meat or in the intestine of healthy humans and animals. *Staph.ylococcus* is an example of gram positive organisms; these are purple and clustered like Grapes. There are many types of *Staph.ylococcus* such as *Staph. aureus* and *Staph. epidermidis. Staph. aureus* colonizes mainly in the nasal passages while *Staph. epidermidis* is an occupant of the skin. Bacteria are found almost everywhere in environment such as air stool, water, sewage, human body, wounds, and other solid surfaces (Hurst *et al*, 2009). Although some are beneficial in the human body, others are not and may cause problems (Rolli and Jenner 1998). When pathogens or opportunistic microorganisms gain access into the body, they can cause infectious diseases, induce antigen-antibody reactions, mix with the normal flora and also may form bio-films (Macowiak, 1982).

Studies on the effectiveness of hand sanitizers have been somewhat conflicting. Some findings suggest that sanitizers are actually better than normal hand washing at killing micro-organisms while others have discovered that hand washing is still superior. The research indicates that there are many variable that would be causing these discrepancies. First of all, the concentration of alcohol – based sanitizers needs to be at least 60% to be effective. Alcohol based sanitizers at

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this concentration or higher are very effective at killing microbes but the alcohol evaporates quickly on the hands and may not be present on the skin long enough for adequate protection. As a result, unless the product can maintain high alcohol concentrations for a long period of time, it is probably not effective as regular hand washing hence, the center for disease control advices using warm water, working the soap into a lather and rubbing it for at least 20 seconds. However, hand washing with soap removes the body's own fatty acid from the skin, which may result in cracked skin that provides an entry portal for pathogens (Cagataz *et al*, 1998, Winnefield *et al* 2000). Also, high quality hand disinfectants contain additional skin care products like emollients. They also do not require the use of water which makes the application easy and uncomplicated.

II. Objectives of the Study

- To isolate potential pathogenic bacteria such as *Staph.ylococcus aureus, Staph.ylococcus epidermidis, Streptococcus specie* and *Shigella specie* from the palms of individuals within Abraka, Delta state.
- To compare the activity of two different alcohol-based hand sanitizers (Dettol® and Lovillea®) and two different toilet soaps (Premier® and Lux®) commonly sold in Abraka, Delta state against the organisms isolated.

a) Significance of the Study

This study serves to broaden the knowledge of the general public on the effect of hand sanitizers and toilet soaps against microorganisms for effective hand hygiene which is a key intervention in disease prevention.

Furthermore, the knowledge might encourage manufacturers of these products in ensuring better compliance to good manufacturing procedures.

b) Scope of the study

The study covers comparison of the effects of two alcohol-based hand sanitizers and two toilet soaps commonly found in Abraka, Delta State.

c) Limitation of the Study

This study was targeted on comparing the effects of some selected hand sanitizers and soaps against isolated bacteria species from the palms of individuals. The toxic effect of these products on the human skin was not determined.

d) Objectives of the Study

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Furthermore, the knowledge might encourage manufacturers of these products in ensuring better compliance to good manufacturing procedures.

f) Scope of the study

The study covers comparison of the effects of two alcohol-based hand sanitizers and two toilet soaps commonly found in Abraka, Delta State.

g) Limitation of the Study

This study was targeted on comparing the effects of some selected hand sanitizers and soaps against isolated bacteria species from the palms of individuals. The toxic effect of these products on the human skin was not determined.

III. Materials and Methods

a) Collection of the Different Alcohol-Based hand Sanitizers and Toilet Soaps

Two commonly used alcohol-based hand sanitizers commercially sold were purchased. One of the sanitizers was purchased from a pharmacy while the other was purchased from a super market, both in Abraka, Delta state Nigeria. The toilet soaps used were also purchased from a supermarket in Abraka. The alcohol-based hand sanitizers bought are; Dettol® and Lovillea® alcohol-based hand sanitizer while the toilet soaps bought are; Premier® soap and Lux® soap.

b) Culture media

The culture media used in the study include: Nutrient agar (Fluka, Germany), Macconkey Agar (Himedia India), Peptone water (Himedia India), Nutrient broth (San. Diego USA), Meuller Hinton Agar (Titan Biotech, India), Mannitol salt Agar (Titan Boitech, India).

c) Specimen

Swab from palms of both hands.

d) Reagents and chemicals used

Ethanol (BOH, India), crystal violet (Avishkar, Germany), Safranine (Avis, Germany), lugol's Iodine (Brema, Nigeria), Hydrogen peroxide, sugars (Glucose, Lactose and Sucrose).

e) Alcohol – based hand sanitizers

- Dettol® (Reckitt Benckiser)
- Lovillea® (PT Mandon, Indonesia)

f) Toilet Soaps

- Premier® (Pz cussons Nigeria Plc. Nigeria)
- Lux® (Unilever Nigeria Plc, ogun state)

g) Microbial Cultures

Staph.ylococcus aureus, *Staph.ylococcus epidermidis*, *Streptococcus specie*, *Shigella specie*.

h) Sample collection of bacterial isolate

Samples were obtained from individuals within Abraka, Delta state. The samples were obtained from the palms of both hands of the individuals using a sterile cotton swab. The swab specimens collected were then transported immediately to the laboratory for handling and analysis. The swab sticks containing the samples were aseptically streaked in different Nutrient Agar plates, after which the plates were incubated at 37°C for 24hours. After incubation, distinct colonies were observed on fifty eight Nutrient agar plates. The distinct colony found on each plate was then inoculated in separate nutrient agar slants prepared in McCartney bottles and incubated at 37°C for 24hours. Thereafter, growth was observed on all the fifty eight nutrient agar slants and the slants were properly stored for subsequent studies.

i) Characterization Based on Colony Morphology and physiology

i. *Macroscopic Identification of Colonies*

The organism identified in the course of this study were *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus species* and *Shigella specie.*

j) Biochemical Tests and Other Identification Tests

The following biochemical tests were carried out to confirm the identified organisms. The test includes;

- Catalase test.
- Coagulase test
- Indole test
- Fermentation test

k) Bacterial susceptibility to alcohol- based hand sanitizers and soaps

Muller-Hinton Agar was prepared according to manufacturer's specifications, sterilized, cooled, 20mls each poured into eight sterile petri dishes and kept for 45 minutes in order to allow it solidify. Thereafter, the test organisms were aseptically inoculated into four different properly labelled petri dishes containing already solidified Muller Hinton agar by using different sterile swab sticks to pick the organisms from prepared overnight broth and streaking the organisms all over the petri dishes. This procedure was carried out using another four properly labeled petri dishes which served as duplicate for the experiment.

A 5mm cork borer was used to bore holes in the solidified agar on each petri dish. Using a 2ml syringe, few drops each of the hand sanitizers, 10mg/ml solution of the soaps, and Dettol® antiseptic (used as control) was added to their respective holes in the petri dish. After 5minutes, all the petri dishes were carefully packed with a masking tape and transferred into the incubator for 24hours at 37°C. Zones of inhibition were observed and recorded after 24hours.

l) Determination of minimum inhibitory Concentration

The determination of the minimum inhibitory concentration of the soaps was carried out to determine the lowest concentration of the soaps that can inhibit the visible growth of the test organisms (*Staph.ylococcus aureus Staph.ylococcus epidermidis*, *Streptococcus specie* and *Shigella specie*) after 24 hours of incubation. The agar dilution technique was used and it involves the following process. Firstly, 19ml of sterilized Muller Hinton agar was poured with 1ml of each dilutions of the soaps (Lux® and Premier® soaps) into eight different petri-dishes and allowed to solidify. Four of the petri-dishes contained 1ml each of the several dilutions (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of one soap (Premier®) plus 19ml each of the sterilized Muller Hinton agar while the other four petri-dishes contained 1ml each of several dilutions (10mg/ml,5mg/ml,2.5mg/ml and 1.25mg/ml) of the other soap(Lux®) plus 19mls each of the sterilized Muller Hinton agar. Thereafter, the test organism were streaked onto the different properly labelled plates seeded with the soap solutions using a flamed wire loop. The plates were packed with a Masking tape and incubated at a temperature of 37°C for 24 hours. After 24hours incubation, the least concentrations of each of the soaps that inhibited the test organisms were taken as the minimum inhibitory concentration.

For the alcohol-based hand sanitizers, different volumes of the hand sanitizers were tested to know if increased volumes of hand sanitizers enhance their effectiveness. Firstly, 19.8ml, 19.5ml, 19ml, 18.5ml and 18ml of sterilized Muller Hinton agar was poured with 0.2ml, 0.5ml, 1ml, 1.5ml and 2ml respectively of the two different alcohol-based hand sanitizers being tested into ten different petri- dishes. Five of the petri-dishes contained different volumes (0.2ml, 0.5ml, 1ml, 1.5ml and 2ml) of one alcohol based hand sanitizer (Dettol®) plus corresponding volumes of sterilized Muller Hinton agar while the other five petri dishes contained different volumes (0.2ml,0.5ml,1ml,1.5ml and 2ml) of the other hand sanitizer (Lovillea®) plus corresponding volumes of the sterilized Muller Hinton agar. The mixture on each of the petri dishes was swirled gently and allowed to solidify. Thereafter, the test organisms were aseptically streaked onto the different prepared plates seeded with the alcohol based hand sanitizers using a flamed wire loop and then incubated at 37°C for 24 hours. After 24 hours of incubation, the least volume the hand sanitizers that inhibited the growth of the test organisms was observed and tabulated.

IV. Result and Discussion

Table 3 : Effect of hand sanitizers and soaps against bacterial isolates

	Hand Sanitizers		Toilet soaps		Control
Test organism	Dettol ®	Lovillea®	Lux® 10mg/ml	Premier® 10mg/ml	Dettol® antiseptic
Staph. aureus	5	3	2	2	35
Staph. Epidemidis	5	4	5	2	50
Streptococcus spp	2	5	6	5	45
Shigella specie	3	2	-	-	36

Key:
Zone of inhibition in millimeter (mm)
(-) sign represents no visible zone of inhibition

Table 4 : Minimum inhibitory concentrations (MIC) of the hand sanitizers and soaps

Table 4a : MIC of Dettol® hand sanitizer

Test organisms	Dettol®				
	0.2ml	0.5ml	1ml	1.5ml	2ml
Staph. aureus	+	+	+	+	-
Staph.epidermidis	+	+	+	+	-
Streptococcus spp.	+	+	+	+	-
Shigella specie	+	+	+	+	-

Table 4b : MIC of Lovillea® Hand Sanitizer

Test organisms	Lovillea® Hand Sanitizer				
	0.2ml	0.5ml	1ml	1.5ml	2ml
Staph. aureus	+	+	+	+	-
Staph. epidermidis	+	+	-	-	-
Streptococcus spp.	+	+	+	+	-
Shigella specie	+	+	+	+	-

Table 4c : MIC of Lux® Soap

	Lux® Soap			
Test organisms	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Staph. aureus	+	+	+	+
Staph. epidermidis	-	-	+	+
Streptococcus spp.	-	-	+	+
Shigella specie	+	+	+	+

Table 4d : MIC of Premier® Soap

	Premier® Soap			
Test organisms	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Staph. aureus	+	+	+	+
Staph. epidermidis	-	-	+	+
Streptococcus spp.	+	+	+	+
Shigella specie	+	+	+	+

Key:
(-) sign indicates no growth (inhibition)
(+) sign indicates growth (no inhibition)

