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Discovering Thoughts, Inventing Future

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Ultravist Studies on the Histology Patterns of the Kidney of Adult Wistar Rats

By Valentine C. Ikamaise, Theresa B. Ekanem, Kebe E. Obeten & Gabriel Udo-Affah
University of Calabar, Nigeria

Abstract- The aim of this study was to assess the effects of ultravist on the histological patterns of the kidney of the Wistar rat. Thirty (30) Wister rats weighing between 182-212kg/BW were divided into three groups of ten (10) animals each. Group A served as the control group, while group B and C served as experimental groups receiving low and high dosage of ultravist (iopromide) which is the Radiographic Contrast Medium (RCM) used for the experiment respectively. Collection of tissues was carried out on rats at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours following administration of RCM on the experimental groups. The sampling from experimental and control groups were carried out simultaneously following administration of low and high dose of RCM to rats from experimental groups. The result obtained for ultravist after one hour administration presented in plates 2 and 4 showed alteration in the high dose animals while plates 3 and 5 showed marked alterations in the cellular morphology of the kidney of the low dose animals.

Keywords: *kidney, wistar rats, ultravist, hyper cellular mesangium.*

GJSFR-C Classification : *FOR Code: 069999*

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Ultravist Studies on the Histology Patterns of the Kidney of Adult Wistar Rats

Valentine C. Ikamaise [α], Theresa B. Ekanem [σ], Kebe E. Obeten [ρ] & Gabriel Udo-Affah [ω]

Abstract- The aim of this study was to assess the effects of ultravist on the histological patterns of the kidney of the Wistar rat. Thirty (30) Wister rats weighing between 182-212kg/BW were divided into three groups of ten (10) animals each. Group A served as the control group, while group B and C served as experimental groups receiving low and high dosage of ultravist (iopromide) which is the Radiographic Contrast Medium (RCM) used for the experiment respectively. Collection of tissues was carried out on rats at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours following administration of RCM on the experimental groups. The sampling from experimental and control groups were carried out simultaneously following administration of low and high dose of RCM to rats from experimental groups. The result obtained for ultravist after one hour administration presented in plates 2 and 4 showed alteration in the high dose animals while plates 3 and 5 showed marked alterations in the cellular morphology of the kidney of the low dose animals. The alterations included swollen or oedematous glomeruli, swollen epithelial cells, loss of Bowman's space and hyper cellular mesangium (proliferation of cells in the mesangium). The implication is that cells in this condition cannot perform the primary functions of secretion and excretion or maintaining the glomerular filtration rate within the normal range of arterial pressure of approximately 80 to 180 mmHg. The consequence of these changes in the cells may lead to a number of physiological manifestations such as acute renal failure.

Keywords: kidney, wistar rats, ultravist, hyper cellular mesangium.

I. Introduction

Ultravist (iopromide) is an injectable radiographic contrast medium. A radiographic contrast medium (RCM) is a substances introduced into the body in order to make an organ, or the surface of an organ, or materials within the lumen of an organ visible on the radiograph. Mostly, the media are of greater radiographic density than the structures they outline; sometimes lower densities are introduced, usually air. (Speck, 1993; Thomsen & Morcos, 2000; Morcos, 2003)

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. They remove excess organic molecules from the blood, and it is by this action that their best-known function is performed: the removal of waste products of metabolism. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove water soluble wastes, which are diverted to the bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the re-absorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin, the last of which indirectly acts on the kidney in negative feedback. (Cotran *et al.,* 2005).

Located at the rear of the abdominal cavity in the retroperitoneal space, the kidneys receive blood from the paired renal arteries, and drain into the paired renal veins. Each kidney excretes urine into a ureter, that empties into the bladder.

The use of RCM could be traced to 1896 and over the years, various types and classes were developed to include ionic monomers, non-ionic monomers, ionic and non-ionic dimmers, and others (Speck, 1993; Marshal, 2006_a).The parent molecule from which RCM for injection is derived is benzene, a toxic water-insoluble liquid. Addition of other elements to obtain a desired RCM may have made the compound less toxic, but hypersensitivity reactions to all groups of RCM for injection are reported stemming from the osmolality of the contrast, the frequency of the injection, the dose and the viscosity of the agent. Ionic dimmers and non-ionic monomers are better tolerated by the body with fewer adverse effects than the ionic monomers (Raport and Levitan, 1974; Katayama et al, 1990; Hoffmann et al, 2000; Marshall, $2006_{a\,\&\,b}$).

Most studies carried out to investigate the safety and risk of contrast media are clinical trials on patients to establish physiological manifestations of effects of contrast medium where observations and classifications of adverse effects are established. These studies revealed the occurrence of adverse effects but observed that reactions are unpredictable and intravenous injection carries a higher risk than intra-arterial injection. Others observed that some reactions are unrelated to the concentration or dose of contrast medium.(Burns et al, 1981; Hoffmann et al, 2000; Meth and Maibach, 2006).

This study therefore, was designed to assess the effects of ultra vist at the cellular level by looking at

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the histological patterns of the kidney for cellular alteration or damage following administration of ultravist (non-ionic RCM) which is currently in use in Nigeria. This may contribute to the understanding of aetiology which will enhance predictability of expected effects following ultravist administration.

II. Materials and Methods

Thirty (30) Wister rats and a brand radiographic contrast medium namely; ultravist (iopromide) were used for the experiment. The body weights of all rats used were determined. The rats were separated into 3 groups with 10 rats in each group. Two groups were experimental groups, and a group was used as a control group. Rats of a particular group were identified with marks. Each group was kept in a separate cage throughout the experimental period. From all groups, collection of tissue was carried out on rats at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours following administration of RCM on the experimental groups. The sampling from experimental and control groups were carried out simultaneously following administration of low and high dose of RCM to rats from experimental groups.

The dose of RCM for each rat was calculated using thisformula – Dosage=$\frac{V_c \times W_r}{W_{man}}$

Where V_c = *Volume of contrast medium*
W_r = *Weight of rat*
W_{man} = *Weight of a standard physiological man (70kg)*

The doses of 60ml and 100ml on a physiological 70kg man are considered low and high doses in RCM administration. Therefore, a volume of 60ml and 100ml respectively were used for the calculation of low and high doses for the rats.

The rats were sacrificed by cervical dislocation and placed on a dissecting board in the supine position for laparotomy. The kidney tissue was removed from the animal with great speed by the use of forceps and scissors.The kidney tissue was fixed in Bouin's fluid after extraction in separate tubes for 24 hours before dehydration, clearing, embeddingand sectioning at 5 micrometer thick using rotary microtome.Thirty (30) minutes following ripening and staining of slides with Haematoxyl in and Eosin (H&E) samples were rinsed and viewed under microscope. The glomerulus, bowman capsule, nucleus, mesangium and the cytoplasm were observed. Histological changes between the experimental and control groups and between the two different brands of contrast media on the photomicrographs were noted. The result obtained for urografin (diatrizoate) shall be reported in another article.

III. Results

Plate 1 showed a photomicrograph of the renal cortex of a control Wistar rat with prominent glomeruli, distinct bowman capsule, prominent cellular mesangium and the cell consists of distinct basophilic nuclei and moderately eosinophilic cytoplasm. The cells are closely packed with sparse interstitium consisting of blood vessels.

Plate 2 showed a photomicrograph of a renal cortex of Wistar rat at 30 minutes after injection of 0.29 ml (high dose) of ultravist with swollen oedematous glomeruli with marked loss of Bowman's space. There is hyper cellular mesangium (proliferation of cells). The cortical renal tubules are lined by swollen epithelial cells. The cells have prominent basophilic nuclei with abundant of eosinophilic cytoplasm.

Plate 3 showed a photomicrograph of the renal cortex of Wistar rat 30 minutes after injection of 0.19 ml (low dose) of ultravist. The glomeruli are swollen with loss of Bowman's space. There is hyper cellular mesangium. The cellular outlines are indistinct.

Plate 4 showed a photomicrograph of the renal cortex of Wistar rat 1 hour after injection of 0.26 ml (high dose) of untravist. There is swollen glomeruli with obliterated Bowman's capsule. Hyper cellular mesangium and cortical renal tubules are lined by swollen epithelial cells with prominent nuclei. The intervening interstitium is sparse.

Plate 5 showed a photomicrograph of the renal cortex of Wistar rat 1 hour after injection of 0.16 ml (low dose) of ultravist. The glomeruli are prominent with distinct Bowman's space showing mild swelling. The mesangium is cellular. The renal cortical tubules are lined by mildly swollen epithelial cells with prominent nuclei. The lumen of the cortical tubule is distinct with sparse interstitium.

Plate 6 showed the photomicrograph of the renal cortex of Wistar rat 2 hours after injection of 0.27 ml (high dose) of ultravist. There are prominent glomeruli with well demonstrated Bowman's capsules and herpercellularmesangium. The cortical tubules are lined by cuboidal epithelial cells which are mildly swollen with prominent nuclei.

Plate 7 showed a photomicrograph of the renal cortex of Wistar rat 2 hours after injection of 0.16 ml (low dose) of ultravist. The glomeruli are prominent with less distinct Bowman's capsules. Hyper cellula rmesangium and moderately swollen glomeruli. The renal cortical tubules are lined by swollen cuboidal epithelium. Some tubules have distinct luminal space.

Plate 8 showed a photomicrograph of the renal cortex 24 hours after injection of 0.30 ml (high dose) of ultravist with prominent glomeruli, distinct Bowman's space. Some of the renal cortical tubules are lined by prominent cuboidal epithelial cells.

IV. Discussion

The result obtained for ultravist (non-ionic monomer – iopromide) after one hour administration presented in plate 2 to plate5 showed marked

alterations in the cellular morphology of the kidney. The alterations included swollen or oedematous glomeruli, swollen epithelial cells, loss of Bowman's space and hyper cellular mesangium (proliferation of cells in the mesangium). The implication is that cells in this condition cannot perform the primary functions of secretion and excretion or maintaining the glomerular filtration rate within the normal range of arterial pressure of approximately 80 to 180 mmHg. The consequence of these changes in the cells may lead to a number of physiological manifestations such as acute renal failure. Gill (2006_a) reported that Acute Renal Failure (ARF) is induced by contrast agents within the duration less than 72 hours after contrast agents' administration. According to him the contrast induced ARF occurs because the contrast agent trigger a rise to more than 25% of the serum creatinine value or has increased the serum value of above 44μml/L (0.5mg/dl).

The results presented in plate 6 to plate 8 for ultravist injection after the duration of 1 hour demonstrate prominent glomeruli, distinct Bowman's capsule and space with close to normal cellular mesangium, though some cells were still swollen within the capsule and in the interstitial tissues. This implies that the elimination of ultravist from the system is rapid and thus supports the findings of Berg et al (1958) and Shellock and Kanal (1999) who found out that 83% of injected dose of contrast medium is eliminated by six hours.

V. Conclusion

The main aim of this study was to assess the effects of radiographic contrast media on the histological patterns of the kidney of the wistar rat using ultravist (iopromide -a non-ionic contrast medium). From the findings of this study the following conclusion can be drawn.

The kidney of Wistar rat is affected by radiographic contrast medium (ultravist) by alteration of the normal histological pattern of the cells (glomerular, Bowman's capsule, tubules, mesangium and interstitium.

The effects of low dose ultravist compared to that of high dose may not be outstanding within the intervals of monitoring.

The effect of radiographic contrast media on kidney cells is rapid and is recoverable.

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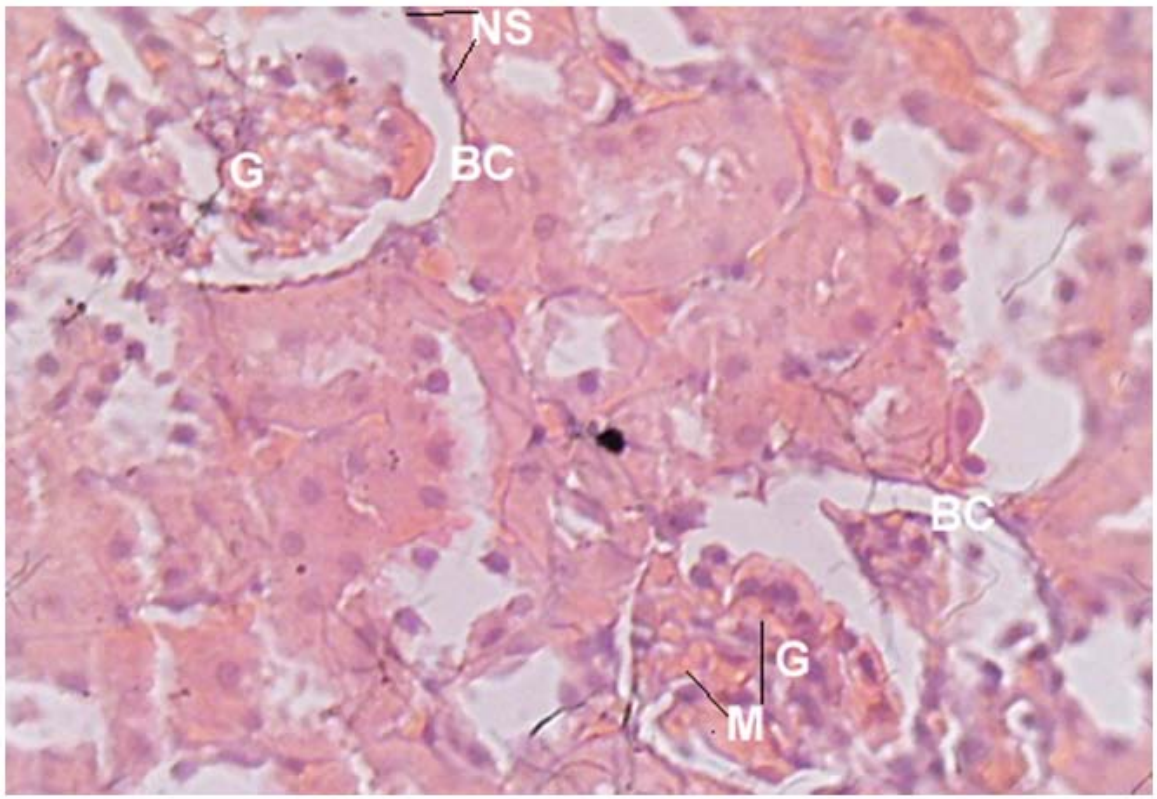

Plate 1 : Photomicrograph of a normal renal cortex of Wistar rat [X400; H&E]
BC- Bowman capsule **G**- Glomerulus M- Mensangial cells **NS**- Nuclei of squamous cells **BS**-Bowman space

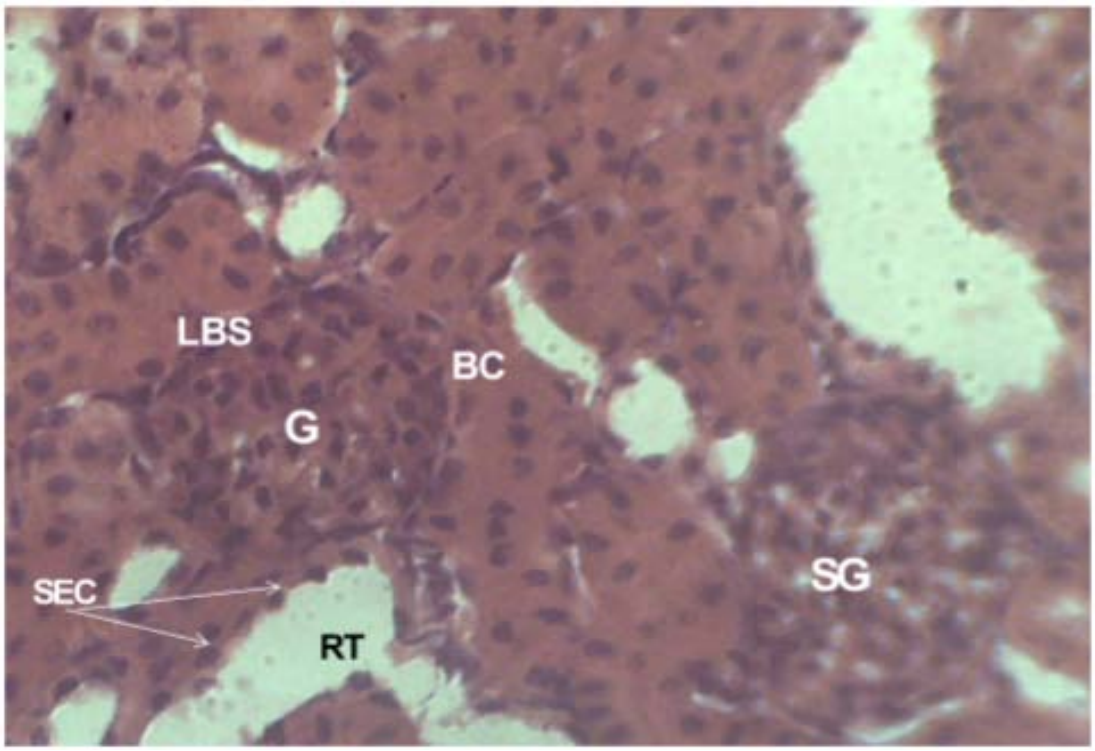

Plate 2 : Photomicrograph of the renal cortex of Wistar rat 30 mins after injection of 0.29ml of ultravist. H&E stain, X400
LBS – Lost Bowman space **SEC** – Swollen epithelial cells **RT** – Renal tubule **SG** – Swollen glomerulus.