V. Discussion

Two alcohol based hand sanitizers (Dettol® and Lovillea®), and two toilet soaps (Lux® and Premier® at 10mg/ml) were tested against four isolated bacterial species. Based on the data obtained from table 3, Dettol® had the highest zones of inhibition i.e. 5mm, 5mm, and 3mm against *Staph.ylococcus aureus, Staph.ylococcus epidermidis and Shigella specie* respectively, with a zone of inhibition (2mm) against *Streptococcus specie*. Lovillea® hand sanitizer had zones of inhibition (3mm, 4mm, 5mm and 2mm) against *Staph. aureus, Staph. epidermidis, Streptococcus specie and Shigella specie* respectively. For the soaps, Lux® soap had the highest zone of inhibition (6mm) against *Streptococcus specie*, with zones of inhibition; 2mm and 5mm against *Staph. aureus and Staph. epidermidis* respectively but there was no zone of inhibition for *Shigella specie*. Premier® soap had the lowest zone of inhibition i.e. 2mm against *Staph. aureus and Staph. epidermidis,* with a zone of 5mm against *Streptococcus* specie and no zone of inhibition against *Shigella specie*. Comparing the activity of the hand sanitizers and toilet soaps, three bacterial isolates (*Staph. aureus, Staph. epidermidis and Shigella specie)* were more susceptible to Dettol® hand sanitizer while *Streptococcus specie* was more susceptible to Lux® soap and least susceptible to Dettol®. Furthermore, Premier® soap had the least activity against *Staph. aureus and Staph. epidermidis*. The soaps had no effect against gram negative *Shigella specie* which indicates that the hand sanitizers were more effective than the toilet soaps against gram negative *Shigella specie*. The result also shows that *Shigella specie* is the most resistant amongst the isolated bacterial species, this is because it displayed the least zones of inhibition to the hand sanitizers and it was the only organism that displayed resistance to the soaps tested. *Streptococcus specie and Staph.ylococcus epidermidis* displayed the highest margin of susceptibility.

From the data obtained from the determination of the minimum inhibitory concentration as shown in table 4 above, *Shigella specie* was the most resistant bacteria because it was not inhibited by any of the soaps and was only inhibited by the hand sanitizers at the highest volume of 2ml. Lux® soap had MIC value at 5mg/ml against *Staph. epidermidis and Streptococcus specie* while Premier® soap had MIC value at 5mg/ml against *Staph. epidermidis* only. Lovillea® hand sanitizer inhibited *Staph.ylococcus epidermidis* only at a lower volume of 1ml while all four organisms were inhibited by the two alcohol based hand sanitizers (Lovillea® and Dettol®) at a volume of 2ml. The most susceptible organism inhibited by the hand sanitizers and soaps (at lower concentrations) was *Staph.ylococcus epidermidis.*

VI. Conclusion

Hand washing is one of the most important steps to avoid spreading germs. Germs can live on the skin, mouth, intestines etc. and can enter the body through openings such as the nose, mouth, and also through breaks in the skin. Today, hygiene is associated with disease prevention and health promotion. Therefore, effective hand hygiene is a key intervention in disease prevention (Aiello *et al*, 2008). The study revealed that *Staph.ylococcus aureus, Staph.ylococcus epidermidis, Streptococcus species* and *Shigella specie* are present on the hands of humans. The result also revealed better efficacy of the alcohol based hand sanitizers in comparison to the toilet soaps because all four bacterial isolates were susceptible to them; with Dettol hand sanitizer having better activity against more bacterial isolates. Also, the soaps had no effect against Gram negative *Shigella specie* which makes them less efficacious. However, the activity of the hand sanitizers against the bacterial isolates was poor compared to that of the antiseptic which was used as control. Therefore, there is need to confirm the concentration of alcohol in hand sanitizers sold in order to verify the 99.9% germ killing ability of these products as claimed by the manufacturers.

The efficacy of alcohol based hand sanitizer is affected by several factors such as the type, concentration and volume of alcohol used, the contact time, (CDC, 2002), the test method (invivo and invitro), target organisms and matrix. (Liu *et al*, 2010.)

VII. Recommendation

The outbreak of epidemic infections such as Ebola hemorrhagic fever (caused by the Ebola virus), HIV, Diarrhea, etc., some of which are highly infectious and can be easily transmitted through infected hands calls for the need to evaluate the effect of antimicrobials such as hand sanitizers as well as soaps commonly used for hand washing. The use soap and water only for hand hygiene can be effective if there is availability of clean water but if not, hand sanitizers are preferable because they are rinse free and as a result, do not require water. However, when hands are visibly dirty, hand sanitizers are not as effective as soap for cleansing. Hence, if hands are visibly dirty, effective hand hygiene can be achieved by first washing hands with soap and water after which hand sanitizers can be used but if not, hand sanitizers are preferably used alone. Furthermore, manufacturers and regulatory authorities should enforce stringent quality control measures during production and routine inspection to ensure the efficacy of these products. Finally, sanitation as a means of proper hygiene is essential for good health benefits for social and economic developmental purposes.

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Localization of Acid Phosphatase in the Different Tissues of Developmental Stages of Indian Major Carp, *Labeo Rohita* (Hamilton)

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Abstract- Acid phosphatases (AcPs) are known to provide phosphate to tissues that have high energy requirements, especially during development, growth and maturation. AcPs is a lysosomal enzyme as it is lytic and destructive in nature. In the present investigation the attempt was made to localize this enzyme in the intestine, liver and kidney during the developing stages of *Labeo rohita*. For the histochemical localization of this enzyme activity, sodium β-glycerophosphate was used as a substrate. The AcPs activities in the developing stages were increased gradually from 24hr hatching to 60mm stage. It was concluded that the presence of AcPsin the yolk sac might be due to its role in the yolk metabolism. Gradual increase in enzyme activity from 10mm onwards in the intestine could be due to maturation of digestive tract and in the liver due to increase in the metabolic load with increase in growth and age.

Keywords: *acid phosphatase, Labeo rohita, developing stage.*

GJSFR-C Classification : *FOR Code: 069999*

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Abstract- Acid phosphatases (AcPs) are known to provide phosphate to tissues that have high energy requirements, especially during development, growth and maturation. AcPs is a lysosomal enzyme as it is lytic and destructive in nature. In the present investigation the attempt was made to localize this enzyme in the intestine, liver and kidney during the developing stages of *Labeo rohita*. For the histochemical localization of this enzyme activity, sodium β-glycerophosphate was used as a substrate. The AcPs activities in the developing stages were increased gradually from 24hr hatching to 60mm stage. It was concluded that the presence of AcPs in the yolk sac might be due to its role in the yolk metabolism. Gradual increase in enzyme activity from 10mm onwards in the intestine could be due to maturation of digestive tract and in the liver due to increase in the metabolic load with increase in growth and age.

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I. Introduction

Labeo rohita is one of the Indian major carp and is an economically important fast growing fish that attends the sexual maturity at the age of two years. A better knowledge of digestive enzyme activities is essential for understanding of the physiology of fish nutrition. The digestion in vertebrates is carried out by the intestinal enterocytes expressing brush border enzymes such as disaccharidase, alkaline phosphatase and transpeptidase (Maroux *et al*., 1973; Semenza, 1986 and Ferraris *et al*., 1992). In recent years, digestive research on fish has focused on the occurrence and activity changes of digestive enzymes during larvae and juvenile stages (Chen *et al*, 2004). Studies on sea bream *Pagrosomus major* (Chen *et al*, 1998) and amur sturgeon *Acipenser schrenckii* (Su & Zhao, 2005) found that types, time of occurrence, and activity levels of digestive enzymes in larvae and juveniles differ significantly. As the stability of enzyme activity can influence the body's biological metabolism and adaptive capacity (Wang *et al*, 2001), it is essential to quantify its activity during the early developmental stages of fish.

Acid phosphatases (AcPs) are known to provide phosphate to tissues that show high energy requirements, especially during development, growth and maturation (Blum, 1970; Hurkadli *et al*., 1985). They are ubiquitous enzymes that catalyze the hydrolysis of orthophosphate monoesters under acidic conditions. Despite a common functional identity, these hydrolases can be differentiated according to structural, catalytic and immunological properties, as well as tissue distribution and sub-cellular location (Suter *et al*., 2001).

The aim of this study was to determine histochemical localization of acid phosphatase, in the different tissues of *Labeo rohita* and their contribution to intracellular digestive processes.

II. Materials and Methods

All the developmental stages of *Labeo rohita* were collected in monsoon season from Government of Maharashtra Fish Seed Center, Pench located 47Km from Nagpur. Live fish seeds were carried to the laboratory in oxygen filled polythin bags. Body length was measured prior to fixation. Acid phosphatase activity was demonstrated by Gomori method(1952). Stages were fixed in ice-cold 10% Neutral buffer formalin (NBF) for 24 hrs. After fixation material transferred to 10% and 20% sucrose for 1hr and 30% sucrose for overnight. The stages were washed with ice cold distilled water and then were cut 10µm thick sections on Leica cryostat at -20°C.Sections were washed with ice cold distilled water and after washing with distilled water, the sections were incubated for 16-20hrs in Gomori medium at 37°C. Sections were washed with distilled water and transferred to dilute yellow ammonium sulphide solution for 1 min, washed in distilled water and mounted in glycerine jelly. Lastly the microphotographs were taken.

III. Result

Acps activity during development of *Labeo rohita* was studied from 24hrs stage upto 60mm stage. It was observed that this enzyme exhibits tremendous variation from early to late stage of development after hatching.

a) 24 hrs to 72 hrs stage

In 24 hrs after hatching, diffuse to granular staining of the enzyme acid phosphatase is observed in the yolk granules which remain weaker in 36 hrs, 48 hrs and 60 hrs after hatching. In all these stages, there is

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progressive absorption of the yolk sac and in about 72 hrs stage, the yolk sac is almost completely absorbed. At this stage, intense diffuse to granular staining is observed in the boundary wall of the yolk sac indicating its active lysis by the enzyme (Fig. 1-5). Intestine also exhibits weak staining upto 36 hrs, which becomes moderate in 48 hrs (Fig. 3). In 60 hrs after hatching (Fig. 4), it becomes intense and in 72 hrs weak granular staining is observed in intestinal epithelium. Liver and Primordial Germ Cells (PGCs) remain unstained for about 72 hrs after hatching. Muscles exhibit poor staining upto 72 hrs (Fig. 5). Mesonephric tubules which are placed in close proximity to PGCs are unstained upto 72 hrs.

b) 10mm to 60 mm stages

Intestinal epithelium which is weakly stained upto 72 hrs, shows moderate, granular diffuse staining in 10 mm stage (Fig. 6) which in 20 mm (Fig. 8) is intensified and it shows intense diffuse to granular staining through successive stages right upto 60 mm stage (Fig. 11, 14, 17 and 20). Muscles blocks are weakly stained for acid phosphatase upto 72 hrs. Acid Phosphatase staining is not noticed in muscles from 10 mm to 60 mm stage. Liver exhibits granular staining in hepatocytes at 10 mm stage (Fig. 7). It becomes dense granular in 30 mm (Fig. 13) and remains intense upto 60 mm stage (Fig. 16, 19, 22). In 10 mm (Fig. 6) and 20 mm stage (Fig. 9), diffuse to granular staining is shown by renal tubules around the lumen. Interstitial haemopoeitic tissues also have some diffuse reactivity. In 30 mm stage (Fig. 12), the staining becomes intense which continues upto 60 mm stages (Fig. 21).From 10 mm to 60 mmstage, PGCs seem to remain unstained upto fingerling stage.

IV. Discussion

AcPs is a typical lysosomal constituent that is a destructive enzyme. The activities of AcPs were not correlated with egg viability and lytic processes did not reduce egg viability (Cara *et al*., 2003). Larva in *Labeo rohita* when hatches out from the egg carry his own bag of food in the form of yolk sac. Yolk is the main energy source for most of the fishes during endogenous feeding period which begins at fertilization and ends at the onset of exogenous feeding by the hatched larvae (Kamler, 1992).

From 72 hrs after hatching, yolk sac is almost completely absorbed. Intense diffuse to granular staining is observed in the boundary walls of the yolk sac, thereby indicating the role of acid phosphatase in this lytic process. Mobilization of yolk reserves in teleostean embryos is reported to occur through the vitelline syncytium that envelops the whole yolk mass after the closer of the blastopore. Yolk is absorbed by phagocytic activity of the inner part of the syncytical layer is degraded into lower molecular weight substances and is transported into the blood (Walzer & Schonenberger, 1979; Shimizu & Yamada, 1980; Buddington & Doroshov, 1986).

When mouth opens and active feeding starts after 72 hrs, initially weak granular staining is observed in the intestinal epithelium, but with feeding activity picking up, intensity of AcPs becomes more and more as evident by intense staining upto fingerling stage. AcPs are the main enzymes along with other digestive enzymes whose activity was detected at the moment of mouth opening during larval development of white sea bream, *Diplodus sargus* (Cara *et al*., 2003). Localization of acid phosphatase in normal and neoplastic tissues and cells continues to be an extremely important endeavor (Cox & Singer, 1999).

Intestine upto 72 hrs exhibits weak to moderate activity for AcPs enzymes. The role of incomplete alimentary tract seems to be minimal till the absorption of yolk sac. Activity of AcPs enzymes intensifies as the development proceeds upto 60 mm stage. Food was first found in the anterior part of the alimentary tract of carp, *Cyprinus carpio* larvae on the third day after hatching (Matlak & Matlak, 1976). Same is observed in *Labeo rohita* also. The variation in the activity of digestive enzymes are correlated either to the developmental events such as functional start of the stomach (22 day after hatching) or to changes in the nature of the diet (Cara *et al*., 2003). AcPs activity in the intestinal epithelium becomes moderate and later intense upto 60 mm stage in *Labeo rohita*.

liver initially is unstained upto 72 hrs for acid phosphatase, but in later stages its staining intensifies. The significance of AcPs activity in the intestine and liver may be connected to various types of food to be broken down by larva and its switching over to varied food as the development proceeds. Liver function becomes important for various metabolic activities. Activity of enzymes in general depends upon number of external factors such as temperature, pH etc. (Powar, 1981). The relationship between metabolic rate and temperature in early ontogenesis was studied in *Esox lucius* by Lindroth (1942, 1946) and in *Clupea harengus* (Holliday *et al*., 1964).

There are some recent reports about enzyme distribution and localization in gastrointestinal tract during the larval period of fish such as in sea bass *Lates calcarifer* (Walford & Lam, 1993), *Theragra chalcogramma* (Oozeki & Bailey, 1995), *Winter flounder* (Baglole *et al*., 1998), *Solea senegalensis* (Ribeiro *et al*., 1999) and in *Siberian sturgeon* (Gisbert *et al*., 1999). They all have pointed a correlation between enzyme activities and maturation of digestive tract. Though digestive enzymes are not studied in ontogeny of *Labeo rohita*, lysosomal and hydrolytic enzyme activities reported in the present study can be correlated with variation in the food, maturation of the tract and

environmental parameters such as temperature and pH etc.

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Photo Plate 1

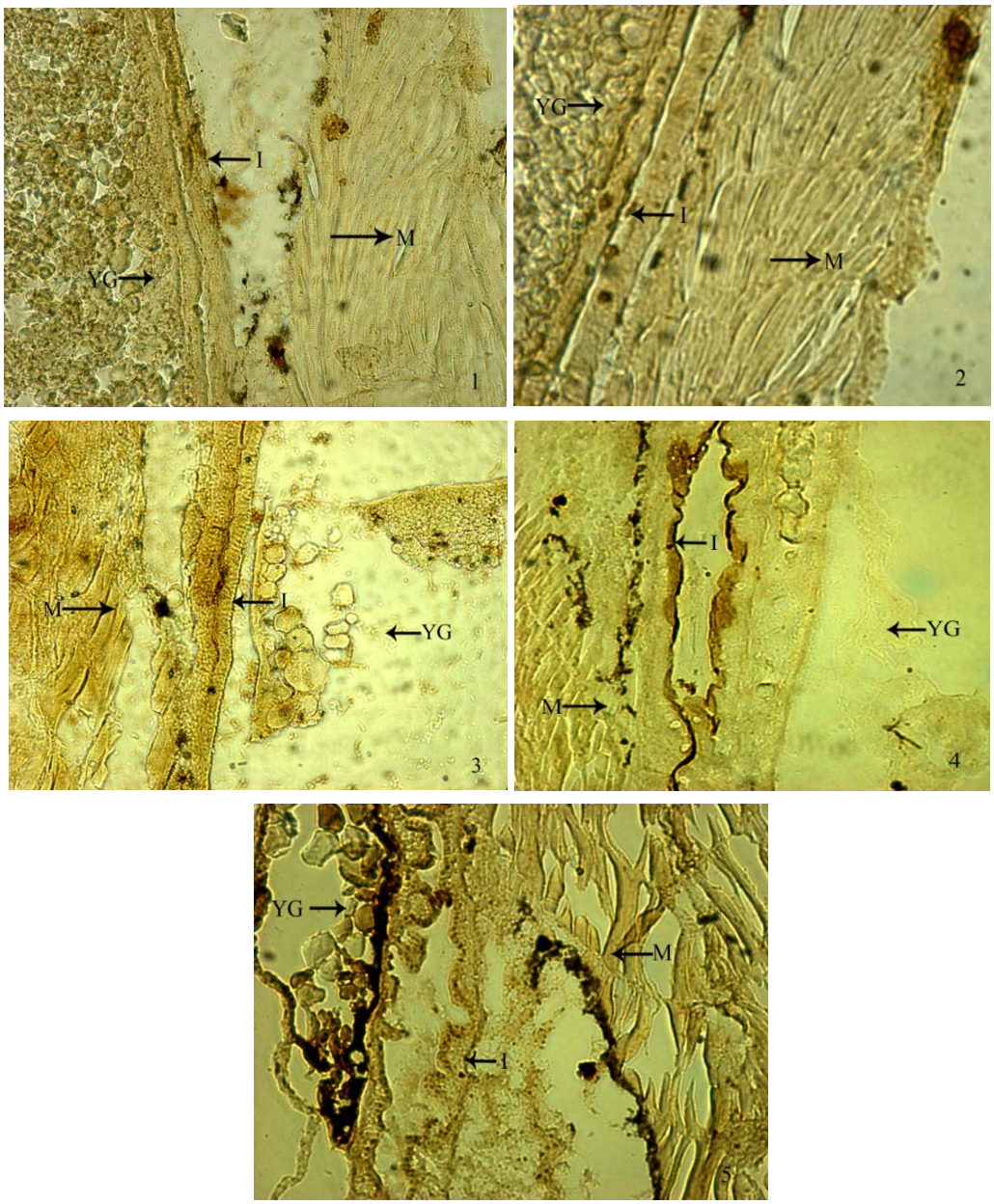

Fig. 1 : Sagittal section of 24 hrs hatching showing acid phosphatase activity in intestine (I) , muscles (M) and yolk granules (YG) X200

Fig. 2 : Sagittal section of 36 hrs hatching showing acid phosphatase activity in intestine (I) , muscles (M) and yolk granules (YG) X200

Fig. 3 : Sagittal section of 48 hrs hatching showing acid phosphatase activity in intestine (I) , muscles (M) and yolk granules (YG) X200

Fig. 4 : Sagittal section of 60 hrs hatching showing acid phosphatase activity in intestine (I) , muscles (M) and yolk granules (YG) X200

Fig. 5 : Sagittal section of 72 hrs hatching showing acid phosphatase activity in intestine (I) , muscles (M) and yolk granules (YG) X200

Photo Plate 2

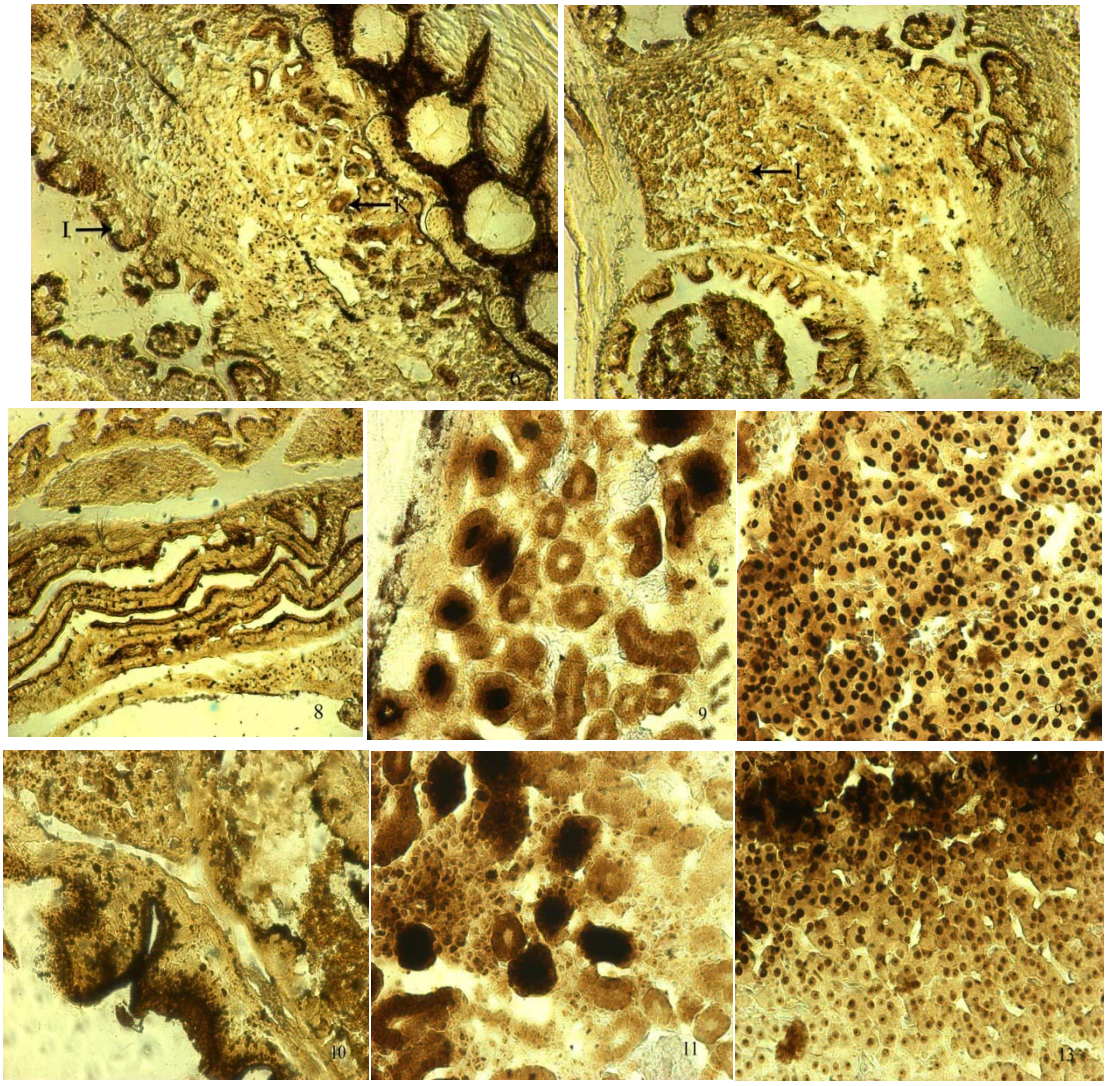

Fig. 6 : Sagittal section of 10 mm stage showing acid phosphatase activity in kidney (K) and intestine (I) X200