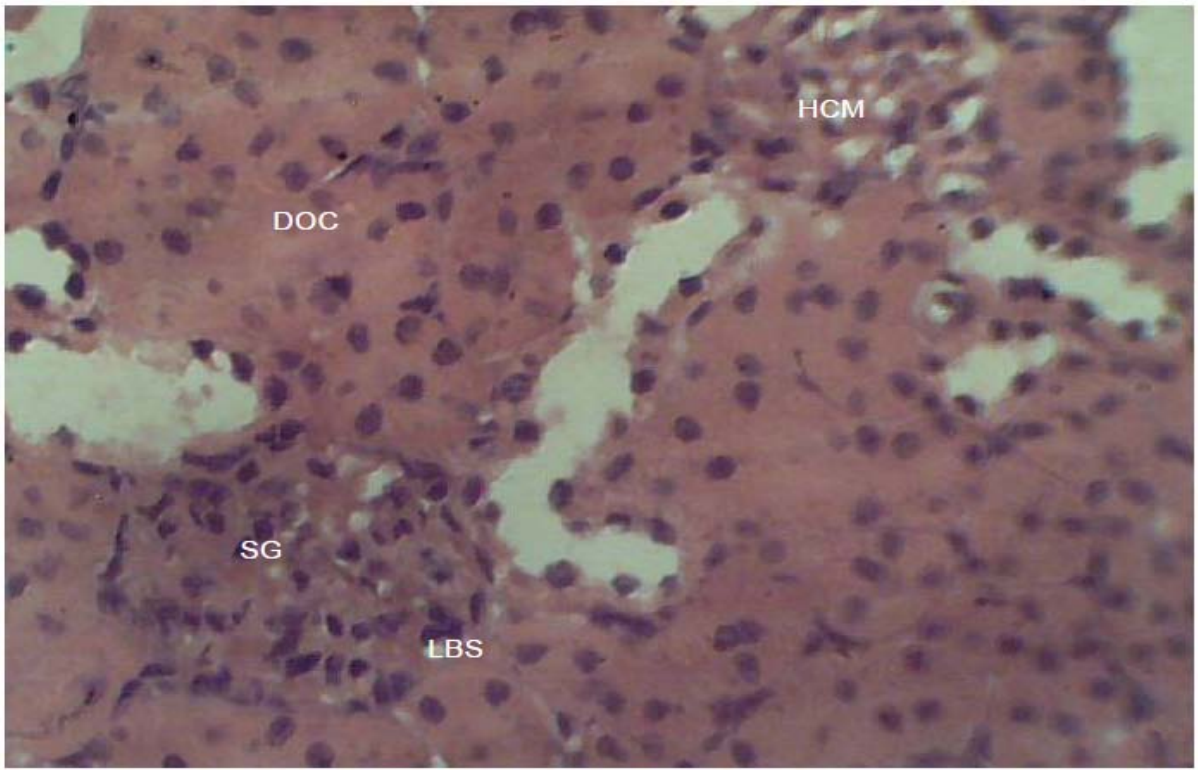

Plate 3 : Photomicrograph of the renal cortex of Wistar rat 30 minutes after injection of 0.19ml of ultravist. H&E, X400
DCO – Distorted cellular outline, **LBS** – Lost Bowman pace, **SG** – Swollen glomerulus, **HCM** – Hyper cellular mesabgium

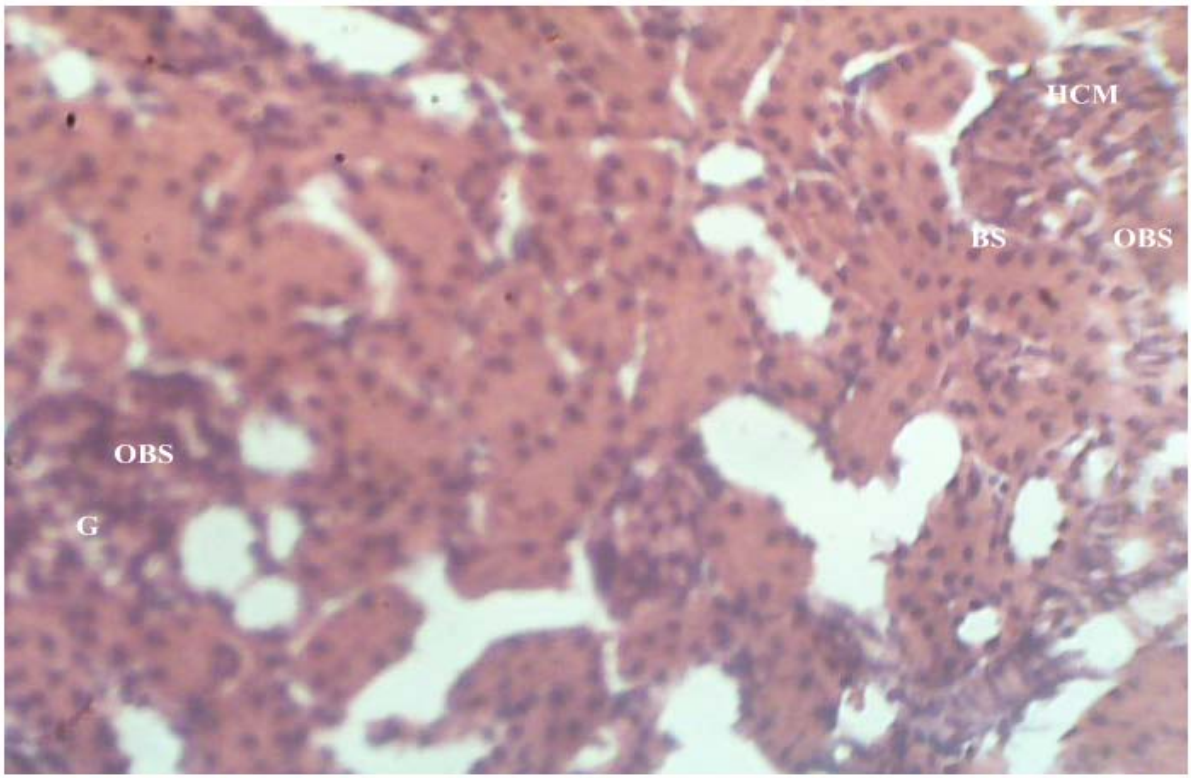

Plate 4 : Photomicrograph of the renal cortex of Wistar rat 60 minutes after injection of 0.26ml of ultravist. H&E stain, X400
HCM – Hypercellularmesangium, **OBS** – obliterated Bowman space, **G**-glomerulus, **BS**- Bowman space

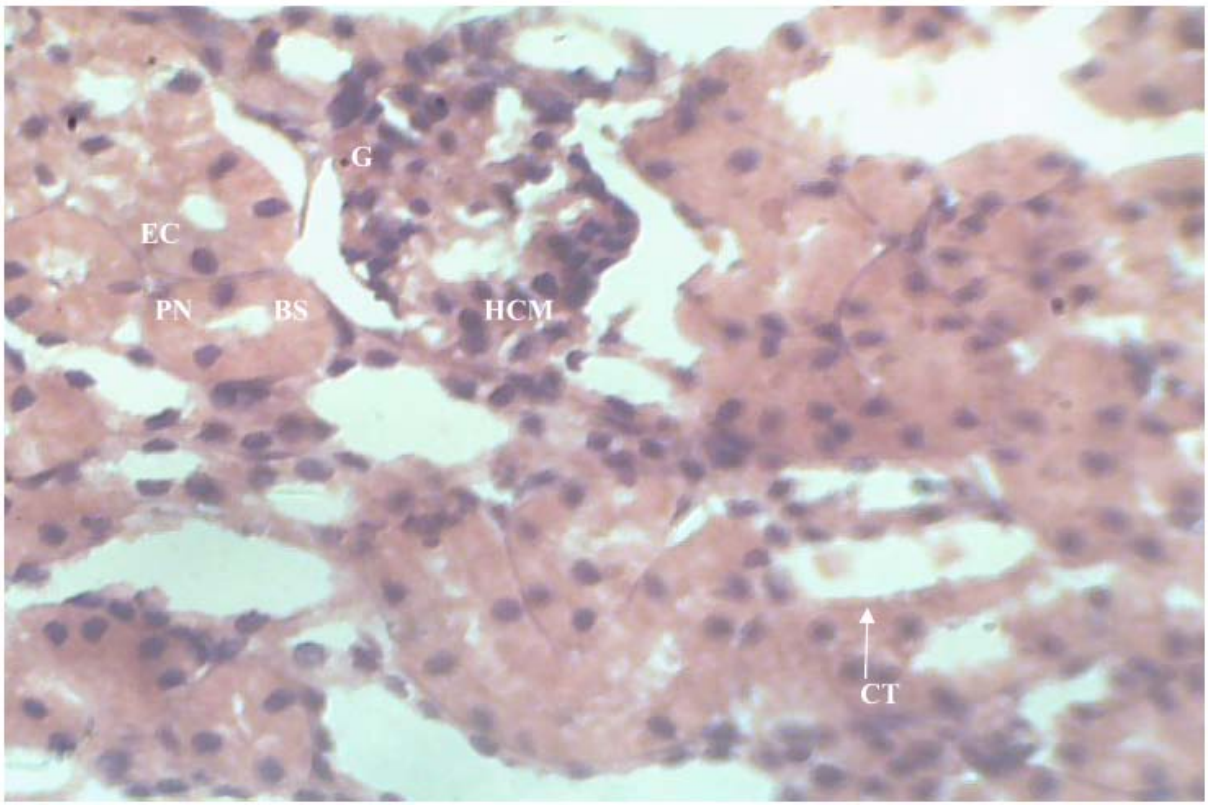

Plate 5 : Photomicrograph of the renal cortex of Wistar rat 60 minutes after injection 0.16ml of ultravist. H&E stain, X400

EC – Epithelial cell, **PN** – Prominent nucleus, **BS** – Bowman space, **HCM** –Hypercellularmesangium, **CT** – Cortical tubule

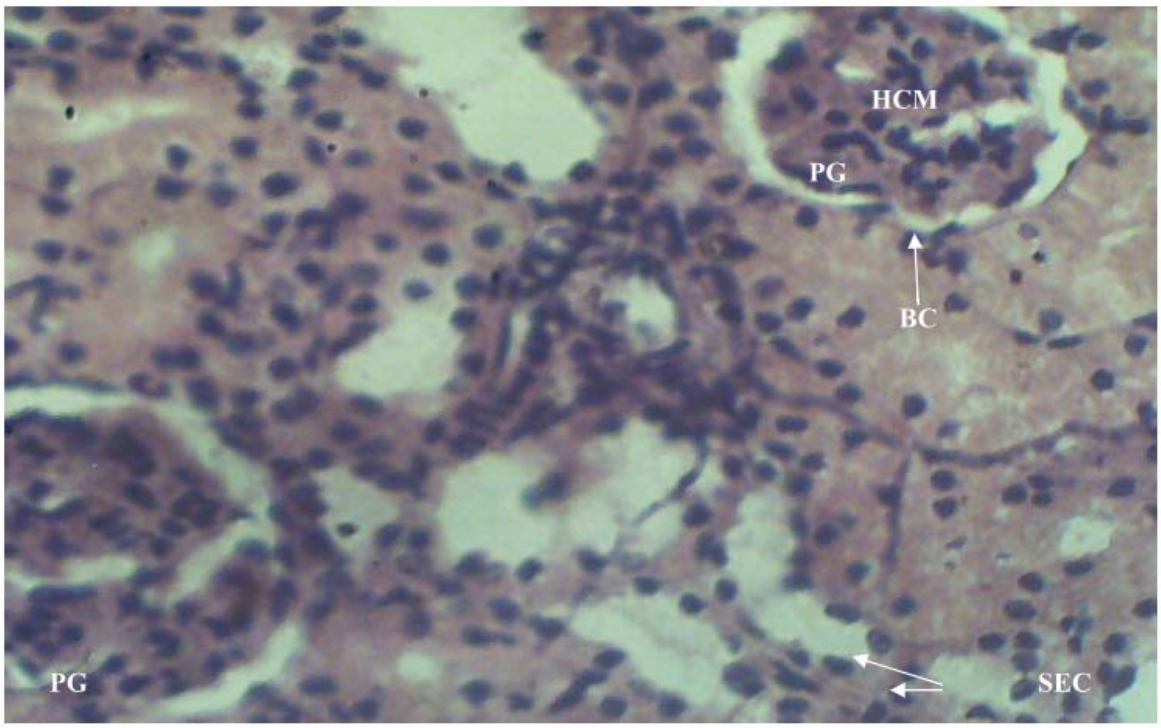

Plate 6 : Photomicrograph of the renal cortex of Wistar rat 2 hours after injection of 0.27 of ultravist. H&E stain, X400.

PG – Prominent glomerulus **BC** – Bowman capsule **SEC** – Swollen epithelial cells

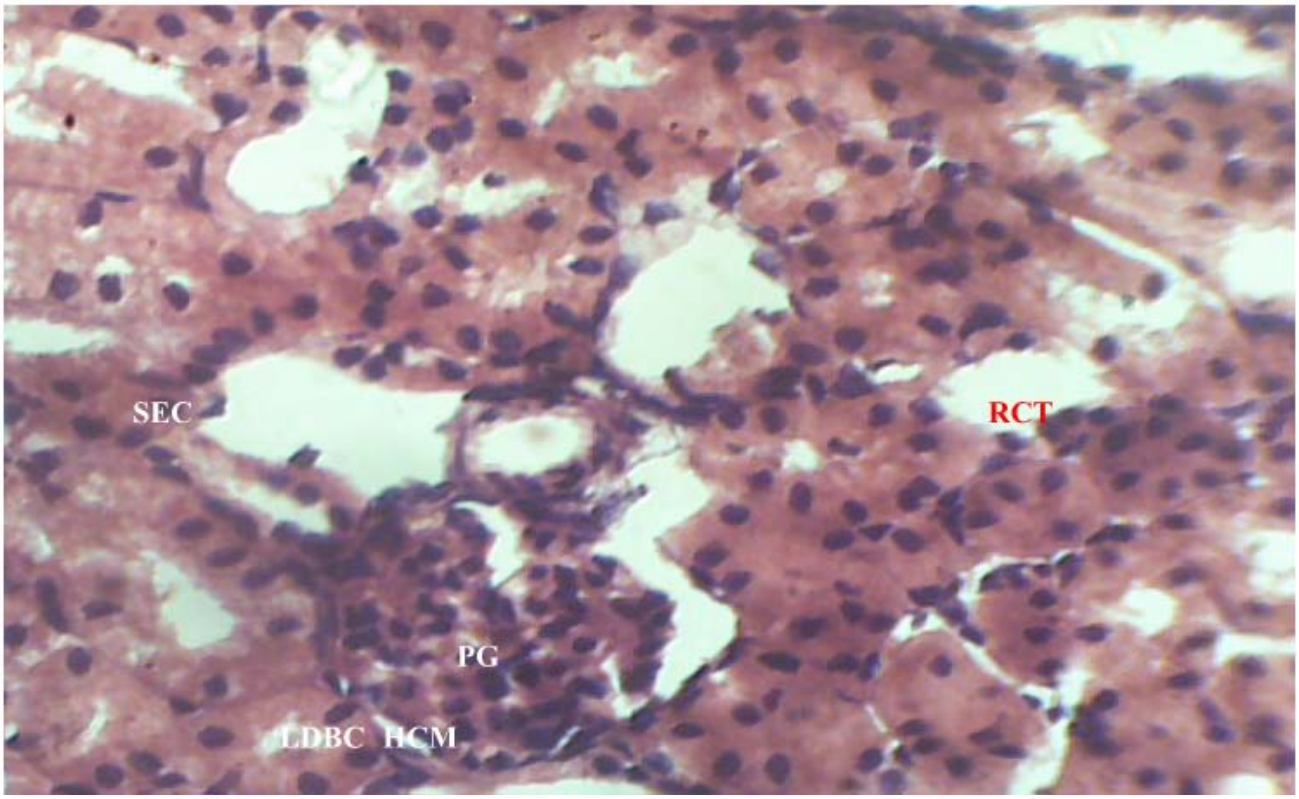

Plate 7 : Photomicrograph of the renal cortex of Wistar rat 2 hours after injection of 0.16ml of ultravist. H&E stain, X400