Fig. 7 : Sagittal section of 10 mm stage showing acid phosphatase activity in liver (L) X200

Fig. 8 : Sagittal section of 20 mm stage showing acid phosphatase activity in intestine (I) X200

Fig. 9 : Sagittal section of 20 mm stage showing acid phosphatase activity in kidney (K) X200

Fig. 10 : Sagittal section of 20 mm stage showing acid phosphatase activity in liver (L) X200

Fig. 11 : Sagittal section of 30 mm stage showing acid phosphatase activity in intestine (I) X200

Fig. 12 : Sagittal section of 30 mm stage showing acid phosphatase activity in kidney (K) X200

Fig. 13 : Sagittal section of 30 mm stage showing acid phosphatase activity in liver (L) X200

Photo Plate 3

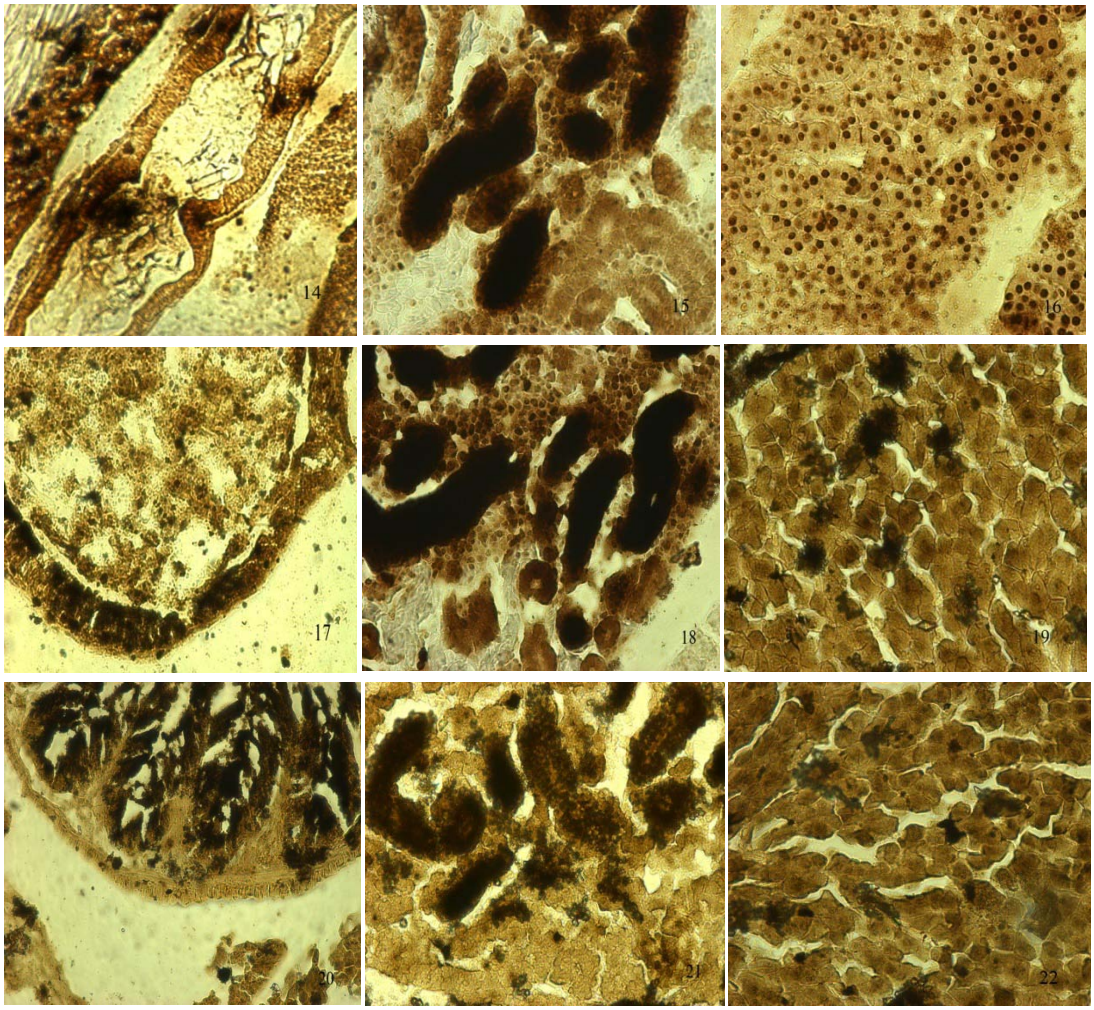

Fig. 14 : Sagittal section of 40 mm stage showing acid phosphatase activity in intestine (I) X200

Fig. 15 : Sagittal section of 40 mm stage showing acid phosphatase activity in kidney (K) X200

Fig. 16 : Sagittal section of 40 mm stage showing acid phosphatase activity in liver (L) X200

Fig. 17 : Sagittal section of 50 mm stage showing acid phosphatase activity in intestine (I) X200

Fig. 18 : Sagittal section of 50 mm stage showing acid phosphatase activity in kidney (K) X200

Fig. 19 : Sagittal section of 50 mm stage showing acid phosphatase activity in liver (L) X200

Fig. 20 : Sagittal section of 60 mm stage showing acid phosphatase activity in intestine (I) X200

Fig. 21 : Sagittal section of 60 mm stage showing acid phosphatase activity in kidney (K) X200

Fig. 22 : Sagittal section of 60 mm stage showing acid phosphatase activity in liver (L) X200

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Palynology of Late Paleocene - Earliest Eocene Outcrop Sediments from Benin Basin, SW Nigeria: Implications for Paleoclimatology and *PETM* Record in the Tropics

By Peter Adegbenga Adeonipekun & Ayobami Oyelami
University of Lagos, Akoka Lagos Nigeria

Abstract- Palynological and sedimentological studies were carried out on Paleocene/Eocene outcrop sediments from Shagamu Quarry Benin basin, SW Nigeria so as to gain insight into the paleoclimate of this important geological period in the tropics. Standard palynological preparation techniques were applied to sub-samples of the outcrop. Another suite of same sediments was sedimentologically prepared and analyzed for lithological inferences. The outcrop samples are made up of a larger shaly section and a very short dolomitic shaly sand unit within the Oshoshun (Akinbo) Formation. Diversity and abundance of palynomorph taxa decreased upward from the late Paleocene to the earliest Eocene in the outcrop area. Four phyto-climatic zones were recognized. The late Paleocene section was wet except a brief dry interval with abundant Poaceae and fungal elements, while the earliest Eocene was dry. Occurrence of *Apectodinium* acme and abundant *Botryococcus* within a marine transgression event perhaps indicate the PETM in the study area.

Keywords: *shagamu, nigeria, benin basin, palynology, paleoclimate, phyto-climatic zones, petm, apectodinium acme, botryococcus abundance, oshoshun formation, marine transgression.*

GJSFR-C Classification : *FOR Code: 040308*

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Peter Adegbenga Adeonipekun [α] & Ayobami Oyelami [σ]

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Keywords: Shagamu, Nigeria, Benin Basin, Palynology, Paleoclimate, Phyto-Climatic Zones, PETM, Apectodinium Acme, Botryococcus Abundance, Oshoshun Formation, Marine Transgression.

I. Introduction

Palynology has been a veritable tool in vegetation reconstruction for paleoclimatic interpretation Faegri and Iversen (1966). That recovered palynomorphs reflect the vegetation of an area to a reasonable extent has been reported by several workers among whom are Faegri and Iversen (1966), Sowunmi (1987, 1981a and b, 1986) and Traverse (1988) as well as Morley and Richards (1993). Sowunmi (1986) used pollen and spores recovered from Niger delta sediments to interpret the paleoclimate of the area from the Eocene to Recent. Sowunmi (1987) later compared palynomorphs from Recent samples with older subsurface samples in the Niger delta and found that the composition of the surface sediment palynomorphs reflected the vegetation of the study area and bore a strong similarity with the subsurface. Morley and Richards (1993) have also used the distribution of charred Gramineae (Poaceae) cuticles to infer the paleoclimatic changes of the Neogene Niger delta. They posited that the abundance of these cuticles from the late Miocene upward indicates dry climate conditions as they were sourced from burnt savanna vegetation. Adeonipekun (2006) also used pollen and spores as well as charred Poaceae cuticles to infer the paleoclimatic changes of Neogene Niger delta. Ige (2009; 2011), Ige and Datta (2011) and Adebayo *et al.* (2012) recently used palynomorphs to reconstruct the paleoclimate of the Niger delta basin. Despite these efforts on the Niger delta sediments, the authors are not aware of any palynology based paleoclimate study on the eastern Benin basin sediments. Most of the works on Benin basin have dwelt on the stratigraphy, geochemistry and structural geology (Rayment, 1965; Adegoke, 1969; Ogbe, 1972; Omatsola and Adegoke, 1981; Coker and Ejedawe, 1987; Elueze and Nton 2004, Adeonipekun *et al.*, 2012). Knowledge of the paleoclimatic conditions from this basin will further enhance the understanding of the paleoclimate of the Paleogene of Nigeria and give further insight into the understanding of the Paleotropical Maximum events in the tropical areas.

The Paleocene–Eocene Thermal Maximum (PETM) at *c.* 55.8 Ma is a short interval of rapid greenhouse warming of global lithosphere, hydrosphere and atmosphere. The work of Jaramillo and Dilcher (2000) on tropical Paleocene – early Eocene sediments shows that there was extinction at the Paleocene – Eocene Thermal Maximum (PETM) after which in the younger Eocene, diversity increased in the Colombian eastern Andes. Harrington and Jaramillo (2007) also reported taxonomic diversity increases in the late Paleocene of the US Gulf Coast, a trend replaced by a marked extinction into the early Eocene. Wing and Currano's (2013) work on plant macrofossils in the Bighorn Basin, Wyoming, United States however recorded radical floristic change during the PETM. This reflects "local or regional extirpation of mesophytic plants, notably conifers, and colonization of the area by

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thermophilic and dry-tolerant species, especially Fabaceae". They posited however that this floristic change reversed itself at the end of the PETM even "though some immigrant species persisted and some Paleocene species never returned". Apart from Jaramillo and Dilcher (2000), Prasad *et al.* (2006) in northern India and Schulte *et al.* (2011) in Dababiya Quarry section Egypt are other works in the tropics on the PETM.

While Prasad *et al.* (2006) utilized dinoflagellate *Apectodinium* acme for recognizing the PETM, Schulte *et al.* (2011) applied sequence stratigraphy to recognize the PETM at the base of the Eocene within a transgressive systems tract (TST). Jaramillo *et al*. (2010) studied the tropical rainforest response to the PETM event using palynomorphs in eastern Colombia and western Venezuela. They "observed a rapid and distinct increase in plant diversity and origination rates, with a set of new taxa, mostly angiosperms, added to the existing stock of low-diversity Paleocene flora". Jaramillo *et al.* (2010) recorded no evidence of aridity and that the rainforest survived the extreme temperature increase of the 200,000 years' event.

Gebhardt *et al.* (2010) is the only work known to the authors on the Benin basin with respect to the terminal thermal events. Gebhardt *et al.* (2010) studied the foraminifera and carbon isotopes Excursion (CIE) of sediments from the Shagamu Quarry, southwest Nigeria and recognized the Initial Eocene Thermal Maximum (IETM) at the Paleocene - Eocene boundary as being characterized by the dominance of dysoxic benthic foraminifera - *Bulimina* and *Nonionella* spp. with concomitant great reduction in planktic and benthic foraminifera. This work did not apply much of the palynological data and that not much of the Eocene section was involved. It rather emphasized more of foraminiferal and Carbon Isotope Excursion indices apart from limiting the thermal events to the late Paleocene.

While fuller and more recent information in literature abounds for the temperate and other regions of the world, there is little information on the Paleocene/Eocene sediments from Nigeria. The excellent recovery of marker palynomorphs, foraminifera and nannofossils by Adeonipekun *et al.* (2012) in the Shagamu Quarry Benin basin is sufficient to make paleoclimatic inference with respect to the PETM.

II. Geology of Benin Basin

Benin basin lies pseudo-parallel to the West African coast beginning from the Ghana ridge onshore and extending through Togo and Benin republics to the Benin Hinge Line in Western Nigeria (Fig. 1). It was formed in response to the separation of the African and South American landmasses and the subsequent opening of the Atlantic Ocean in the Jurassic – lower Cretaceous (Omatsola and Adegoke, 1981).

The oldest dated sediments onshore Benin Basin is lower Cretaceous (Omatsola and Adegoke, 1981) while the oldest known outcrop is the Maastrichtian part of the Abeokuta Formation that sits unconformably on the basement complex. The Abeokuta Formation, as shown by onshore drilled wells however, sits conformably on the basement complex. This Neocomian - Paleocene Abeokuta Formation has been assigned a group status and sub-divided by Omatsola and Adegoke (1981) into three formations – Ise, (oldest); Afowo; and Araromi (youngest). Sitting on top of the Abeokuta Formation conformably are the Paleocene/Eocene limestone; marine shales and sandy shales; and claystones of the Ewekoro, Oshosun, and Ilaro Formations respectively in ascending order. Late Tertiary sediments of Benin Formation terminate the stratigraphic sequence with shallow marine – none marine gravel, sand and sandy clay that sit unconformably on the Paleocene / Eocene sequence (Table 1).

Table 1 : Stratigraphy of Benin basin (Omatsola & Adegoke, 1981; Ogbe, 1972)

AGE	FORMATION (Ogbe, 1972)	FORMATION (Omatsola & Adegoke, 1981)
m. Miocene-Recent	BENIN	BENIN
Lower Eocene	ILARO	ILARO
Lower Eocene	OSHOSHUN	OSHOSHUN
Paleocene/Eocene	AKINBO	OSHOSHUN
Paleocene	EWEKORO	EWEKORO
Senonian/Paleocene	ABEOKUTA	ARAROMI
Senonian	ABEOKUTA	AFOWO
Neocomian-Senonian	ABEOKUTA	ISE

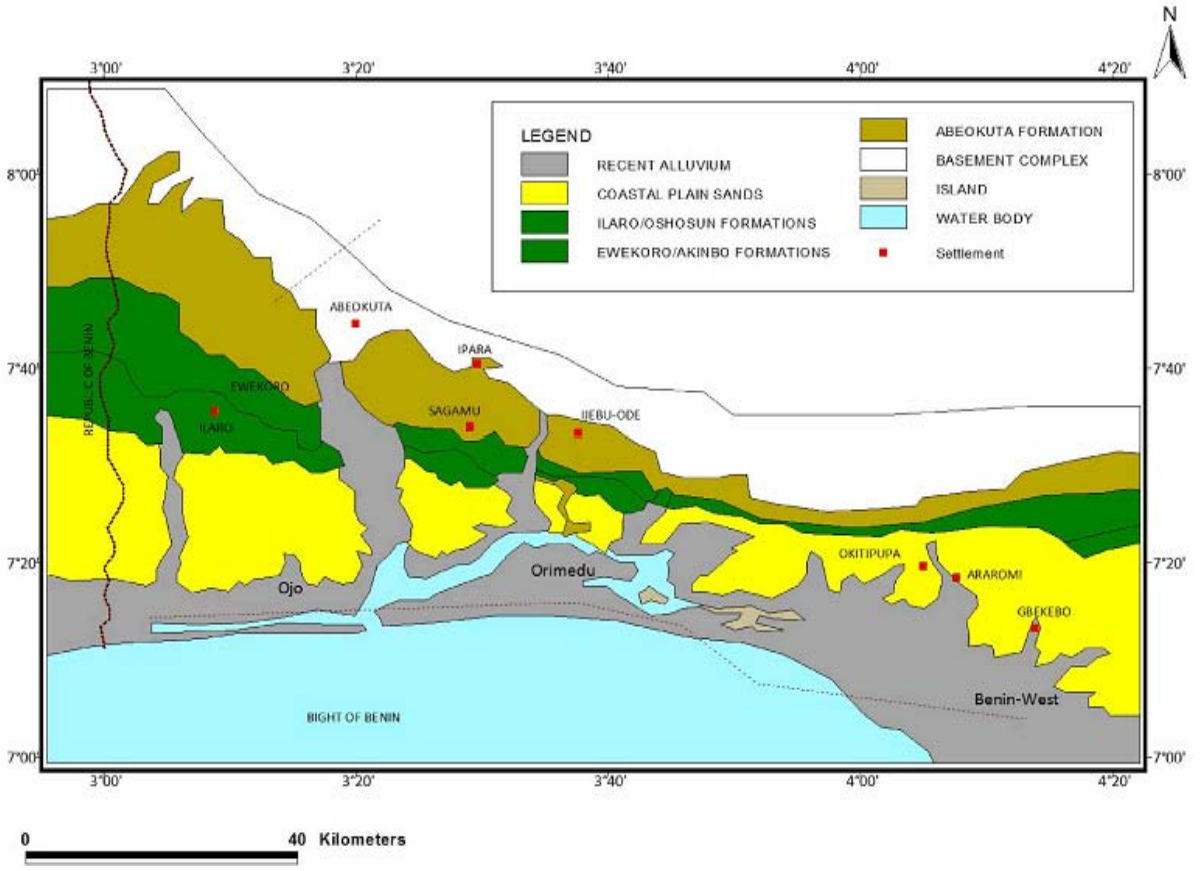

Fig. 1 : Geological map of the study area (SHAGAMU)

III. Vegetation of Eastern Benin Basin

Two main types of pseudo-parallel vegetation types (savanna and rainforest) exist in the eastern Benin basin in southwest Nigeria. They are the Guinea savanna; the Lowland rainforest, Freshwater swamp forest and a thin strand of Mangrove as well as Beach vegetations. These are extensions from the other parts of southern Nigeria as described by Keay (1959) Fig. 2. The Guinea savanna has characteristic plants such as *Daniella oliveri, Irvingia gabonensis, Isoberlinia dalziella, Afzelia africana*, *Terminalia glaucuscens* and *Elaeis guineensis*. Constituting the Guinean Lowland rainforest are Meliaceae, *Ceiba*, Sapotaceae, *Triplochiton*, *Calpocalyx*, *Celtis*, *Canthium*, *Mimosa*, Ceasalpinaceae and Papilionaceae (Keay, 1959). Taxa such as *Pandanus, Calamus, Uapaca, Crudia*, *Cyperus, Nymphaea, Symphonia, Cleistopholis, Raphia* and shrubs, ephiphytes as well as ferns dominate the Freshwater swamp forest. The Beach vegetation has plant taxa such as *Ipomoea, Dalbergia, Sporobolus, Eugenia, Hibiscus and Phoenix* while the thin strand of Mangrove contains relics of *Rhizophora* spp., *Avicennia germinans* and *Acrostichum aureum*.

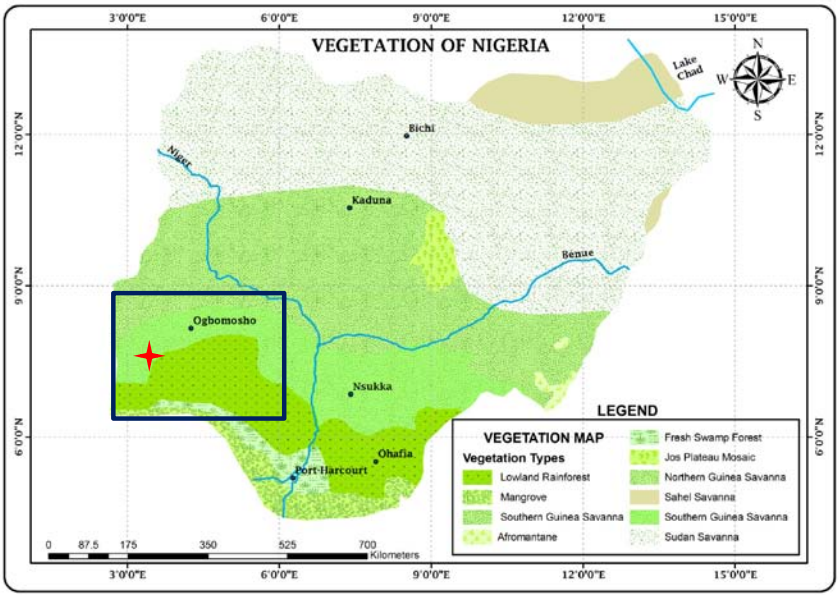

Fig. 2 : Vegetation Map of Nigeria showing the location of studied Quarry with a red star

IV. Materials and Methods

Ten outcrop samples from Shagamu Quarry, Shagamu, south west Nigeria were collected at an average of 0.5 m interval from the Oshoshun (Akinbo) Formation. Twenty-five grams each of sediments was palynologically treated with the use of HCl, HF, and acidified $ZnCl_2$ solution for heavy mineral separation. Samples were studied using Olympus microscope CH2 Model, and photomicrographs of some important palynomorphs were taken using Motic Camera 2.0 as shown in Adeonipekun *et al*. (2012). Identification of recovered palynomorphs was done using several published papers and atlases on the Paleogene of Nigeria such as Germeraad *et al.* (1968), Sowunmi (1986; 1987), Adegoke *et al.* (1991) and Jan du Chene *et al.* (1978). Recovered palynomorphs were assigned to palynoecological groups of Poaceae, Aracaceae, Freshwater Swamp forest, Mangrove, and Pteridophyte spores while charred Poaceae cuticles were used to substantiate paleoclimatic deductions. These were further grouped into Wet paleoclimate and Dry paleoclimate indicating palynomorphs. The inverse relationship between Poaceae and Pteridophyte spores forms the basis of the paleoclimatic deductions. Poaceae are open vegetation dwellers while Pteridophytes are humid moist habitat dwellers. Abundant Aracaceae, Mangrove, and Freshwater swamp forest pollen substantiated wet paleoclimate condition while abundance of charred Poaceae cuticles support inferred dry paleoclimatic condition. Spearman's correlation statistical tool was applied to the recovered palynomorph data with their correlation features noted.

Subsamples of the outcrop were gently crushed and dried so as to visually study the color, sphericity and sorting of the sand particles. The shale: sand: calcite ratio and their features were identified and recorded. Accessory minerals considered are pyrites, mica flakes, carbonaceous detritus and shell fragments. The work of Selly (1976) guided all lithological deductions (Table 2). The sediments belong to the Oshoshun Formation as described by Omatsola and Adegoke (1981).