SEC – Swollen epithelial cells **LDBC** – Less distinct Bowman space **HCM** – Hypercellularmesangium
RCT – Renal cortical tubule

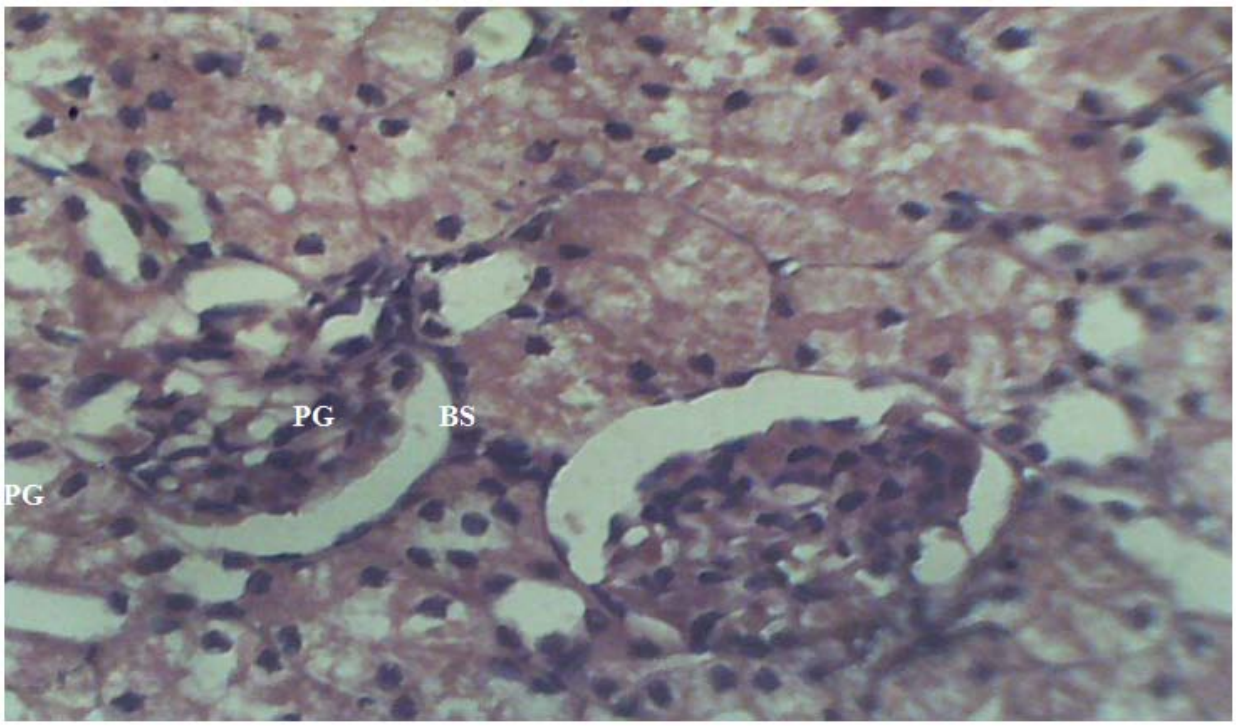

Plate 8 : Photomicrograph of the renal cortex of Wistar rat 24 hours after injection of 0.30ml of ultravist. H&E stain, X400.

PG – Prominent glomerulus **BS** – Bowman space.

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Classification and Ordination of Vegetation in Shahbaz Gari, District Mardan

By Musharaf Khan, Farrukh Hussain & Shahana Musharaf
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Abstract- A survey of natural vegetation of Shahbaz Ghari, District Mardan was undertaken in 2013-2014. Total 48 plant species in 9 communities were observed by Hierarchical Cluster Analysis and Detrended Correspondence. Plant species of each community are presented together which showed the correlation between species. The results represent that group A1a have 6 species, group A1b (2 species), A2 (3 species), group B1a (9 species) group B1bi (13 species), group B1bii (6 species), group B2 (9 species) and total important value was 53.9, 27.4, 37.6, 43, 44.9, 36.6 and 56.5 respectively. The Detrended Correspondence Analysis showed that the highest weighted mean species scores 2.88% was presented by *Stellaria media* followed by *Boerhaavia procumbens* (2.76%) and *Chenopodium album* (2.75%). Classification and ordination techniques provided very similar results based on the floristic composition and communities similarity. The results produced the source for the mapping division of vegetation.

Keywords: *cluster analysis, species weight, community structure, vegetation.*

GJSFR-C Classification : *FOR Code: 069999*

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Keywords: cluster analysis, species weight, community structure, vegetation.

I. Introduction

Ordination techniques are commonly used in phytosociology. This may be done either by arranging the points along the axis or by forming the disperse diagram with two or more axis. Detrended Correspondence Analysis (DCA), an indirect gradient analysis technique in which the distribution of species is not controlled by environmental variables rather, it focuses to analyze the pattern of species distribution. Khan, (2013) stated that analysis of plant species data by Cluster analysis and DCA is used to express the reasons of vegetation"s changes. Ordination techniques are widely used by the ecologists to study the relationship between vegetation and environment. He *et al*., (2007) using the Detrended correspondence analysis in the Alxa Plateau of Inner Mongolia, China. Khaznadar et al., (2009) analyze the distribution of plants species and environmental factors. Ahmad, (2009) studied the herbaceous vegetation by TWINSPAN in Margalla Hills National Park, Islamabad, Pakistan. El-Bana *et al*., (2009) studied *Juniperus phoenicea* and associated vegetation at three mountains in Egypt by TWINSPAN and DCA analysis techniques. Jabeen and Ahmad, (2009) conducted a study to analyze the vegetation and environment data by PCOrd 5 and CANOCO 4.5 of Ayub National Park, Rawalpindi. Ahmad et al., (2010) conducted a study along motorway (M-2), Pakistan using multivariate techniques. Classification and ordination is an invaluable method for vegetation survey (ElGhanim *et al*., 2010). Khan and Hussain, (2012) studied classification and ordination distribution patterns in Tehsil Takht-e-Nasratti. The community structure and distribution patterns of research area have not been given due attention till the date by the plant ecologists, and hence poorly understood (Khan, 2013). In the present study, an effort has been made to investigate and analyse correlation of communities with key environmental factors. The particular objectives of present study include quantifying the vegetation Shahbaz ghari, District Mardan using Clustering and ordination techniques for upcoming conservation and providing base line data of ecological important area.

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II. Research Area

The district lies from 34°23"N 72°17"E. The elevation of the valley is 1000 to 2056m above sea level. The total area of the valley is 5 kilometers sq. Shahbaz Garhi is situated on the junction of three ancient routes i.e. Kabul to Pushkalavati, Swat through Buner and Taxila through Hund on the bank of Indus River. The town was once a thriving Buddhisy city surrounded by monasteries and stupas. (Khan *et al*., 2014).

III. Materials and Methods

The phytosociological expeditions were carried out in 2013 -2014. Quadrat method was used to study and analyse the vegetation dynamic as well as to collect the primary data for statistical analyses (Figure 1). A total of 9 sites were laid in the study area. 10 Quadrats were laid in each selected sites having best representation of floral biodiversity and geographic extent of the area.

IV. Vegetation Attributes

Vegetation attributes including frequency, density and cover were recorded. The importance value of each species was compiled adding relative density (RD), relative frequency (RF) and relative cover (RC) following Hussain (1989).

V. Community Named

On the basis of the highest importance values of the first three dominant species from each layer, the communities were established and named. Plants from the premises of sampling points as well as isolated vegetation patches were also collected to record maximum number of species and their distribution patterns. Collected samples were pressed, dried and transported to herbarium of Federal Government College Mardan, Khyber Pakhtunkhawa, Pakistan, where they were identified and classified following Stewart (1961) and Nasir and Ali (1970-94).

VI. Data Analysis

All the communities and species data as well as the field area, were used for the analysis. The PC- ORD ver. 4.16 (McCune & Mefford, 1999) i.e. HCA and DCA, were used for classification and ordination analysis.

VII. Results

In investigated area collectively 48 plant species consisting of 7 shrubs and 41 herbs constructing *Cenchrus-Zizyphus-Saccharum* (CZS) community from 9 sites in which 9 communities i.e. *Fumaria-Rumex-Xanthium* (FRX) community, *Cynodon-Solanum-Sonchus* (CSS) community, *Cynodon-Sorghum-Alhagi* (CSA) community, *Ajuga- Malvastrum-Calotropis* (AMC) community, *Cynodon –Convolvulus-Cyperus* (CCC) community, *Ajuga-Saccharum-Chenopodium* (ASC) community, *Alhagi-Rumex-Euphorbia* (ARE) community, *Saccharum-Cannabis-Xanthium* (SCX) community and *Achyranthus-Ajuga-Euphorbia* (AAE) community were found.

VIII. Hierarchical Cluster Analysis (HCA)

For ordination and classification, important values of floristic data and communities were analyzed by cluster analysis techniques. For agglomerative clustering data were analyzed using different similarity indices, quantitative and work on abundance data. Three similarity indices such as Jaccard's index (JI), Sørensen's index (SI), Correlation' index (CI) on basis of ward's methods were used for agglomerative clustering. Classification by cluster analysis was stopped at the characteristic level so that the size of stands would express ecological significance through their plant life structure on basis of species IV in community. The result of classification was presented in tree like structure i.e. dendrogram, together with the indicator species used by the software for every level of division. The following groups were originated.

a) Faction A

This association is formed at cluster cycle 44 and combined group 4 into group 1 at level 1.8148E+04. This association is formed with the combination of 2 sub group i.e. A1 and A2.

i. *Faction A1*

This association is formed at cluster cycle 42 and combined group 19 into group 1 at level 1.4735E+04. This association is again divided into 2 sub group i.e. A1a and A1b.

a. Faction A1a

This association is formed at cluster cycle 37 and combined group 7 into group 1 at level 1.0032E+04. In this association total 6 species i.e. *Achyranthus aspera*, *Calotropis procera*, *Centaurea calcitrapa*, *Launea procumbens*, *Solanum surattense* and *Sonchus asper* were present. The species present in this faction comprises 53.9 important values (Figure 2; Table 1).

b. Faction A1b

This association is formed at cluster cycle 23 and combined group 21 into group 19 at level 3.4117E+03. In this association total 2 species i.e. *Cynodon dactylon* and *Cyperus scarlosus* were present and total important value was 27.4 (Figure 2;Table 1).

ii. *Faction A2*

This association is formed at cluster cycle 30 and combined group 24 into group 4 at level 6.0752E+03. In this association total 3 species i.e. *Alhagi maurorum, Euphorbia hirta* and *Saccharum spontaneum* were present. The total important value comprises by a fraction was 37.6 (Figure 2; Table 1).

b) Faction B

This association is formed at cluster cycle 46 and combined group 8 into group 2 at level 2.5270E+04. This association is formed with the combination of 2 sub group i.e. B1 and B2.

i. *Faction B1*

This association is formed at cluster cycle 45 and combined group 3 into group 2 at level 2.1210E+04. This association is formed with the combination of 2 sub group i.e. B1a and B1b (Figure 2).

a. *Faction B1a*

This association is formed at cluster cycle 41 and combined group 6 into group 2 at level 1.3319E+04. In this association total 9 species i.e. *Ajuga bractiosa, Boerhaavia procumbens, Cenchrus ciliaris*, *Chenopodium album, Malva neglecta, Solanum nigrum, Sonchus arvensis, Stellaria media* and *Tribulus terrestris* were present. The total important value of fraction was comprises 43 (Figure 2).

b. *Faction B1b*

This association is formed at cluster cycle 43 and combined group 17 into group 3 at level 1.6337E+04. This association is formed with the combination of 2 sub group i.e. B1bi and B1bii (Figure 2).

- *Faction B1bi*

This association is formed at cluster cycle 40 and combined group 5 into group 3 at level 1.2386E+04. In this association total 13 species i.e. *Ajuga parviflora, Amaranthus viridis, Capsella bursa-pestoris, Cassia occidentalis, Chenopodium murale, Cyperus rotundus, Datura metel, Euphorbia prostrate*, *Gallium aparine, Malvastrum coromandelianum, Parthenium hysterophorus, Riccinis communis* and *Silybum marianum* were present. In this fraction total important value 44.9 was contributed by 13 species (Figure 2; Table 1).

- *Faction B1bii*

This association is formed at cluster cycle 38 and combined group 23 into group 17 at level 1.0771E+04. In this association total 6 species i.e. *Convolvulus arvensis, Euphorbia helioscopia, Oxalis corniculata*, *Sorghum halepense, Taraxacum officinale* and *Withania somnifera* were present. The species in the fraction contain the total important value was 36.7 (Figure 2; Table 1).

ii. *Faction B2*

This association is formed at cluster cycle 39 and combined group 10 into group 8 at level 1.1564E+04. In this association total 9 species i.e. *Cannabis sativa*, *Carthamus oxycantha, Chrozophora oblique, Cymbopogon distans, Fumaria indica, Heliotropium europaeum, Rumex dentatus, Sonchus auriculata* and *Xanthium strumarium* were present. The total important value 56.5 was comprises by this fraction (Figure 2; Table 1).

IX. Detrended Correspondence Analysis (DCA)

Second method used was ordination analysis that employed abundance data using the 'Domin scale' without any transformation. Preliminary analysis using Detrended Correspondence Analysis (DCA) suggested that ordination using DCA provided more robust and interpretable results and in terms of species and communities turnover or standard deviation (s.d). Detrended Correspondence Analysis (DCA) was performed to describe compositional gradients in the vegetation. DCA was performed using a default value for detrending and rescaling. The Detrended Correspondence Analysis showed that the highest weighted mean species scores 2.88% was presented by *Stellaria media* followed by *Boerhaavia procumbens* (2.76%) and *Chenopodium album* (2.75%) while the lowest weighted mean species scores 0.83% was presented by *Withania somnifera* followed by *Calotropis procera* (1.36%) and *Sonchus asper* (1.37%). At AX1 the 7 species values was high than 200. The highest values was found by *Sonchus auriculata* (341) followed by *Carthamus oxycantha (*297), *Fumaria indica (*268), *Rumex dentatus* (262), *Xanthium strumarium* (235), *Heliotropium europaeum* (218) and *Chrozophora oblique* (209). In AX1 the 6 species values was less than 50. The lowest values was presented by *Withania somnifera* which was zero followed by *Gallium aparine* (2), *Malvastrum coromandelianum* (13), *Convolvulus arvensis* (20), *Amaranthus viridis* (32) *and Taraxacum officinale* (36). Between 50 and 200 values the 35 species were present (Figures 2; 3). At AX2 the 5 species was high values above 200. The highest values 290 was found in *Datura metel* followed by *Riccinis communis* (234), *Malvastrum coromandelianum (*214), *Gallium aparine* (203) and *Silybum marianum* 196.

In AX2 the 6 species values was less than 50. The lowest values zero was presented by *Malva neglecta* followed by *Oxalis corniculata* (15), *Withania somnifera* (16), *Tribulus terrestris* (32), *Saccharum spontaneum* (32) and *Solanum nigrum* 46. The 37 species were present between 50 and 200 values (Figures 2; 4). At AX3 the 10 species was high values above 200. The highest values 279 was presented by *Euphorbia prostrate followed by Stellaria media* (271), *Amaranthus viridis* (255), *Sonchus arvensis* (251), *Cenchrus ciliaris* (244), *Capsella bursa-pestoris* (236), *Parthenium hysterophorus* (233), *Cyperus rotundus* (223), *Silybum marianum* (217) and *Chenopodium album* 207. Only single specie i.e. *Sonchus auriculata* have a zero values. The 37 species were present between 50 and 200 values (Figures 3; 4). Among communities the weighted mean communities scores was high 26.7% of FRX while low 2.98% of CSA at Axis 1. At Axis 2 the highest weighted mean community's scores was 32.7 % of AMC and low -7.3% of CSA. 22.9% was the highest weighted mean communities scores presented by SCX and low zero percent in FRX (Figure 6).