Table 2 : Palyno-ecological Groups, Lithology and age with *Botryococcus* and *Apectodinium Values* Adeonipekun *et al.* (2012)

DEPTH (m)	LITHO	AGE	SAMP. NO.	*M. annu.*	C.P.C.	Areca.	F.SW. FR	Spores	Mang.	Botry.	Apect.
4	SHALE	EOCENE	53A	2	0	1	0	0	0	2	0
6	SHALE/ SAND		51A	0	4	1	0	0	1	18	0
8	SHALE	PALEOCENE	49A	2	1	1	0	2	o	27	1
8.5			48A	1	1	1	0	2	0	4	1
9.5			46A	1	0	0	0	0	0	6	2
10			45A	0	0	5	0	6	1	0	11
10.5			44A	0	1	4	4	2	0	4	3
11			43A	4	0	2	1	0	0	0	1
12			41A	0	0	1	0	0	2	8	1
12.5			40A	0	0	14	13	11	2	0	12
TOTAL				10	7	30	18	23	6	69	32

M. annu. – *Monoporites annulatus* (Poaceae); C.P.C – Charred Poaceae cuticles; F. SW. FR – Freshwater Swamp Forest; Mang. – Mangrove; Areca. – Arecaceae; Botry. – *Botryococcus*; Apect – *Apectodinium* dinocyst, PETM Interval - Red colored symbol ; Important Palynomorph events - Red colored font

V. Results

A total of 23 pollen and seven pteridophyte taxa were recovered along with continentally derived charred Poaceae cuticles and fungal spores. Diversity and abundances of pollen and spores decreased from the Paleocene section to almost zero values in the earliest Eocene from the studied area in spite of little difference in lithology (Fig. 3). Pollen diversity and depth correlate positively with significant level of 0.045 and correlation coefficient of 0.642. Diversity of spores correlates positively with depth but it is insignificant (Tables 3 and 4). Good correlation was also observed between Pollen abundance and depth with Spearman's correlation coefficient value of 0.654. This is not the case with Spore abundance which is characterized by weak and insignificant positive correlation. Spore and Pollen abundances also correlate positively well but insignificantly. All these point to a floristic change and taxonomic extinction that have been reported for this climatic phenomenon when there was increased carbon dioxide in the hydrosphere and atmosphere which led to temperature increase of approx. 6 ^{0}C (Jaramillo and Dilcher, 2000; Harrington and Jaramillo, 2007; Jaramillo *et al*., 2010; Wing and Currano, 2013).

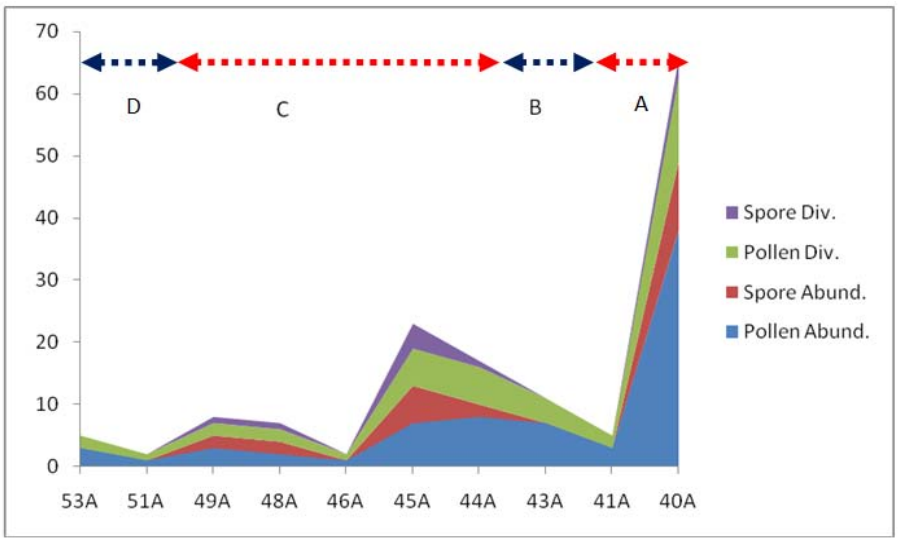

Fig. 3 : Sporomorph statistics and recognized phyto-climatic zones of study area (A, B, C, and D)

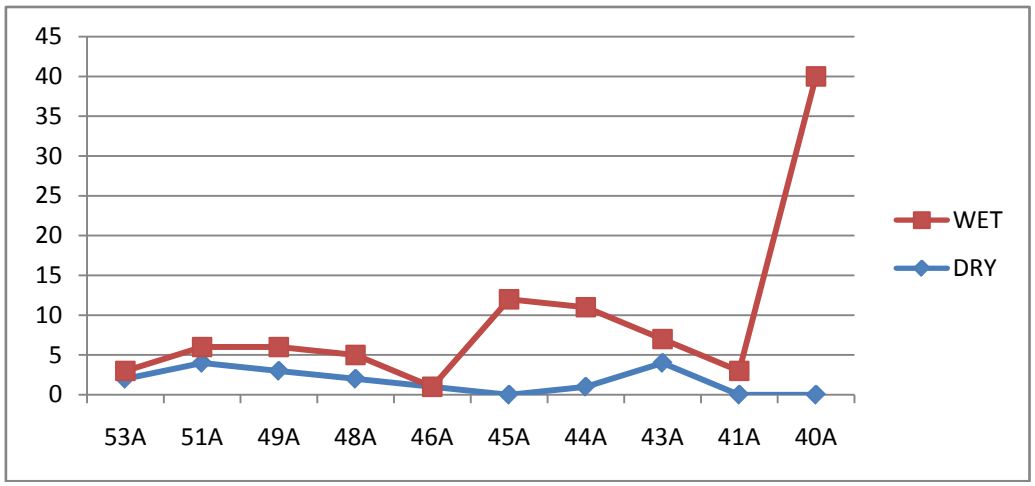

Fig. 4 : Relationship between Wet, and Dry paleoclimatic groups across the studied column

Table 3 : Correlation details: Pollen and Spore abundances with depth

			Depth (m)	Pollen Abundance	Spore Abundance
Spearman's rho	Depth (m)	Correlation Coefficient	1.000	.654*	.321
		Sig. (2-tailed)	.	.040	.365
		N	10	10	10
	Pollen Abundance	Correlation Coefficient	.654*	1.000	.608
		Sig. (2-tailed)	.040	.	.062
		N	10	10	10
	Spore Abundance	Correlation Coefficient	.321	.608	1.000
		Sig. (2-tailed)	.365	.062	.
		N	10	10	10

**. Correlation is significant at the 0.05 level (2-tailed).*

Table 4 : Correlation details: Pollen and Spore diversity values with depth

			Depth (m)	Pollen Diversity	Spore Diversity
Spearman's rho	Depth (m)	Correlation Coefficient	1.000	.642*	.269
		Sig. (2-tailed)	.	.045	.453
		N	10	10	10
	Pollen Diversity	Correlation Coefficient	.642*	1.000	.708*
		Sig. (2-tailed)	.045	.	.022
		N	10	10	10
	Spore Diversity	Correlation Coefficient	.269	.708*	1.000
		Sig. (2-tailed)	.453	.022	.
		N	10	10	10

**. Correlation is significant at the 0.05 level (2-tailed).*

Table 5 : Correlation details: Phyto-climatic phases with depth

			Depth (m)	Wet Phase	Dry Phase
Spearman's rho	Depth (m)	Correlation Coefficient	1.000	.652*	-.582
		Sig. (2-tailed)	.	.041	.077
		N	10	10	10
	Wet	Correlation Coefficient	.652*	1.000	-.526
		Sig. (2-tailed)	.041	.	.119
		N	10	10	10
	Dry	Correlation Coefficient	-.582	-.526	1.000
		Sig. (2-tailed)	.077	.119	.
		N	10	10	10

**. Correlation is significant at the 0.05 level (2-tailed).*

Palynoecological and subsequent phyto-climatic groupings enabled the recognition of four Phyto-climatic zones A – D from the base of the studied section. The Zone A (40A – 41A) is rich in Pteridophyte spores, Aracaceae and Freshwater swamp forest pollen, but lacks Poaceae and charred Poaceae cuticles. From the floral assemblages, it represents a wet paleoclimatic condition (Table 2 and Figs. 3 and 4). Zone B (43A) is represented by a depth, with characteristic abundance of Poaceae, low proportions of Aracaceae and Freshwater swamp forest with absence of Pteridophytes. This record indicates a dry paleoclimate condition. Following this is the extensive Zone C (44A – 49A) with abundant occurrences of Pteridophytes, Arecaceae, Mangrove and Freshwater swamp forest pollen. Charred Poaceae cuticle is very low to absent while Poaceae also occurred in relatively low proportions. The high diversity and abundance of sporomorphs within this zone indicate a pronounced wet paleoclimatic episode. Zone D (51A and 53A) recorded high proportions of Poaceae, charred Poaceae cuticles and low occurrence of Aracaceae as well as none occurrence of Pteridophytes and Freshwater swamp forest pollen. This assemblage indicates a dry paleoclimate condition.

Several species of dinoflagellates and microforaminiferal wall linings were also recovered which have been extensively dealt with in Adeonipekun *et al*. (2012). The studied section was dated Paleocene/ Eocene by Olowu (1996) using calcareous nannofossils, and by Adeonipekun *et al.* (2012) using *Proxapertites cursus, P. operculatus, Retistephanocolpites williamsii, Longapertites vaneendiburgi, L. marginatus, Dictyophillidites harrisi* and *Foveotriletes margaritae.* These pollen and spore markers were corroborated by marker dinoflagellates such as *Phelodinium, Hafniasphaera, Apectodinium, Areoligera* and *Cordosphaeridium*; and foraminiferal *Morozovella, Globigerina velascoensis, Acarina nitida and Bulimina subfosiformis.* Zone D is the only phyto-climatic zone that falls within the early Eocene while the rest (C - A) falls within the late Paleocene/earliest Eocene. This Eocene section (53A and 51A) is interpreted as a dry paleoclimatic phase while the Paleocene part (40A – 41A) and Paleocene/earliest Eocene part (44A – 49A) are interpreted as wet phases with a brief profound dry phase indicated at sample 43A by abundant fungal elements (Appendix 1).

Samples 51A - 53A recorded high proportions of Poaceae, charred Poaceae cuticles and fungal elements with non occurrence to low record of Pteridophyte spores, Aracaceae and Freshwater swamp forest pollen. Samples 44A – 49A and 40A – 41A contain abundant Pteridophyte spores, Arecaceae, and Freshwater swamp forest as well as Mangrove with low proportions of charred Poaceae cuticles and Poaceae. Sample 43A however recorded abundant Poaceae and none occurrence of Pteridophytes as well as poor recovery of Freshwater swamp forest (Table 2 and Appendix 1).

Dinoflagellate *Apectodinium* spp. has two peaks within the studied section. One (in 45A within a Transgressive systems tract - TST) is very close to the recognized maximum flooding surface 56. 8 Ma at sample 46A and another at sample 40A at the base of the Oshoshun Formation close to the 57.5 Sequence Boundary as recognized by Adeonipekun *et al.* (2012) Fig. 5. *Apectodinium* spp. are important markers associated with the maximum flooding surface of the PETM. Prasad *et al.* (2006) used the coincidence of *Apectodinium* acme, negative carbon isotope excursion and palynofacies to recognize the PETM in India. Aubry *et al.* (2007) have also associated *Apectodinium* species' global proliferation with the PETM. The reported extinction of planktic and benthic foraminifera (Nagy *et al.,* 2013) at the PETM has also been linked to algal bloom since the algae had no foraminiferal fauna to feed on them Hansen (1990). This faunal extinction was recorded in the latest Paleocene/earliest Eocene sections of the studied outcrop with the greatest impact recorded within sample 48A Adeonipekun *et al.* (2012) Table 2.