X. Discussion

The investigated area comprised of 48 species in the 9 communities. The environmental factors, habitat and different plant life determined communities' structure. Plants communities are useful in classification, naming and identification of vegetation structure. The results showed that vegetation structure is diverse in the area. Muller Dumbois and Ellenberg, (1974) stated that plant community structure interpret and analyze the plant life at diverse revelation. The factors which influenced plant life structure are unplanted settlements, overgrazing, erosion, land sliding, habitat destruction, poverty and anthropogenic activities. During research work it is noticed that grazing rate and erosion is high due to which natural vegetation is diverse. Khan and Hussain, (2012) stated that the animal palatability effect the vegetation structure. Khan *et al.,* (2014) stated that the investigated area was under heavy biotic pressure due to deforestation and over grazing. Brinkmann *et al*., (2009) evaluated the vegetation reaction to ecological

situation of open woodlands along an altitudinal and animal palatability preference. Soil is essential that has continued life on earth and it also helps the plants' growth that increased the competition of grazing animals and human. The soil of research area is clay. Vegetation changed the physical and chemical properties of soil. It improves the soil infiltrations, structure and prevents erosion. Shameem *et al*., (2011) and Buckman and Brady, (1967) described that the resources of soil is limited and its physical and chemical properties are restricted mostly by humus and clay. It is noticed that with the passage of time human transportation and population are increase in research area which effect the vegetation structure. According to Turner *et al*., (2004) and Shameem *et al*., (2011) stated that the distinctive habitation altered due to increasing human transportation and population. Several research works dealing with different features of plant life from diverse parts of the state have been taken out from time to time (Stewart, 1982; Dar *et al*., 2001). The investigated area presents a limited number of animal and plant species. The investigated area is more suitable for the legume plant due the presence of high content of sand particles in the area. Plant growth somewhat indirectly manipulated through soil structure. It also effects the seedling growth which is very sensitive to physical condition of soil texture. The rigid compacted layer slows down the growth of the seedling for root cannot penetrate easily in such soil.

XI. Conclusion

This study pointed out that climatic environment of area has restricted mobilization of area and association of plant was changed with the change of environment and population. Plant ecologists have commonly been aware that vegetation shows an inconsistency over a broad variety of particular scales and area. Therefore, it is needed that we apply the multivariate techniques i.e. HEC and DCA methods for studying the degree of vegetation differences.

XII. Acknowledgements

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Figure 1 : Taking a Quadrat for shrubs vegetation study in research area

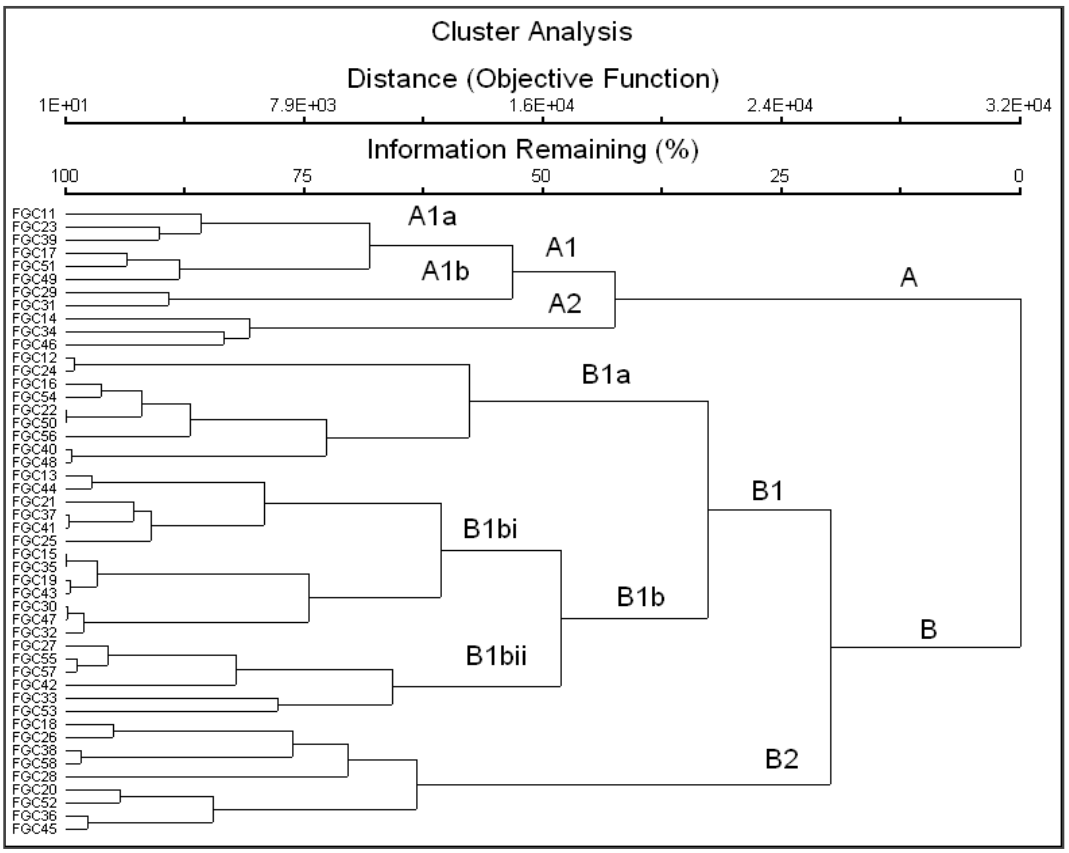

Figure 2 : Two way cluster dendrogram showing grouping of different plant species into association of Shahbaz Gari, District Mardan

Table 1 : Mean relative importance value of plant species in different associations distinguished through cluster analysis of Shahbaz Gari, District Mardan

Group	Sub group		Species	VN	FRX	CSS	CSA	AMC	CCC	ASC	ARE	SCX	AAE	IV	TIV
A	A1	A1a	*Achyranthus aspera* L.	FGC211	-	19.6	17	13.8	11.1	-		12	25	10.9	53.9
			Calotropis procera (Wight.) Ali.	FGC217	-	23.5	16.9	17.8	13.8	-	10.6			9.17	
			Centaurea calcitrapa L.	FGC223	-	20	-	-	14.7	-		6.9	13.7	6.14	
			Launea procumbens Roxb.	FGC239	-	24.2	-	16.5	17.4	-	15.8	13.7	13.1	11.2	
			Solanum surattense Burm.f.	FGC249	-	26.4	15.3	6.43	-	-	18		13	8.79	
			Sonchus asper (L.) Hill.	FGC251	-	26.3	19.2	13.1	-	-		10		7.63	
		A1b	*Cynodon dactylon* L. Pers.	FGC229	21.2	32.5	26.9	-	26	-	14.4		13	14.9	27.4
			Cyperus scarlosus R.Br.	FGC231	18	22.2	18.7	-	20.8	-	17.8	15.2		12.5	
	A2		*Alhagi maurorum* Medic.	FGC214	16.6	22	24.2	12		20.1	25	11.9	10.1	15.8	37.6
			Euphorbia hirta L.	FGC234		22.1		6.62		20.7	21.4	9.7	17.4	10.9	
			Saccharum spontaneum L.	FGC246		22.1	15.5			25.1	15.5	20.6		11	
B	B1	B1a	*Ajuga bractiosa* Wall. Benth.	FGC212				20.7		26.1			24.1	7.87	43
			Boerhaavia procumbens Banks ex Roxb.	FGC216				12.7		15.1				3.08	
			Cenchrus ciliaris L.	FGC222						18.8	13.4	7.46		4.41	
			Chenopodium album L.	FGC224				13.1		22.6			13.9	5.51	
			Malva neglecta Wallr.	FGC240			17.3			18.3				3.96	
			Solanum nigrum L.	FGC248			17.8			16.1			11.8	5.08	
			Sonchus arvensis L.	FGC250						20.1	14.3	11.7		5.11	
			Stellaria media (L.) Cry.	FGC254						19.8		9.4	7	4.02	
			Tribulus terrestris L.	FGC256		18.3				17				3.92	
		B1bi	*Ajuga parviflora* Benth	FGC213				15.3	13.7		9.5		14.7	5.91	44.9
			Amaranthus viridis L.	FGC215					12.2			11.5		2.63	
			Capsella bursa-pestoris Medic.	FGC219					12.9			15.2	16.5	4.95	
			Cassia occidentalis L.	FGC221	16.3			16.9	16		9.5			6.53	
			Chenopodium murale L.	FGC225					12.3		13.1			2.82	
			Cyperus rotundus L.	FGC230				13.5				14.1		3.07	
			Datura metel L.	FGC232				7.41						0.82	
			Euphorbia prostrata L.	FGC235					8.82			16.3		2.79	
			Gallium aparine L.	FGC237				10.9	15					2.88	
			Malvastrum coromandelianum(L.) Garcke.	FGC241				18.2	18.4					4.06	
			Parthenium hysterophorus L.	FGC243					11			9.1	8.1	3.13	
			Riccinis communis L.	FGC244				12.1					16.9	3.22	
			Silybum marianum (L.) Gaertn.	FGC247				9.88				9.2		2.12	
		B1bii	*Convolvulus arvensis* L.	FGC227			20.6		23.4			13.5		6.39	36.7
			Euphorbia helioscopia Mewski.	FGC233	17.7		14.9		9.65				21.1	7.05	
			Oxalis corniculata L.	FGC242			17.2				15.6	12.4		5.02	
			Sorghum halepense (L.) Persoon.	FGC253			25.9	11.2	20		16.8		16.9	10.1	
			Taraxacum officinale Weber.	FGC255			17.5	10.7	12.8			6.97		5.33	
			Withania somnifera (L) Dunal.	FGC257			15.2		10.1					2.81	
	B2		*Cannabis sativa* L.	FGC218	16.9			16.9		12.4	16.7	18.6	10.7	10.3	56.5

		Carthamus oxycantha M. Bieb.	FGC220	24.6					20.1				4.97	
		Chrozophora oblique (Vahl) A. Juss.	FGC226	21.7			8.86			15.4	12.6		6.51	
		Cymbopogon distans (Nees ex Steud.)Watson.	FGC228	22.8	20.8		15.4					9.5	7.61	
		Fumaria indica (Hausskn) Pugsley.	FGC236	28.1					15.4	15.6			6.57	
		Heliotropium europaeum L.	FGC238	20.7							15.2	13	5.43	
		Rumex dentatus L.	FGC245	27.6						21.6			5.46	
		Sonchus auriculata L.	FGC252	20.9									2.32	
		Xanthium strumarium L.	FGC258	26.9					12.4		16.7	10.5	7.4	

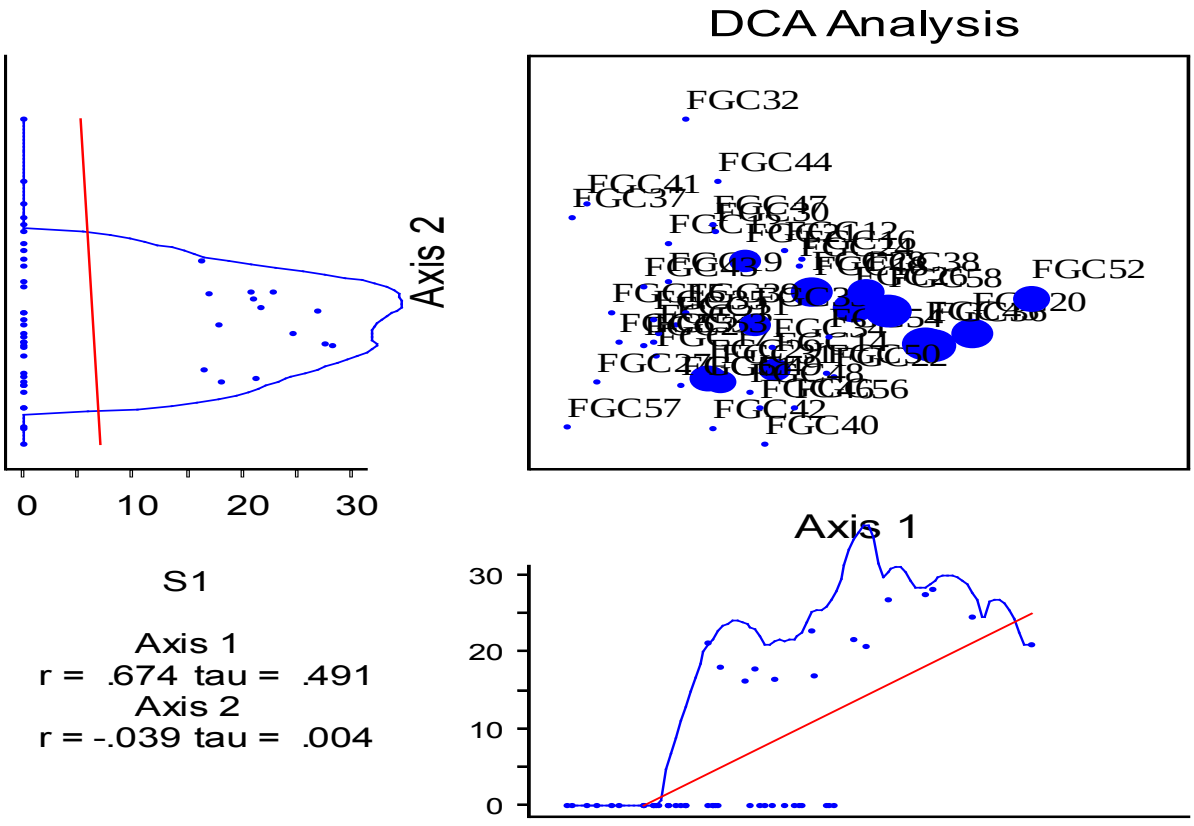

Figure 3 : Sample scores at Axis 1 and Axis 2, which show weighted mean species scores

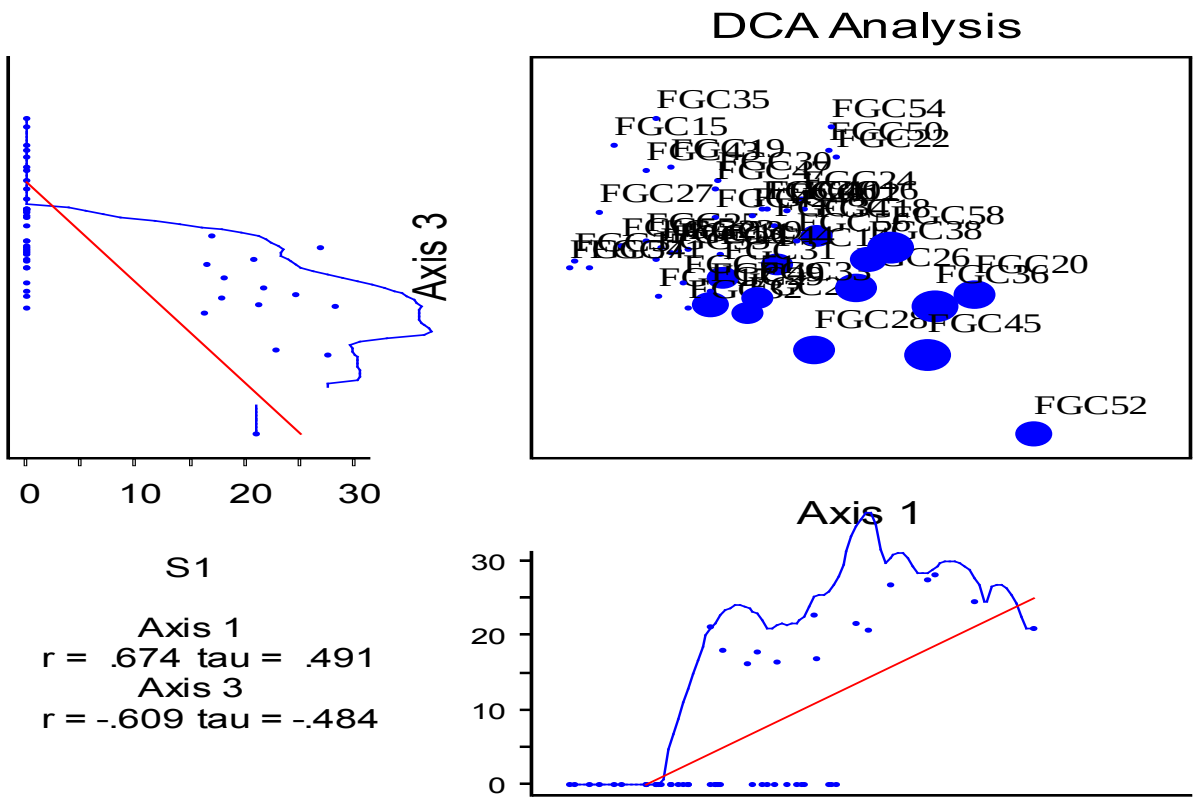

Figure 4 : Sample scores at Axis 1 and Axis 3, which show weighted mean plant species scores.