Two lithological units were recognized; the short solely shaly-sand (51A) and the extensive shaly units. The shaly-sand unit is dolomitic with high proportions of calcite and few carbonaceous detritus and shell fragments. These accessory minerals are absent within the shaly section. The sample number with their corresponding depths in meters are as follows: 53A -6m; 51A-7m; 49A-8m; 48A-8.50m; 46A-9.50m; 45A-10m; 44A-10.50m; 43A-11m; 41A-12m; 40A-12.50m. Details of the depositional environments within the shaly and shaly sand lithology have been recognized in Adeonipekun *et al.* (2012) as varying from inner neritic to middle neritic with occasional foray into outer neritic settings.

VI. Discussion

a) Floristic features

The late Paleocene/early Eocene has been reported as the warmest paleoclimatic period in the Paleogene and it is popularly referred to as Paleotropical Maximum or Paleocene - Eocene Thermal Maximum (PETM). At this geologic period, present temperate areas experienced tropical-like paleoclimate with high temperature and therefore tropical-like vegetations (Traverse, 1988). Even the present Canadian Arctic area recorded warm temperate vegetations. Sluijs *et al*. (2006) reported temperature increase from ~18 °C to over 23 °C during the PETM in the North Pole Arctic region thus recording a subtropical Ocean temperature in the Arctic Ocean. Sluijs *et al*. (2013) recently found also that "continental air and sea surface temperatures warmed from 27–29 °C to ~35 °C" during the PETM from deep sea core sediments.

Jaramillo and Dilcher (2000) worked on tropical Paleocene – early Eocene sediments and reported extinction at the PETM after which in the younger Eocene, diversity increased in the Colombian eastern Andes. Harrington and Jaramillo (2007) also reported taxonomic diversity increases in the late Paleocene of US Gulf Coast, a trend replaced by a marked extinction into the early Eocene. Wing and Currano's (2013) work on plant macrofossils in the Bighorn Basin, Wyoming, United States however recorded "radical floristic change" during the PETM. Much of what happened in the early Eocene of our study area could however not be ascertained as this section is rather short. It is however clear that there was a significant reduction in diversity and abundance of pollen from the latest Paleocene into the earliest Eocene where Pteridophyte spores disappeared to suggest a drier paleoclimatic condition than the late Paleocene after the PETM (Fig 3).

The Paleocene part though with a brief dry interval at 43A, was of wetter climatic condition with regular occurrence of Pteridophyte spores, Mangrove, Arecaceae and Freshwater swamp forest pollen while Poaceae and charred Poaceae cuticles recorded great reduction in proportions. This agrees with the transgressive phase report of Nagy *et al*. (2013) for the PETM. However within sample 43A, Poaceae value soared to a maximum with no record of spores, all within a monotonously shaly section. This represents the only short dry paleoclimatic phase within the late Paleocene section studied. From Table 5, the significant Spearman's correlation coefficient value of 0.652 confirms that there is a positive correlation between depth (m) and wet phase. i.e with increase depth, wet phase indicator palynomorphs increased in proportion. Dry phase indicator palynomorphs however have insignificant negative correlation values with depth since with increase depth, dry phase indicators decreased. These indices point to a generally wet late Paleocene and generally dry earliest Eocene paleoclimates in the study area.

The Eocene part involved in this study is short thus precluding making conclusions with respect to diversity trends beyond the top of studied section. Also only one outcrop was involved in the study. In spite of these, Jaramillo *et al.*'s (2010) conclusions that the tropical rainforest was not affected by the PETM events in eastern Colombia and western Venezuela where it was reported to have survived the high temperatures and high proportion of atmospheric carbon dioxide, may not be applicable to the Benin basin sediments. Previous findings indicating floral extinction and migration by Jaramillo and Dilcher (2000), Harrington and Jaramillo (2007) and Wing and Currano (2013) are supported by results from this present study. The conclusion of lack of evidence for aridity in the tropical rainforest areas within the PETM is supported by findings from our work, however two dry paleoclimatic phases recognized in this present study most likely serve as terminal boundaries of the PETM interval. A short one was recorded in the late Paleocene - Zone B at sample 43A, while the other was recorded in the early Eocene - Zone D at interval 51A – 53A. Pteridophyte spores disappeared from this Eocene part with corresponding increase in Poaceae and charred Poaceae cuticles (Table 2; Figs 3 &4). Results from present work agree with Garel *et al*. (2013) record of dry episodes just before the PETM and moister climate during it in Normady, France from geochemical and palynofacies studies.

Despite the monotony of lithology, paleoclimatic records deducible from paleo-vegetational changes were recognizable. This shows that facies change does not always represent paleoclimatic change while the fact that it is monotonous also does not mean that paleoclimate had not changed (Table 2).

VII. The Paleocene - Eocene Thermal Maximum (PETM)

Various authors have attempted the recognition of the PETM in different areas of the world tying it to eustacy since increased CO_2 would have led to increased sea level with melting of ice in the polar region and consequent sea transgression globally. Prasad *et al.* (2006) recognized the PETM onset at the peak of *Apectodinium* spp. in Northeastern India. Sluijs *et al*. (2006) however reported from Arctic Ocean sediments that the Paleocene–Eocene Thermal Maximum indicated a sea level rise deducible from the temperature rise from 18 ^{0}C – 23 ^{0}C. Schulte *et al.* (2011) recorded its recognition at the base of the Eocene within a transgressive systems tract (TST) in Dababiya Quarry section, Egypt. Aleksandrova and Shcherbinina (2011) working on sediments from Nasypnoe section in Eastern Crimea, Ukraine reported that nannofossil and dinocyst distribution suggests that "a drastic sea-level fall preceded the PETM and occurrence of two transgressive episodes during it". Rivandi *et al.* (2013) reported the possible presence of the PETM at the end of a high stand systems tract (HST) that is characterized with deposition of shale and marl facies. The end of the HST specifically at the sequence boundary represented by a paleosol was interpreted as a major sea level fall in the latest Paleocene. Associated with this paleosol according to Rivandi *et al.* (2013) is the river channel sediments (conglomerate). Minelli *et al.* (2013) recognized the PETM within a sea level rise interval (TST of Serraduy Sequence) in the Ager Basin of Central Pyrenees, Spain. Sluijs *et al*. (2013) also most recently recorded a sea level rise during the PETM from deep sea core sediments. Nagy *et al.* (2013) observed that the PETM acme coincides with the maximum flooding surface (MFS) of the Gilsonryggen depositional sequence in Spitsbergen Norway. Its transgressive phase was initiated by local tectonics, while the eustatic sea-level rise of the PETM was superimposed on this transgression.

Gebhardt *et al.* (2010) from the study of the foraminifera and carbon isotopes Excursion (CIE) of sediments from the Shagamu Quarry, southwest Nigeria recognized the Initial Eocene Thermal Maximum (IETM) at the Paleocene - Eocene boundary being characterized by dominance of dysoxic benthic foraminifera - *Bulimina* and *Nonionella* spp. with concomitant great reduction in planktic and benthic foraminifera. Frieling *et al.* (2011) working on the tropical sediments from Nigeria also observed a coincidence of the PETM with the Carbon Isotope Excursion (CIE) interval where *Apectodinium* was absent but abundantly present before their recognized interval of CIE. Nagy *et al.* (2013) reported that the faunal extinction at the PETM was co-eval with hyposaline and hypoxic conditions caused by continental influx into the marine environments. High kaolinite indicating high humidity was also recorded within the climatic anomaly. Samples 48A and 49A in the present work recorded high humidity with relatively high pteridophyte spores' values (see Table 2 and Appendix 1). Freshwater alga *Botryococcus* recorded an unprecedented upsurge at sample 49A in the earliest Eocene within the HST interval recognized at the terminal marine transgression (Adeonipekun *et al.,* 2012) associated with the PETM in the Shagamu Quarry outcrop. With samples 44A - 49A showing high humidity and particularly Sample 48A having all the reported faunal and floral features of the PETM (extinctions with high humidity), we propose this interval as the PETM in the Shagamu Quarry, Southwest Nigeria. These samples represent the terminal TST and HST recognized by Adeonipekun *et al* (2012) see Fig. 5, and is defined in the present work as the wet paleoclimatic Zone C (Fig. 2). Incidentally, the marker dinoflagellate for the Paleocene/Eocene boundary - *Hafniasphaera septata* applied by Adeonipekun *et al.* (2012) has its top within Sample 48A. The extinction of calcareous foraminifera and other planktonic organisms has also been linked to *Botryococcus* pulse since their death would have provided nutrients for the alga thereby leading to a bloom Hansen (1990). The two dry paleoclimate phases recognized also have one preceding the PETM (Phyto-climatic Zone B) and the other terminating it (Phyto-climatic Zone D). From Appendix 1, *Botryococcus*, a freshwater alga commonly recovered from marine sediments recorded an unprecedented abundance from sample 49A upwards.

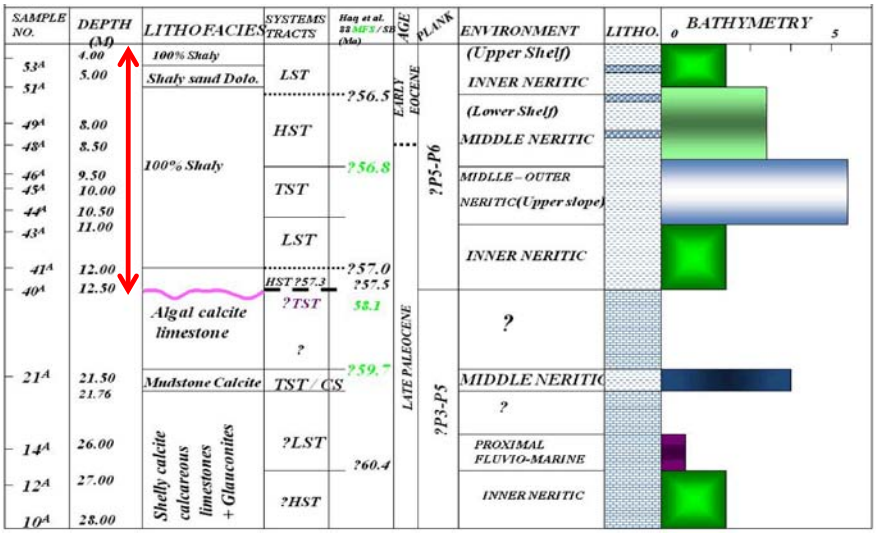

Fig. 5 : Sequence Stratigraphy of Shagamu Quarry (Adeonipekun et al. 2012). Red symbol indicates the studied *section* in the present work

Within much of the early Eocene section studied, the climate was extremely dry (Phyto-climatic Zone D) with no record of pteridophyte spore while recovery of Poaceae and charred Poaceae cuticles were relatively high. This record is synonymous with palynological features of low sea level (LST)/ later part of Highstand Systems tract (HST) in sequence stratigraphy of deltaic sedimentary basin in the tropics (Morley, 1995; Adeonipekun, 2006).