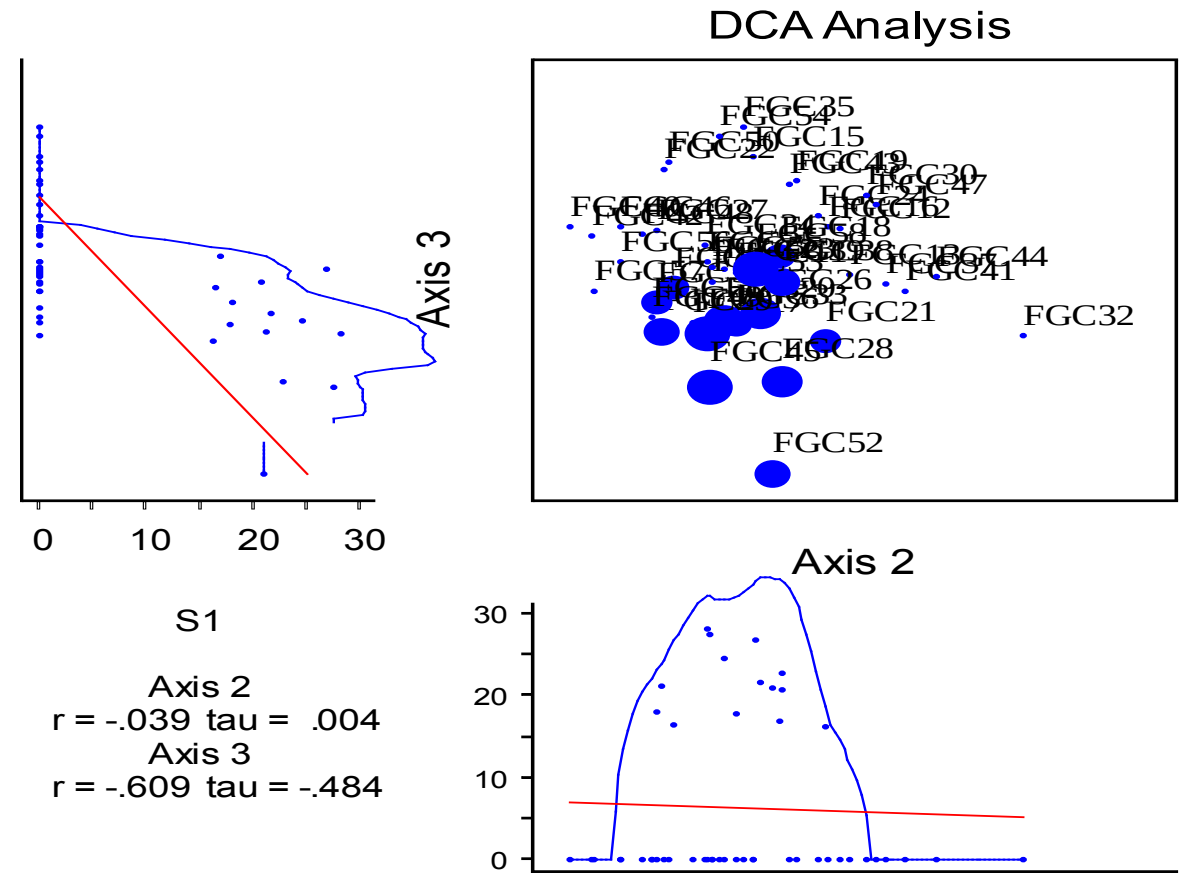

Figure 5 : Sample scores at Axis 2 and Axis 3, which show weighted mean plant species scores

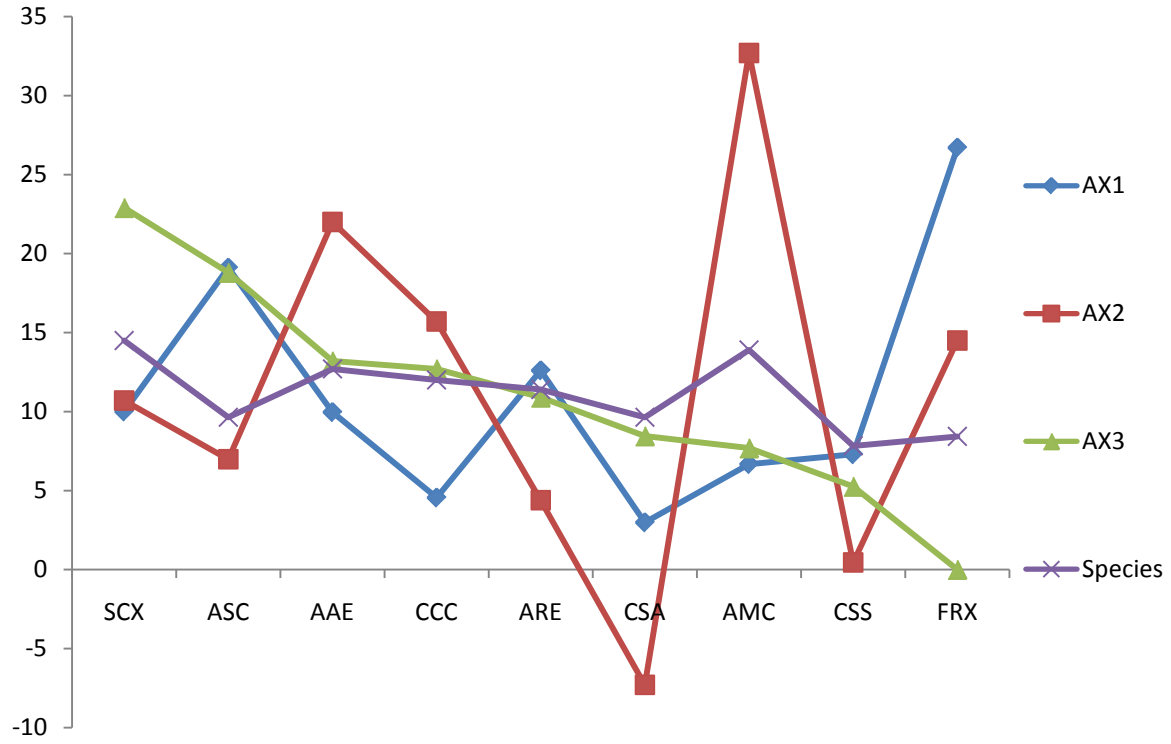

Figure 6 : Communities scores at Axis 1, Axis 2 and Axis 3, which showed weighted mean communities scores.

Figure 7 : Group photo during research study

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Report of Newly Recorded Eight Scleractinian Corals from Middle and South Andaman Archipelago, India

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Zoological Survey of India, India

Abstract- Eight species of scleractinian corals *viz*. *Acropora azurea* Veron, and Wallace 1984 belongs to the family Acroporidae, *Favia vietnamensis* Veron, 2000 belongs to the family Faviidae, *Turbinaria irregularis* Bernard, 1896 under the family Dendrophylliidae, *Psammocora vaughani* Yabe and Sugiyama, 1936 and *Coscinaraea wellsi* Veron and Pichon, 1980 belong to the family Siderastreidae, *Halomitra meierae* Veron and Maragos, 2000 under the family Fungiidae, *Lobophyllia flabelliformis* Veron, 2000 and *Mussismilia braziliensis* (Verrill, 1867) belong to the Mussidae family are reported as new distributional record to Indian waters from Andaman and Nicobar Islands. This present paper deals with the taxonomical features, occurrence and status evaluation of these eight newly recorded species along with their previous distribution.

Keywords: *biodiversity, biogenic, new record, andaman and nicobar islands.*

GJSFR-C Classification : *FOR Code: 279999*

REPORTOFNEWLYRECORDEDEIGHTSCLERACTINIANCORALSFROMMIDDLEANDSOUTHANDAMANARCHIPELAGOINDIA

Strictly as per the compliance and regulations of :

Report of Newly Recorded Eight Scleractinian Corals from Middle and South Andaman Archipelago, India

Tamal Mondal [α], C. Raghunathan [σ] & K. Venkataraman [ρ]

Abstract- Eight species of scleractinian corals *viz. Acropora azurea* Veron, and Wallace 1984 belongs to the family Acroporidae, *Favia vietnamensis* Veron, 2000 belongs to the family Faviidae, *Turbinaria irregularis* Bernard, 1896 under the family Dendrophylliidae, *Psammocora vaughani* Yabe and Sugiyama, 1936 and *Coscinaraea wellsi* Veron and Pichon, 1980 belong to the family Siderastreidae, *Halomitra meierae* Veron and Maragos, 2000 under the family Fungiidae, *Lobophyllia flabelliformis* Veron, 2000 and *Mussismilia braziliensis* (Verrill, 1867) belong to the Mussidae family are reported as new distributional record to Indian waters from Andaman and Nicobar Islands. This present paper deals with the taxonomical features, occurrence and status evaluation of these eight newly recorded species along with their previous distribution.

Keywords: biodiversity, biogenic, new record, andaman and nicobar islands.

I. Introduction

The coral reef ecosystem is one of the most productive ecosystems of this planet and stood second in rank after Tropical Rainforests. The complexity of food chain and its resemblance thrive the naming of coral reef ecosystem as Rainforests of Sea [1]. The distributional pattern of corals indicates very minimum coverage i.e.0.2% of world's ocean floor, despite of this narrow affiliation, it support enormous microhabitat with an estimated quantification of 25% of all marine species [2]. The coverage and support ratio has been contiguously demonstrating the widespread significance of coral reef biodiversity for sustaining the marine biodiversity. Andaman and Nicobar Islands with its intrinsic ecological attributes sustain a wide range of marine faunal communities from intertidal region to the greater depth of the sea. The presence of mostly fringing type reefs in the continental shelf region of these islands harbours optimum level of scleractinian corals. The life supporting environmental clues for the scleractinian corals mostly available in tropical waters [3]. Scleractinian corals are baseline animals for the development of balanced marine ecosystem [4]. Andaman and Nicobar Archipelago signify the natural inherent and pristine environment for the recruitment and development of scleractinian corals. The biological, ecological, sociological, economical and etc. roles of scleractinian lives are undreamed off either directly or indirectly. The present paper deals with the addition of eight newly recorded species in Indian waters from Andaman and Nicobar Islands with their global and regional status as well as earlier record on distribution.

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II. Material and Methods

An extensive study was carried out in Middle and South Andaman Archipelago of Andaman and Nicobar Islands during March and November 2014 to document the scleractinian corals. Undersea species investigation was carried out by employment of Self Contained Underwater Breathing Apparatus (SCUBA) diving. Underwater recording of individual species was made by underwater digital camera (Canon Powershot G15). Sampling of small portion of colonies were also made to study the corallite structures for better understanding of the morphological features under stereo zoom microscope (Leica, M 205 A). Species individual photos were identified in conjunction with Veron and Pichon [5-7], Veron *et al.* [8] Veron and Wallace [9], Veron [10] and Wallace [11]. On completion of detailed taxonomical characters, the specimens were registered in National Zoological Collections and deposited at Zoological Survey of India, ANRC, Port Blair.

III. Results

Eight species of scleractinian corals were recognized as new to Indian waters from Andaman and Nicobar Islands on the basis of morphological characterization. The detailed morphometric description is given below with distributional range.

Systematics

Family: ACROPORIDAE Verrill, 1902
Genus: Acropora Oken, 1815

a) ***Acropora azurea*** Veron, and Wallace 1984 (Fig. 1)

Material Examined: Five colonies of the said species were observed at Neil Island (Lat. 11°51.115'N and Long. 93°02.689'E) of South Andaman at the depth of 9 m to 14 m on 27.iv.2014. One small portion of the

colony was sampled for taxonomical studies (Reg. No.: ZSI/ANRC-10593).

Description: Colonies are the combination of irregularly arranged clumps of fine branches and branchlets. Those are arising basically from a solid base. Radial corallites are not regularly arranged. The radial corallites are appressed and ended with small nariform rounded openings. Axial corallites are small and tubular.

IUCN Red List category and criteria: Not Evaluated, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Australia, Indonesia, Taiwan, Province of China and Viet Nam.

Family: FAVIIDAE Gregory, 1900

Genus: Favia Oken, 1815

b) ***Favia vietnamensis*** Veron, 2000 (Fig. 2)

Material Examined: Two colonies were observed at Neil Island (Lat. 11°50.556'N and Long. 93°00.508'E) of South Andaman at the depth of 15 m to 17 m on 26.iv.2014.

Description: Colonies are massive in structure but the appearance is usually small. Corallites are irregularly shaped and deeply excavated to form the colony. Septa are well marked and irregular in length. Developed paliform lobes are prominent. Colonies are usually fleshy during *in situ* condition.

IUCN Red List category and criteria: Near Threatened, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Australia, Cambodia, Indonesia, Japan, Kenya, Malaysia, Mozambique, Papua New Guinea, Philippines, Singapore, Taiwan, Province of China, Tanzania, United Republic of Thailand and Viet Nam.

Family: DENDROPHYLLIIDAE Gray, 1847

Genus: Turbinaria Oken, 1815

c) ***Turbinaria irregularis*** Bernard, 1896 (Fig. 3)

Material Examined: Two colonies were observed at Neil Island (Lat. 11°51.115'N and Long. 93°02.689'E) of South Andaman at the depth of 10 m on 27.iv.2014.

Description: Colonies are encrusting plates. The outer marginal areas are free and irregular. Corallites are also irregularly arranged in colony and exsert. The openings of the corallites are small. The coenosteum is usually smooth and uniform between adjacent corallites.

IUCN Red List category and criteria: Least Concern, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* American Samoa, Australia, British Indian Ocean Territory, Cambodia, Comoros, Djibouti, Egypt, Eritrea, Guam, Indonesia, Israel, Japan, Jordan, Madagascar, Malaysia, Maldives, Mauritius, Mayotte, Micronesia, Mozambique, Northern Mariana Islands, Oman, Palau, Papua New Guinea, Philippines, Réunion, Samoa, Saudi Arabia, Seychelles, Singapore, Solomon Islands, Somalia, Sudan, Taiwan, Province of China, Thailand, Viet Nam and Yemen.

Family: SIDERASTREIDAE Vaughan and Wells, 1943

Genus: Psammocora Dana, 1846

d) ***Psammocora vaughani*** Yabe and Sugiyama, 1936 (Fig. 4)

Material Examined: Five colonies were observed at Haddo NSRY Jetty adjoining area (Lat. 11°40.667'N and Long. 92°42.902'E) of South Andaman at the depth of 4 m on 07.iii.2014.

Description: Colonies are sub-massive and can be seen as small colonial patch. Corallites are arranged in groups within shallow depressions. Septo-costae are thick. They are arranged in neat and have granulated margins.

IUCN Red List category and criteria: Near Threatened, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Fiji, Guam, Indonesia, Japan, Kiribati, Marshall Islands, Micronesia, Nauru, Northern Mariana Islands, Palau, Papua New Guinea, Philippines, Solomon Islands, Taiwan, Province of China, Tuvalu, Vanuatu, Wallis and Futuna.

Family: SIDERASTREIDAE Vaughan and Wells, 1943

Genus: Coscinaracea Milne Edwards and Haime, 1848

e) ***Coscinaraea wellsi*** Veron and Pichon, 1980 (Fig. 5)

Material Examined: One colony was observed in Ship wreck at North Bay (Lat. 11°43.006'N and Long. 092°45.465'E) of South Andaman at the depth of 9 m on 21.iii.2014.

Description: Colonies are small and thin plate like structure. The plate margins are lobed and irregular in orientation. The laminae are overlapping. Corallites are irregularly distributed. The columellae are with deep, The septo-costae are thick and granulated.

IUCN Red List category and criteria: Least Concern, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Australia, British Indian Ocean Territory, Cambodia, Comoros, Djibouti, Egypt, Eritrea, Fiji, French Polynesia, Indonesia, Israel, Japan, Jordan, Kenya, Kiribati, Madagascar, Malaysia, Maldives, Marshall Islands, Mauritius, Mayotte, Micronesia, Mozambique, Myanmar, Nauru, New Caledonia, Norfolk Island, Palau, Papua New Guinea, Philippines, Réunion, Saudi Arabia, Seychelles, Singapore, Solomon Islands,

Somalia, Sri Lanka, Sudan, Taiwan, Province of China, Tanzania, Thailand, Tuvalu, Vanuatu, Viet Nam and Yemen.

Family: FUNGIIDAE Dana, 1846

Genus: Halomitra Dana, 1846

f) ***Halomitra meierae*** Veron and Maragos, 2000 (Fig. 6)

Material Examined: Eleven colonies were observed at off Neil Island (Lat. 11°55.300'N and Long. 93°05.613'E) of South Andaman at the depth of 25 to 32 m on 25.iv.2014. One colony was sampled for taxonomical studies (Reg. No.: ZSI/ANRC-10830).

Description: Colonies are free-living, circular in outline and are flat. Colony is with a central area of parallel septo-costae surrounded by a border of peripheral septo-costae. The septo-costae are perpendicular to the colony margin. Mouths are distinguishing in the central area. The structures of the mouths are small. Septo-costae are arranged in two or three different orders. Septal teeth are prominent and arranged. The teeth are usually granularly dentate and sides of walls are granular. The costae are arranged upto the colony margin. Costal pits are presents. Costal spines are echinose.

IUCN Red List category and criteria: Not Evaluated.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Indonesia and Irian Jaya.

Family: MUSSIDAE Ortmann, 1890

Genus: Lobophyllia de Blainville, 1830

g) ***Lobophyllia flabelliformis*** Veron, 2000 (Fig. 7)

Material Examined: One colony was observed in Ship wreck at North Bay (Lat. 11°43.006'N and Long. 092°45.465'E) of South Andaman at the depth of 9 m on 21.iii.2014.

Description: Colonies are dome-shaped and flabello-meandroid. The valleys are closely compacted and elongated. Due to presence of fleshy polyps, it can be encountered as *Symphyllia* at *in situ* condition. Valleys are well separated. The septal dentition is well marked and strong.

IUCN Red List category and criteria: Vulnerable, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India-Andaman and Nicobar Islands; *Elsewhere-* Australia, Indonesia, Japan, Malaysia, Papua New Guinea, Philippines, Singapore, Solomon Islands, Taiwan, Province of China, Thailand and Viet Nam.

Genus: Mussismilia Ortmann, 1902

h) ***Mussismilia braziliensis*** (Verrill, 1867), (Fig. 8)

Material Examined: One colony was observed at North Passage Island (Lat. 12°18.288'N and Long. 92°54.830'E) of South Andaman at the depth of 6 m on 28.xi.2014.

Description: The colony is large, massive and dome shaped in organization. Cerioid arrangement of corallites is distinctive characterization of this species. The shapes of the individual corallites are variable for each. The corallites are seen as very compact in their developmental pattern also. Septa are round in shape while the dentations are seems to be bead like.

IUCN Red List category and criteria: Data Deficient, 2014.

Occurrence in Andaman and Nicobar Islands: Rare.

Distribution: India- Andaman and Nicobar Islands; *Elsewhere-* Brazil.

IV. Discussion

Scleractinian corals are living with the formation of a variety of interlinking network of faunal communities and close symbiotic association of zooxanthellae [12] mostly shallow depth region (up to 60 m) and encounter as the main source of primary productivity [13] while the azooxanthellate corals are reported from the greater depth. The pattern of vertical narrow range of distribution and presence of microscopic algae i.e. zooxanthellae continues the life process by photosynthesis and respiration and also manage the entire reef energy cycle either from physical energy of oceanic circulatory process or carbon cycle of associated faunal creatures (www.reef.edu.au). Due to the proximity of mammoth beneficial aspects, the scleractinian corals have been considered under great concern of study from the past to recent date. The wide range of species diversity from 1488 to 1520 [14, 15] and their morphological plasticity depending on the geographical as well as physiological deviation leads to immense emphasis on taxonomical studies [16-18]. The taxonomical works on scleractinian corals in India was initiated during 1847 [19] and carried out very scarcely till 1960s. During the period of around next 30 years, active taxonomical works were made by several workers and a total of 199 species of scleractinian corals were recorded as Indian scleractinian database [20-32] In 2003, Venkataraman *et al.* described a total of 208 species of scleractinian corals from all the major four reef areas of India while Andaman and Nicobar Islands represented 177 species of scleractinian corals among them [33]. Due to extensive taxonomical exploration of Zoological Survey of India, a total of 591 species of scleractinian corals are reported from Indian waters while Andaman and Nicobar Islands represents 563 species with two new species of corals, of which one species of coral under the family Fungiidae [34-37]. The addition of eight species of scleractinian corals as new record to Indian waters from Andaman and Nicobar Islands will strengthen the species database of these islands as well as India. Presently reported eight species are found only from the above mentioned study areas and very rare in occurrence to the Andaman and

Nicobar Islands. *Lobophyllia flabelliformis* Veron, 2000 belong to the family Mussidae, was evaluated as Vulnerable (VU) species according to the IUCN Redlist category and criteria while two species were evaluated as Near Threatened. More extensive studies are required in future in Andaman and Nicobar Islands to explore the distributional range of new scleractinian corals as well as the ecological studies to evaluate the regional occurrence and status to compare with the global ratio. The emerging knowledge on scleractinian corals will be helpful to conservation the marine biodiversity with active management plan and strategies.

V. Acknowledgements

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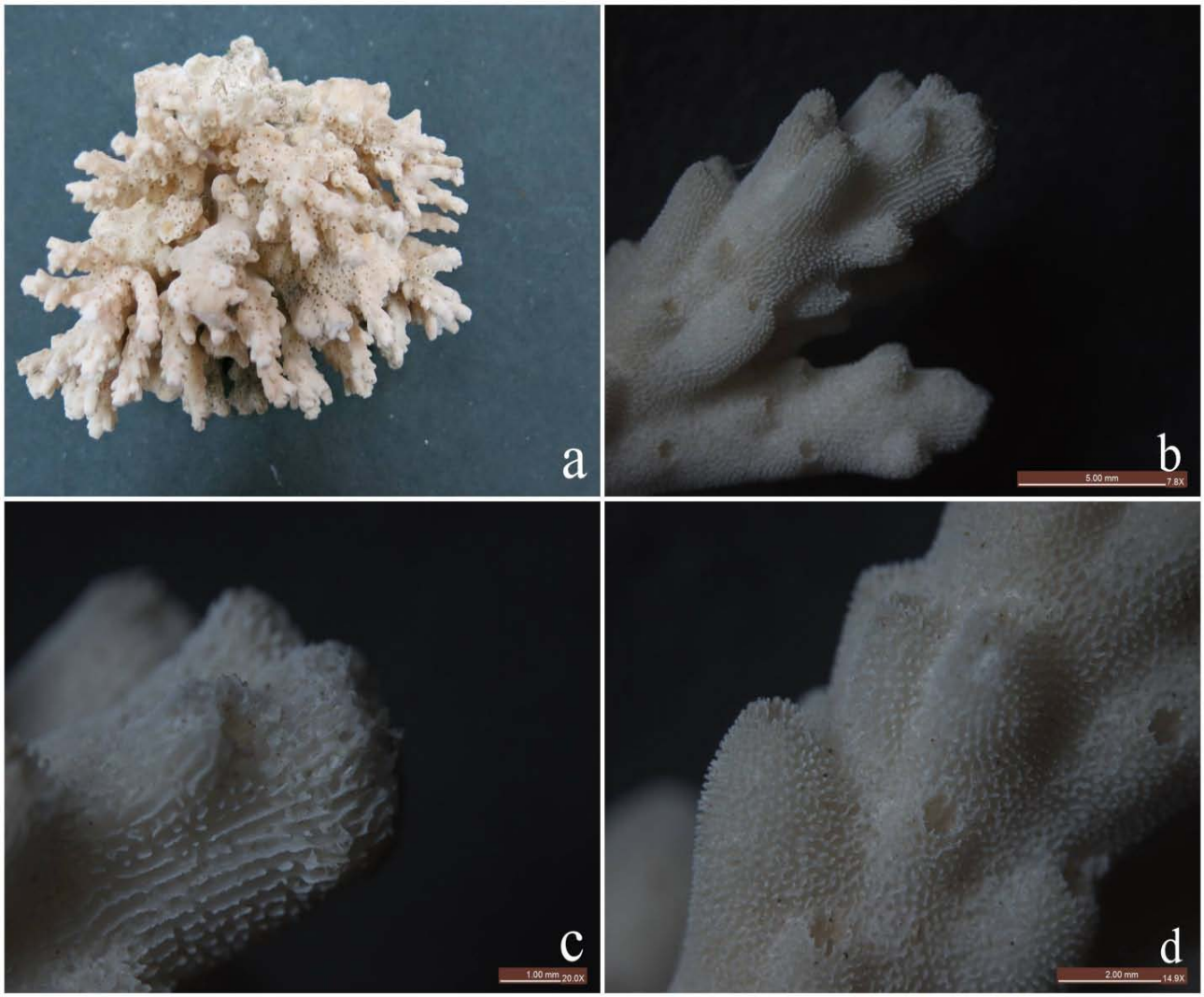

Fig. 1 : *Acropora azurea* Veron, and Wallace 1984
a Small portion of a colony; b- Branches of colony; c- Axial corallites; d- Radial corallites

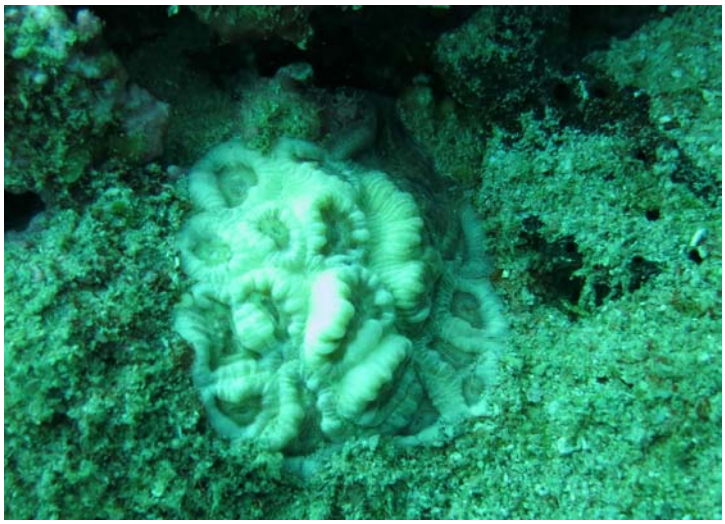

Fig. 2 : *Favia vietnamensis* Veron, 2000

Fig. 3 : *Turbinaria irregularis* Bernard, 1896

Fig. 4 : *Psammocora vaughani* Yabe and Sugiyama, 1936

Fig. 5 : *Coscinaraea wellsi* Veron and Pichon, 1980

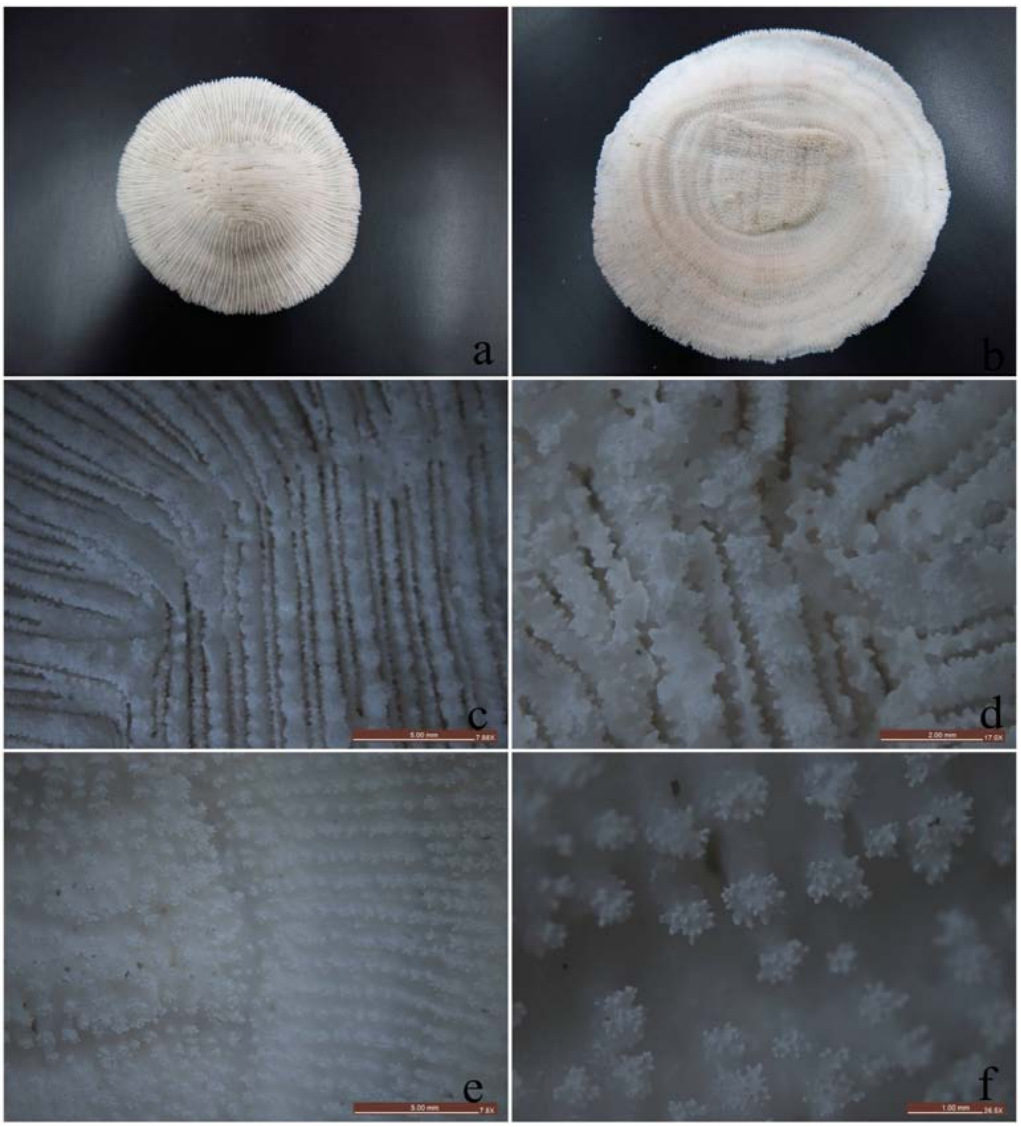

Fig.6 : *Halomitra meierae* Veron and Maragos, 2000

a- Dorsal side of coralla; b- Ventral side of coralla; c- Septal arrangement; d- septal teeth; e-Costal arrangement; f- Costal spines