VIII. Conclusion

Palynological and sedimentological analyses of sediments from the Shagamu Quarry outcrop in the Eastern Benin basin revealed that diversity and abundance of plants decreased from the late Paleocene into the earliest Eocene. Four phyto-climatic zones (A – D, dry B and D; wet A and C) were recognized with the Paleocene/Eocene boundary falling within the extensive wet Zone C. The PETM was recognized within the wet Zone C marked by acme of *Apectodinium*, abundance of *Botryococcus*, high humidity from abundant pteridophyte spores and high floral diversity. It was further tied to the 56.8 Ma marine events with the TST recording high humid wet climate and the HST containing the bloom in algae. Two dry paleoclimatic phases bound the PETM at both the top and bottom.

IX. Acknowledgements

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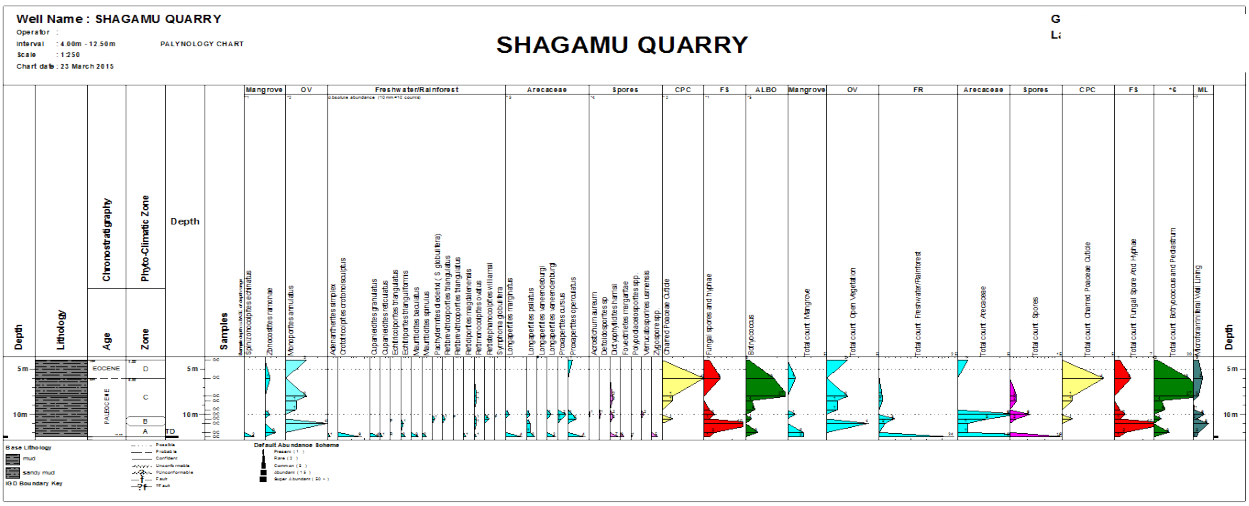
Appendix 1



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Algae Composition and Physico- Chemical Paramaters of Awon Reservoir, Oyo State, Nigeria

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Abstract- The algae composition and physico-chemical parameters of Awon Reservoir were investigated for a period of four months (wet and dry seasons), this was done to examine the effect of seasonal variation on phytoplankton. The algae comprised a total of twenty-two species belonging to four divisions: bacillariophyta, chlorophyta, euglenophyta and cyanophyta. Navicula meniceculus, Gyrosigma scalproides, Closterium archerianum and C. dianae were the dominant species. The physico-chemical parameters determined were higher in the dry season than wet season. The values of physico-chemical obtained fell within the maximum allowable limit set by United Environment Protection Agency.

Keywords: algae, awon reservoir, physico-chemical parameters, seasons, diatoms, green algae, euglenophyte.

GJSFR-C Classification : FOR Code: 060799

Strictly as per the compliance and regulations of :

Algae Composition and Physico- Chemical Paramaters of Awon Reservoir, Oyo State, Nigeria

Akinyemi, S. A. [α] & Sodamade, A. [σ]

Abstract- The algae composition and physico-chemical parameters of Awon Reservoir were investigated for a period of four months (wet and dry seasons), this was done to examine the effect of seasonal variation on phytoplankton. The algae comprised a total of twenty-two species belonging to four divisions: bacillariophyta, chlorophyta, euglenophyta and cyanophyta. Navicula meniceculus, Gyrosigma scalproides, Closterium archerianum and C. dianae were the dominant species. The physico-chemical parameters determined were higher in the dry season than wet season. The values of physico-chemical obtained fell within the maximum allowable limit set by United Environment Protection Agency.

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I. Introduction

Algae have long been used to assess environmental conditions in aquatic habitants throughout the world(Kolkwitz and Marsson, 1908). More recently, the sensitivity of many algal species to pH has been employed to assess problems with acid deposition. (Smotto, 1995; battarbee etal., 1999). Government agencies throughout the world now use algae to monitor and assess ecological conditions in many types of aquatic ecosystems (e,g Weber, 1973; Dixit and Smol, 1992; Bahls, 1993; Whitton and Rott, 1996 Stevenson and Bahles, 1999 Akin-Oriola, 2003). Thus, characterization of algal assemblages has been important in environmental assessment, both in indicating changes in environmental conditions that impair or threaten ecosystem, health and in determining if algae themselves are causing problems. Algal biossessement complements physical and chemical data by providing corroborative evidence for environmental change.

Algological studies as well as physio-chemical parameters of lentic system in Africa are few. Related works include that of Battarbee etal., (1999) Briand etal (1978), Charles and Smol (1988), Dixit et al (1999) Healey and Hendzel (1980) and Akin-oriola (2003).

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In this study, data on the algal composition as well physico-chemical parameters are used to examine the prevailing conditions in Awon Reservoir. This study is carried out in order to contribute to the existing phycological information on Awon Reservoir.

II. Description of the Study Area

Awon Reservoir is located between 3.89º-3.91ºE of Greenwich meridian and Latitude 7.88º-7.90ºN of the equator and in the North western part of Oyo Township.The reservoir was constructed in 1962 mainly to supply portable water to the people of Oyo and its environs. It has a total storage capacity of 750$m^3$and average depth of 1.8m. The temperature of the study ranges from 27-30^{0}C. The human activities going on around the reservoir include laundry, bathing, farming swimming and fishing.

III. Materials and Method

Collection of Samples

The collection of data was carried out during the four months sampling period (September-December 2011). Duplicate water samples were collected for both the chemical and biological analysis using one litre sampling plastic bottles each.

The algae water sample was centrifuged using cortex centrifuge at 1,500 RPM and the filtrate was decanted to produce 10ml concentrate and it was preserved in 4% formalin. The counts were made using Olympus student N107 microscope. Taxonomic keys employed in the identification included Patrick and Reimer (1966, 1975) and Prescott (1964) and 1982).

IV. Physico-Chemical Parameter

pH was measured using (model WTM pH 90) meter. The dissolved oxygen content was determined using oxygen meter, lead, phosphate and nitrate were determined using the methods described in APHA (1998).

V. Results

The algae floral consisted of twenty-two species belonging to four divisions namely bacillariophyta, cholorophyta, cyanophyta and euglenophyta.

Table 1 presents monthly changes in the algae species of Awon reservoir. The abundance of the phytoplankton varied according to the months during the study period. The division bacillariophyta was represented by dominant species such as *Navicula meniseculus, Gyrosigma scalproides Pinnularia otiensis and Tabellaria fenestrata. Navicula meniseculus* has the highest number of cell individual in September 2011 (30 cells per litre) while the lowest was recorded in November 2011 (8 cells per litre) (Table 1).Generally reasons for having dominant species of diatoms in the resevior may be attributed to high levels of nutrient (especially silicate) as well as temperature regime.

Among the chlorophyta, *Closterium archerianum and C. dianae* formed the dominant species. The highest number of cells was recorded for *Closteriumarcherianum*in September 2011. Cyanophyta was represented by *Anabaena spiroides* while *Euglena convoluta* represented division Euglenophyta (Table 1)

Table 1: Monthly changes of phytoplankton species (No. of Cell per litre)

Division	September 2011	October 2011	November 2011	December 2011
Bacillariophyta				
Navicula meniseculus	30	19	08	09
N. rhycocephala	02	01	01	01
N. exigua	03	02	01	01
N.decusis	04	01	02	03
N. crypotephala	03	02	03	04
Gyrosigma Scalproides	17	12	06	10
Gyrosigma sp	03	02	01	01
Pinnularia biceps	06	06	03	04
p. otiensis	10	08	02	05
p.debesii	08	02	04	01
Pinnularia spp	02	01	01	01
Tabellaria fenestrate	09	03	01	01
Tabellaria sp	01	03	04	03
Synedra sp	01	02	03	04
Achanthes ap	04	02	03	03
Aulacoseira sp	05	03	03	03
Nitszchia sp	03	02	01	02
Gomphonema sp	02	01	01	01
Chlorophyta				
Closterium archerianum	23	10	11	02
C. dianea	26	17	13	01
Cyanophyta				
Anabaena spiroides	01	01	03	02
Euglenophyta				
Euglena convolute	02	01	03	04

pH value ranged between 7,2-6.0, Dissolved Oxygen ($mg1^{-1}$) fluctuated between 5.6-4.6 concentration of Phosphate ($mg1^{-1}$) ranged between 0.2-0.08, Sulphate ($mg1^{-1}$) ranged between 3.60-2.00, Nitrate ($mg1^{-1}$) fluctuated between 0.40-0.31 and Iron ranged between 3.64-3.31 (ppm). (Table 2).

Table 2 : Monthly changes of physic-chemical parameters of Awon Reservoir.

	September 2011	October 2011	November 2011	December 2011
pH	6.0	6.2	6.6	7.2
DO (mg/1)	4.6	5.0	5.1	5.6
PO_4 (mg/1)	0.09	0.08	0.2	0.1
SO_4 (mg/1)	2.10	2.00	3.10	3.60
NO_3 (mg/1)	0.31	0.32	0.36	0.40
Fe (ppm)	3.64	3.31	3.41	3.60

VI. Discussion

Generally the species composition is in line with the community of algae known to dominate Nigeria lentic system (Akinyemi and Nwankwo, 2003). Monthly differences were observed in the occurrence of algae. The highest number of algae recorded during the wet months (Table 1) could be attributed to availability of nutrients. Generally, high diversity of algae has been

associated to moderate nutrients level in water as well as moderate pH level (Egborge and Sagay, 1979).

The effect of nutrient on phytoplankton has been examined in many freshwater ecosystems and it was found that nutrient regulates the seasonal distribution of phytoplankton (Akinyemi 2000). Also fluctuations in the amount of phytoplankton may be attributed to the activities of predators that feed directly on phytoplankton. Also species such as *Closterium archerianum, C. dianae Navicula meniseculus, Gyrosigma scalproides, Pinnularia otiensis and Tabellaria fenestrata* were better represented, compared to species of algae like *Anabaena spiroides* and *Euglena convoluta*(Table 1).

The difference in the species composition in this study may be due to differences in physic-chemical factors such as pH, dissolved oxygen, nitrate, phosphate and sulphate concentration. pH range was neutral to slightly alkaline during the study period. Generally, nitrate and phosphate levels were very low (Egborge and Sagay, 1979).

VII. Conclusion

Research findings show that Awon reservoir consisted of algae species and moderate amount of nutrients that compared favourably with other fresh water bodies. The values of nutrients in the reservoir are within the WHO standards, an indication that the water is fit for drinking and other human activities.In addition, the presence of different species of algae like *Closterium, Navicula, Tabellaria*suggest that the water body is healthy. However, this monitoring should be carried out periodically so as to find out the prevailing conditions in the water bodies.

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TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

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15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

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19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
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Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
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- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

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The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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