Fig. 7 : *Lobophyllia flabelliformis* Veron, 2000

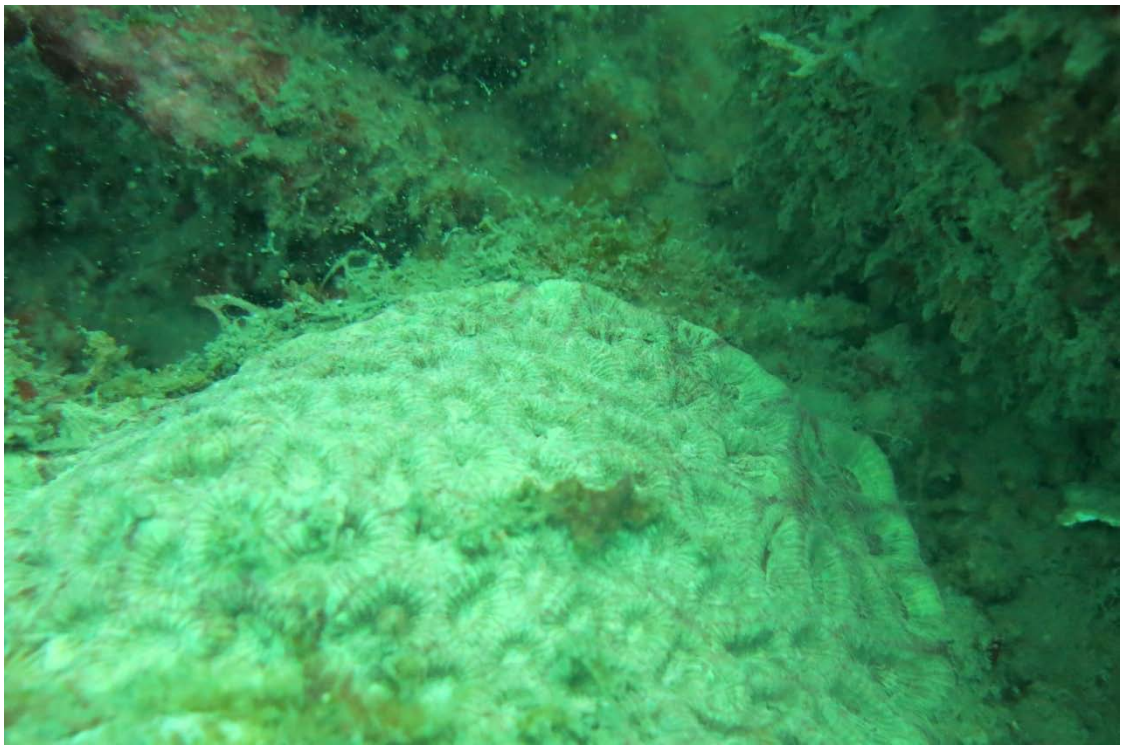

Fig. 8 : *Mussismilia braziliensis* (Verrill, 1867)

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Statistical Analysis of Some Selected Zooplakton Composition Dwelling Two Pan Marine Ecosystems with A Reference to the Abiotic Factors

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Abstract- Physical and chemical factors of marine ecosystems were measured in Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern and Southern Khobar during 2012 & 2013 to study their effect on the distribution of the zooplankton densities. Seasonal variations of water transparency (Secchi disc reading in cm) ; water temperature (°C) ; total dissolved solids (mg/l) ; total alkalinity (mg/l) ; total hardness (mg/l) ; salinity (g/l) ; chlorides (mg/l) ; total Nitrogen (mg/l) and Tributyl tin (µgg-1) were measured. Samples of the four pan marine beaches were taken at each site, using screw top polypropylene bottles of 1000 ml. capacity. The bottles were transported filled with distilled water and washed out and refilled with sea water at each site. Closing the bottles have been done underneath the water surface so as to make sure that they were completely full. These samples were returned to the laboratory and analyzed within twenty-four hours.

Keywords: water transparency; water temperature; total dissolved solids; total alkalinity; total hardness; salinity; chlorides; total nitrogen; tributyl tin; relative densities; zooplankton.

GJSFR-C Classification : FOR Code: 060899

Strictly as per the compliance and regulations of :

Statistical Analysis of Some Selected Zooplakton Composition Dwelling Two Pan Marine Ecosystems with A Reference to the Abiotic Factors

Abdullah Bedeer Hussein [α] & Gaber Ahmed Saad [σ]

Abstract- Physical and chemical factors of marine ecosystems were measured in Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern and Southern Khobar during 2012 & 2013 to study their effect on the distribution of the zooplankton densities. Seasonal variations of water transparency (Secchi disc reading in cm) ; water temperature (°C) ; total dissolved solids (mg/l) ; total alkalinity (mg/l) ; total hardness (mg/l) ; salinity (g/l) ; chlorides (mg/l) ; total Nitrogen (mg/l) and Tributyl tin (µgg-1) were measured. Samples of the four pan marine beaches were taken at each site, using screw top polypropylene bottles of 1000 ml. capacity. The bottles were transported filled with distilled water and washed out and refilled with sea water at each site. Closing the bottles have been done underneath the water surface so as to make sure that they were completely full. These samples were returned to the laboratory and analyzed within twenty-four hours. The present study provided information about the seasonal abundance of major groups of zooplankton namely, Bryozoa, Cnidaria, Rotifera, Nematoda, Annelida, Amphipoda, Copepoda, Isopoda, crustaceanlarvae, Scaphopoda, Bivalvia, molluscan-larvae, and ascidian larvae. All zooplankton studied were previously described and identified (see Saad, 2015).The seasonal fluctuations in the abiotic factors and the distribution of relative densities of zooplankton collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt & Northern and SouthernKhobar of the Arabian Gulf – Saudi Arabia have been statistically analyzed. One-way analysis of variance (ANOVA) at $P<0.0001$, Bartlett's test for equal variances, Tukey's Multiple Comparison Test, Dunnett's Multiple Comparison Test and Newman-Keuls Multiple Comparison Test at $P > 0.05$ and $P < 0.001$ were applied. This study concluded that all abiotic facors of Mediterranean Sea, Egypt are suitable for dwelling of zooplankton than those present in the Arabian Gulf, Saudi Arabia.

The zooplankton densities in the four pan marine ecosystems all study periods were Anfoushy >Abu Qir >Northern Khobar >Southern Khobar.

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Keywords: water transparency; water temperature; total dissolved solids; total alkalinity; total hardness; salinity; chlorides; total nitrogen; tributyl tin; relative densities; zooplankton.

I. Introduction

Planktonic fauna referred to as nektons arefeebly floating microscopic organisms in aquatic habitats. The majority of these groups areentirely planktonic throughout their lifetimeespecially those that belong to the annelids, rotifers, nematodes, crustaceansand echinoderms (Yakub, 2004; Ayodele and Adeniyi,2006; Okogwu and Ugwumba, 2006; Lawal-Are etal., 2010). Planktonic fauna referred to as benthos are bottom- dwellers through- out their lifetime especially those that belong to hydrozoans, anthozoans, gastropods, bivalvians and adult stage of ascidians. Meanwhile, many higher aquatic invertebrateshave developmental stages that are planktonic. Theseinclude the eggs, larvae and other developmentalstages such as shrimps, crabs,oyster, echinoderms and ascidians. Thesedevelopmental life stages are often collected whensamples of zooplankton are taken from the naturalmarine or marinewater bodies (Lawal-Are et al., 2010).

Physico-chemical parameters and quantity of nutrients in water play a significant role in the distributional patterns and species composition of plankton (Scasso, et al. 2001;FAO, 2006; Okogwu 2010). In aquatic habitats, the environmental factors include various physical properties of water such as solubility of gases and solids, the penetration of light, temperature, and density. The chemical factors such as salinity, pH, hardness, phosphates and nitrates are very important for growth and density of zooplankton and some higher consumer depend on their existence. The term "Water quality" refers for the physical, chemical and biological parameters of water and all these characteristics directly or indirectly influences the survival and production of aquaculture species (Boyd, 1998; Boyd and Tucker,1998). The Seasonal variation in the ecological parameters exerts a profound effect on

the distribution and population density of zooplankton (Odum, et al. 1971). A combination of light, temperature, oxygen and other abiotic variables, food resources, competition and predation affectsthe seasonal faunal composition of zooplankton. Many reviews emphasized this vital distribution of zooplankton in other ecosystems (not our study localities), Chakrabarty et al., (1959); Edmondson (1959); Nasar (1977); Rajapaska and Fernando (1982);;Dumont and Van De Velde (1977);Stich & Lampert, 1981; Sharma (1983); Sladecek (1983); Patil and Gouder (1985); Hudec (1987);Rao et. al, (1994); Primicerio & Klemetsen, 1999; Primicerio, 2000; Nogueira (2001);Nagdali &Gupta (2002); Sampaio et al. (2002); Kehayias et al., 2004; Kessler & Lampert, 2004; Arora and Mehra (2003a); Arora and Mehra (2003b); Ugale et al., (2005); Sreelatha (2007);Jyoti, et al. 2009 ; Larson, et al. 2009;Doulka & Kehayias, 2011; Ikbel, et al. 2012). Seasonal stratification is considered to be the primary factor affecting zooplankton species composition and biomass succession (Ortega-Mayagoitia et al., 2000; Ringelberg, 2010).

Studies on plankton distribution and hydrography attracted the interest of many authors (Panikkar and Rao, 1973; Nair et al 1978; Rao 1979; Qasim 1977 &1982; Dejen et al., 2004). Nektonic zooplankton are micronekton (size range, 0.02-l cm) as larvae of nematods, annelids, crustaceans, molluscs & echinoderms and macronekton (size range, 2-10 cm) like fishes (Ahmad and Ashok, 2013). Nekton are those organisms that have developed powers of locomotion so that they are not at the mercy of prevailing sea currents or wind-induced water motion. Pelagic nekton usually have stream-lined shapes that make their propulsive efforts more effective. Most nekton are specialized invertebrates evolved the ability to swim (and hunt) actively in the water column as cephalopods (squids, octopus, cuttlefish, nautilus) and arthropods (shrimp, prawns, some crabs). Mesopelagic nekton seldom exceed 10 cm, have large light-sensitive eyes, uniformly black photophores and provided with light-producing organs. Abyssal pelagic have species-specific pattern of photophores, small with flabby, soft, nearly transparent flesh supported by weak exoskeleton. Benthic invertebrate populations are important factors and frequently used to evaluate overall ecosystem health (Flint, 1979; Rosenberg & Resh. 1993; Reynoldson el al., 1995) because these communities are important to material cycling and secondary production, moreover, they are sensitive to environmental contaminants. Benthic fauna feed mostly upon detritus including sedimentary phytopJankton and zooplankton organisms. The bottom fauna, in turn, furnishes a direct food supply for some aquatic organisms including fish. Populations of the benthic organisms attain marked fluctuations at both spatial and temporal scales in relation to changes in the physical and chemical factors of water (Cyrus & Wepener, 1993; Reynoldson et aL, 1995; Dermott & Kerec, 1997; Palmer & Poff, 1997; Vivier & Cyrus, 1999; Breneman el al, 2000; Nalepa el ai, 2000; Bass & Potts, 2001; Dermott, 2001) and/or biotic interactions such as predation and competitive exclusion (Gómez-Gutiérrez, el al. 1995 & 1996 ; Guimarães,et et. 2009).Benthic meiofauna and macrofauna exhibit similar patterns across the seasons and these patterns are in part related to plankton dynamics in the overlying water column. The signature seasonal pattern is one of increased abundance and biomass (Grassle et al., 1985; Rudnick et al., 1985). It is likely that the increase in biomass and abundance in spring is primarily a response to the deposition and accumulation of organic matter from the winter-spring phytoplankton bloom (zooplankton predation during this time is largely minimal due to cold water temperatures). However, Rudnick et al. (1985) suggested that rapidly increasing sediment temperatures during this time (from 2˚C to approximately 13˚C by May) may also strongly affect benthic communities. It is also possible that the seasonal dynamics of Narragansett Bay benthic communities are affected by other factors (predation) (Frithsen, 1989), and ultimately these temporal patterns are probably affected by multiple factors working in concert.

Zooplankton play a key role in the ecosystem structure due to their quick response to abiotic conditions, especially in impacted marine or marine habitats (Levinton 1995; Neumann-Leitão et al. 1999). Little is known about the seasonal faunal composition of zooplankton in marine habitats of Abu Qir Bay, Egypt or the Arabain Gulf, Saudi Arabia. The primary aim of this study was to make a general ecological survey of the invertebrate fauna (nektonic and benthic zooplankton). The secondary aim was to investigate the differences in the invertebrates distribution along the Abu Qir Bay and Anfoushy, Egypt and the Arabain Gulf, Saudi Arabia, the effect of the chemical and physical properties of the water on the distribution of the zooplankton, the seasonal qualitative and quantitative changes in the zooplankton of each study locality, to compare zooplankton of the Abu Qir Bay and Anfoushy, Egypt with those of the Arabain Gulf, Saudi Arabia. Observations of this study were made on temporal variations in richness, community similarities, abundance, species diversity, dominance and evenness of zooplankton in the four study localities.

II. Materials and Methods

a) Physical and Chemical Factors

The following physical and chemical factors were measured in the fourpan marinesites (according to Hofmann, 1977; Abbasi, 1998; Boyd, 1998)to study their effect on the distribution of the zooplankton .

Seasonal variations of transparency (Secchi disc reading in cm); water temperature (°C); total dissolved solids (mg/l); total alkalinity (mg/l); total hardness (mg/l); salinity (g/l); chlorides (mg/l); total Nitrogen (mg/l) and Tributyl tin (μgg-1). Samples of the four panmarine beaches were taken at each site, using screwtop polypropylene bottles of 1000 ml. capacity. The bottles weretransported filled with distilled water and washed out and refilledwith Sea water at each site. Closing the bottles have been doneunderneath the water surface so as to make sure they were completely full.These samples were returned to the laboratory and analyzed withintwenty-four hours.

Water transparency or light penetration in water was measured with Secchi disc. It was dipped into the water on a calibrated line until it disappeared. The depth at which it disappeared and also the depth at which it reappeared when rose was recorded. The average of these two readings is called Secchi disc reading.Secchi disc reading (cm) = (A + B) / 2 where A = Depth at which Secchi disc disappears ; B = Depth at which Secchi disc reappears and 2 = standard value of equation.

Temperature (°C) of water was measured by dipping a mercury thermometer and recorded during sampling period.

Total dissolved solids (TDS), TBT and salinity were measured with WTW 320 conductivity meter. Water samples were placed into clean beakers, conductance cell of the meter was immersed into sample solution. The resistance was measured in μs/cm or mS/cm, depending upon the concentration of salts in sample, similarly the readings of salinity and total dissolved solids were noted with the conductivity meter by changing mode of measurement to salinity and TDS. The cell was rinsed in a beaker with distilled water after each reading. The calibration measurement was performed in 0.00702 NKCl solutions. This solution has a specific conductance of 100 μmhose at 25 °C.

Determination of Alkalinity has been carried out as follows: Hydrochloric acid (HCl) 0.01 N was prepared by adding 9.0 ml of 36% HCl in 1.0 liter of distilled water in volumetric flask. Two indicators, methyl orange and phenolphthalein were used. Methyl orange was prepared by dissolving of 0.5 g of Methyl orange in 50 g of d.d water. 0.5 g of phenolphthalein in 50 ml of 95% ethyl alcohol and 50 ml water. Dilute sodium hydroxide (NaOH) was added drop wise until faint color appeared. Sample (10 ml) was taken with the help of pipette in the conical flask, 4-5 drops of phenolphthalein indicator was added, if the colour developed pink, the sample was titrated with 0.01N hydrochloric acid, until the colour disappeared. The volume consumed was noted and was labeled as p- alkalinity. In same sample 2-3 drops of methyl orange indicator solution was added. The titration was continued until end point with change of colour from orange to brick red. The volume consumed was noted and amount mg/l was calculated as m-alkalinity.

Alkalinity (mg/l) = (N × M × 50,000) / V Where: N = Normality ; M = Mean ; V =Volume of sample and 50,000 = Standard value of equation.

Determination of Hardnesshas been carried out as follows: 0.372 g of ethylenediaminetetra-acetic acid disodium salt (EDTA) was dissolved in 100 ml of distilled water (0.1 N) and was used as a titrant. Buffer solution was prepared by dissolving 16.9g of ammonium chloride in 143 ml in volumetric flask. 1.179 g of magnesium carbonate, 12.6 H2O in 50 ml d.d. water. These two reagents were mixed to make 250 ml as a final volume of buffer solution, Erichrome black-T, the indicator was used in the form of tablets. Sample (10 ml) was taken in to conical flask with the help of pipette, 0.5 mg of buffer tablet (Erichrome black-T) and 1 ml of conc. ammonium hydroxide (NH4OH) was added as indicator and then titrated with 0.1N (EDTA) solution at the end point the colour turned from red to green then the reading was noted from burette.

Hardness (mg/l) = (N × M × 50,000) / V Where: N = Normality of titrant 0.1 N ; M = Mean of three readings ; V = Volume of sample and 50,000 = standard value of equation.

Determination of Chlorides has been carried out as follows: Silver nitrate AgNO3 (E-Merck) solution of 0.1 N was used as a titrant and was prepared by dissolving 1.6987g of Ag NO3 in 100 ml of d.d water and potassium chromate (5% K2CrO4 in water) was used as indicator. Sample (10 ml) was taken in conical flask and 2-3 drops of potassium chromate solution added. It was titrated with 0.1N AgNO3 till the colour change from yellow to brick red, the reading was noted from burette.

Chloride (mg/l) = (N × M × 35450) / V Where: N = Normality of titrant (0.1 N) ; M = Mean of three readings ; V = Volume of sample ml and 35450 = Standard value of equation.

Determination of total Nitrogen has been carried out using Kjeldhal method. For total nitrogen determination, the solutions were prepared by diluting 50g of NaOH in 100 ml d.d. water (50% NaOH), 10g copper sulphate (CuSO4) in 100ml d.d water (10% CuSO4) and boric acid (H3BO3) solution by adding 01 ml of mixed indicator solution. In 10 ml sample, 4ml conc. H2SO4 and 0.4 ml CuSO4 was added. The mixture was kept on flame and when it boiled, the flame was turned off to allow the sample to cool. The sample was transferred to the Kjeldhal flask and was diluted by adding the same quantity of d.d water into the flask. The sample was made alkaline by adding 2-3 drops of phenolphthalein and 50% NaOH to turn colour of the sample in to pink. Indicator H3BO3 25 ml was taken in 100 ml conical flask. When sample was collected in indicator solution, then was titrated with H2SO4 (0.01N)

solution, finally the color turned from green to red and reading was recorded from burret.

Total Nitrogen mg/l = (A-B × 2.80) / V Where: A= Volume of titrant consumed in sample ; B= Volume of titrant consumed in blank ; V= Volume of sample and 2.80 = Standard value of equation.

b) Animals

Zooplankton were collected from two pan marine ecosystems. During (2009 - 2010) planktons were collected monthly from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt. During 2012 – 2013 planktons were collected from the Northernand SouthernKhobar, Arabian Gulf – Saudi Arabia. All planktons were transported alive in plastic aquaria containing well aerated sea water to the laboratory and placed in large glass aquaria containing well aerated sea water. Randomly selected adults of planktonic communities were dissected in sea water. Ascidian larvaehave been washed and then grown at 20°C (Hofmann,*et al.* 2008 ; Saad, 2002). Zooplankton were then described and prepared for photomacroscopy or / and SEM study.

c) Methods of collection

The zooplankton collection involves primarily the filtration of water by net, collecting the water in bottles/ water samplers or by pumps. The sampling success would largely depend on the selection of a suitable gear; mesh size of netting material, time of collection, water depth of the study area and sampling strategy. The gear used keeping in view the objectives of the investigation (see Sameoto, et al. 2000, Dhargalkar and Verlecar, 2004, Paturej and Gutkowska, 2014 for review). There are three main methods of zooplankton collection used, which are as follows:

d) Bottles / water samplers

This method was used mainly for collecting smaller forms or micro zooplankton. The water is collected at the sampling site in bottles or water samplers of 5 to 20 litre capacity. The sterile bottles should be preferred. Surface water can be collected by scooping water into the bottle of suitable size. While collecting the water samples, there should be minimum disturbance of water to prevent avoidance reaction by plankton. The water samplers with closing mechanisms are commonly used for obtaining samples from the desired depths. The micro zooplankton are then concentrated by allowing them to settle, centrifuging or fine filtration. The advantage of this method was that it is easy to operate and sampling depths are accurately known. The disadvantage is that the amount of water filtered is less. The macro zooplankton and rare forms are usually not collected by this method and so it is unsuitable for qualitative and quantitative estimations.

e) Pumps

The gear is normally used on board the vessel/boat. The sampling can also be carried out from a pier. In this method, the inlet pipe is lowered into the water and the outlet pipe is connected to a net of suitable mesh size. The net is particularly submerged in a tank of a known volume. This prevents damage to the organisms. The zooplankton is filtered through the net. A meter scale on the pump records the volume of water filtered. This method was used for quantitative estimation and to study the small scale distribution of plankton. The frictional resistance of the sampled water in the hose can cause turbulence; damaging the larger plankton especially the gelatinous forms, ctenophores and siphonophores etc. The advantage of the method is that the volume of the water pumped is known. Again the continuous sampling is possible. However, the sampling depth is limited to a few meters and it is difficult to obtain samples from deeper layers.

f) Nets

The most common method of zooplankton collection is by a net. The amount of water filtered is more and the gear is suitable both for qualitative and quantitative studies. The plankton nets used are of various sizes and types. The different nets can broadly be put into two categories, the open type used mainly for horizontal and oblique hauls and the closed nets with messengers for collecting vertical samples from desired depths. Despite minor variations, the plankton net is conical in shape and consists of ring (rigid/flexible and round/square), the filtering cone and the collecting bucket for collection of organisms. The collecting bucket should be strong and easy to remove from the net. The netting of the filtering cone is made of bolting silk, nylon or other synthetic material. The material should be durable with accurate and fixed pore size. The mesh should be square and aperture uniform. The mesh size of the netting material would influence the type of zooplankton collected by a net. The nets with finer mesh would capture smaller organisms, larval stages and eggs of planktonic forms while those with coarse netting material are used for collecting bigger plankton and larvae. Sometimes combinations of nets with mesh of different pore sizes weree used. There is a great variety of mesh available from the finest to the coarse pore sizes.

g) Macroscopic observation

Plaktons were prepared for both macroscopic techniques or / and scanning electron microscopy. They were fixed for 24 hr in buffered 2.5% glutaraldehyde and post fixed for 30 min. in 1% osmium tetroxide. Washing was two times in 0.1 M phosphate buffer, followed by four times in 0.4 M glycerol and two times in PPTA (15 min.). Specimens with hard exoskeleton were washed many times in distilled water and subjected to dilute

nitric acid for decalcification of exoskeleton or the cuticle. Specimens were fixed in neutral 10 % formalin or Bouin. Then washed in distilled water for 24 hrs, dehydration through ascending series of ethyl alcohol, alternated by another dehydration series of tertiary butyl alcohol (used as a softening agent). All zooplankton were stained with Evans stain or Nile blue or Borax carmine to observe its internal structures since they are mostly transparent. Samples were placed on glass slides with embedding mixture of PBS / glycerol / DABCO. Others were dissected with microneedles and incised longitudinally to ease its identification. Immediate viewing and photographing were performed under an Axiomicroscope (ZEISS-Axiophot). The description of almost all zooplankton was carried out on live stages under Axiomicroscope since they are minute, microscopic and transparent. Evan Blue stain was added to the live stages and described alive while movement. The photos did not clarify all described structures.

h) Scanning electron microscopy (SEM)

Samples of larvae were dried by means of the critical point method, mounted using carbon paste on an Al-stub and coated with gold up to a thickness of 400 Å in a sputter-coating unit (JFC-1100E). Observations of larvae morphology in the coded specimens were performed in a Jeol JSM-5300 scanning electron microscope operated between 15 and 20 KeV.

i) Statistical analysis

Analysis of variance (ANOVA) is a broad group of techniques for identifying and measuring different sources of variation within the data set. It consists of a set of procedures by which a variance of the random variable is broken down by certain sources of variation of its value. With the components of variance, depending on the sources, one can conclude if there is a significant difference between the values of dependent variable for different levels of the observed factor variables. In the present study, a one-way analysis of variance is used to compare each abiotic factor and each zooplankton density in the four study areas seasonally which have different levels of one variable.

If the above-mentioned assumptions for ANOVA are not met, the Turkey`s Multiple Comparison Test, Bartlett's test for equal variances and Dunnett's Multiple Comparison Test were used for determining whether three or more independent samples originate give a clear cut differences. When this test leads to significant results, at 1 North one sample differs from the others. A principal component analysis is a standard tool in modern data analysis. It is a simple, nonparametric method for extracting relevant information out of confusing data sets. Principal component analysis is concerned with the interpretation of the variance and covariance structure of the original set of variables through a small number of their linear combinations. The general objectives of principal component analysis are data reduction and interpretation. In order to reduce the number of variables. For more details about methodology of calibrations. However, One-way analysis of variance (ANOVA) at $P<0.0001$, Bartlett's test for equal variances, Tukey's Multiple Comparison Test, Dunnett's Multiple Comparison Test and Newman-Keuls Multiple Comparison Test at $P > 0.05$ and $P < 0.001$ were applied. see (Dijana, et al.2012 for review).

III. Results

In order to define particular pan marine fauna of zooplankton, it is important to analyze accurately as many physical and chemical characteristics of water as possible before preceding the biological studies. The measurements of these characteristics provide valuable information about the marine habitat. Some of the important Physicochemical factors of pan marine habitats have been analyzed:

a) Water transparency

During 2009 – 2010 (Anfoushy & Abu Qir, Egypt)and 2012-2013 (North Khobar & South Khobar, Saudi Arabia) the transparency values of marine water are given in (Tables1-4 ; Histograms 1 & 2) for pan marine habitats in different seasons of the year. Low transparency of water during 2012 was found in North Khobar during the months of February- May (17-20 cm).Tukey's Multiple Comparison Test showed the loSouthern Mean Dif between South Khobar vs Abu Qir at$P < 0.001$during 2009 & 2012. During 2010 & 2013 the loSouthern Mean Dif between North Khobar vs Abu Qir at $P < 0.01$.

The highest value of water transparency was observed during April-May in Abu Qir (173-191 cm)in 2009 and (120 cm) during October 2010.

Tukey's Multiple Comparison Test showed the highest Mean Dif between North Khobar and South Khobar -10,17 at $P > 0.05$ during 2012 andDunnett's Multiple Comparison Test showed -5,083 at $P > 0.05$ during 2013 for the same two marine localities.

Tables 1 & 2 : Seasonal variation of water transparency (Secchi disc reading in cm) inthe fourmarine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	45	53	64	91
February	40	45	50	72
March	68	70	85	148

April	71	64	128	191
May	36	68	180	173
June	50	62	85	107
July	30	46	67	83
August	44	53	80	91
September	64	76	100	150
October	39	47	120	128
November	70	79	60	110
December	75	91	105	160

Table 2 Analyzed				
Water transparency 1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	15,76			
R squared	0,518			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	15,32			
P value	0,0016			
P value summary	**			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	38770	3	12920	
Residual (within columns)	36080	44	819,9	
Total	74850	47		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-10,17	1,23	P > 0.05	-41.41 to 21.08
North Khobar vs Anfoushy	-41	4,96	P < 0.01	-72.25 to -9.753
North Khobar vs Abu Qir	-72,67	8,791	P < 0.001	-103.9 to -41.42
South Khobar vs Anfoushy	-30,83	3,73	P > 0.05	-62.08 to 0.4134
South Khobar vs Abu Qir	-62,5	7,561	P < 0.001	-93.75 to -31.25
Anfoushy vs Abu Qir	-31,67	3,831	P < 0.05	-62.91 to -0.4199

Tables 3 & 4 : Seasonal variation of water transparency (Secchi disc reading in cm) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	22	30	35	60
February	17	25	40	90
March	25	29	65	100
April	15	22	50	60
May	20	28	45	30
June	32	40	55	70
July	25	30	50	65
August	40	55	65	80
September	45	30	70	55
October	28	35	39	120
November	30	40	60	75
December	29	25	40	65

Table 4 Analyzed				
Water transparency 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	24,11			
R squared	0,6218			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	14,77			
P value	0,002			
P value summary	**			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	15140	3	5047	
Residual (within columns)	9210	44	209,3	
Total	24350	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-5,083	0,8606	P > 0.05	-19.46 to 9.293
North Khobar vs Anfoushy	-23,83	4,035	P < 0.01	-38.21 to -9.457
North Khobar vs Abu Qir	-45,17	7,647	P < 0.01	-59.54 to -30.79

b) Temperature

The water Temperature values are given in (Tables 5 & 8 ; Histograms 3 & 4) for pan marine habitats in different Seasons of the year. Low water Temperature of water during 2012 was found in Abu Qir during the months of December - January (8-9 °C). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar vs South Khobar -3 at P > 0.05 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -4,083at P > 0.05.

The highest value of water Temperature was observed during July-October in South Khobar (38-40°C) in 2012 and (39-45°C) during July-October 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar and Abu Qir 9,083at P > 0.05 during 2009 & 2012 and 10,83 at P < 0.01during 2010 & 2013 for the same two marine localities.

Tables 5 & 6 : Seasonal variation of water temperature (°C) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	13	11	12	9
February	15	19	15	12
March	20	24	19	14
April	24	27	24	20
May	29	31	26	23
June	32	35	28	27
July	34	38	31	31
August	35	40	25	28
September	30	36	22	22
October	36	38	27	12
November	30	33	14	10
December	27	29	11	8

Table 6 Analyzed				
Water temprature 1				
One-way analysis of variance				
P value	0,0018			
P value summary	**			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	5,901			
R squared	0,2869			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	0,6831			
P value	0,8772			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1086	3	362,1	
Residual (within columns)	2700	44	61,35	
Total	3786	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-3	0,9382	P > 0.05	-10.78 to 4.783
North Khobar vs Anfoushy	5,917	1,85	P > 0.05	-1.867 to 13.70
North Khobar vs Abu Qir	9,083	2,841	P < 0.05	1.300 to 16.87

Tables 7 & 8 : Seasonal variation of water transparency (Secchi disc reading in cm) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	17	21	13	11
February	20	26	17	13
March	25	30	20	15
April	31	34	26	22
May	33	38	25	26
June	35	40	27	29
July	40	45	30	35
August	36	41	27	30
September	32	37	24	21
October	37	39	21	14
November	33	36	17	12
December	29	30	13	10

Table 8 Analyzed				
Water temprature 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			

F	12,23			
R squared	0,4548			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	1,732			
P value	0,6298			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1836	3	612,1	
Residual (within columns)	2201	44	50,03	
Total	4037	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-4,083	1,414	P > 0.05	-11.11 to 2.945
North Khobar vs Anfoushy	9	3,117	P < 0.01	1.972 to 16.03
North Khobar vs Abu Qir	10,83	3,752	P < 0.01	3.805 to 17.86

c) Total Dissolved Solids (TDS)

The Total Dissolved Solids (TDS) values of marine water are given in (Tables 9 & 12 ; Histograms 5 & 6) for pan marine habitats in different seasons of the year. Low Total Dissolved Solids in water during 2012 was found in North Khobar during the months of October - December (1955-2780mg/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar vs South Khobar -4932 at P > 0.05 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -22,17 at P > 0.05.

The highest value of Total Dissolved Solids was observed during November-December in South Khobar (5301-5593mg/l) in 2012 and (5200-6034mg/l) during May-June 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 1347 at P > 0.05 during 2009 & 2012 and Mean Dif between North Khobar vs Abu Qir 2024at P < 0.01during 2010 & 2013.

Tables 9 & 10 : Seasonal variation of water total dissolved solids (TDS) (mg/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	2901	4260	2034	2220
February	4410	44774	2052	2498
March	2800	4285	2990	2669
April	3570	4791	2066	2222
May	4491	5074	2032	2221
June	4906	5066	2954	2814
July	3760	4987	1873	1520
August	3100	4976	2064	2999
September	2900	4971	1773	2954
October	1955	5220	1533	1941
November	2540	5301	1188	2152
December	2780	5593	1393	2387

Table 10 Analyzed				
Total dissoved solids1				
One-way analysis of variance				
P value	0,039			
P value summary	*			

Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	3,036			
R squared	0,1715			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	130,2			
P value	P<0.0001			
P value summary	***			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	3,04E+08	3	1,01E+08	
Residual (within columns)	1,47E+09	44	33380000	
Total	1,77E+09	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-4932	2,091	P > 0.05	-10670 to 809.3
North Khobar vs Anfoushy	1347	0,5709	P > 0.05	-4395 to 7088
North Khobar vs Abu Qir	959,7	0,4068	P > 0.05	-4782 to 6701

Tables 11 & 12 : Seasonal variation of water total dissolved solids (TDS) (mg/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	2771	3422	3176	2398
February	3285	3865	2883	2165
March	5832	4033	3223	2054
April	4975	4914	3999	2032
May	5222	5200	3243	2487
June	6243	6034	3041	2398
July	3278	4196	3976	2534
August	4734	4165	2222	2265
September	5039	5021	3086	1554
October	3646	3270	1176	2175
November	3581	3990.0	2237	2796
December	2972	3734	2899	2431

Table 12 Analyzed				
Total dissoved solids 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	18,24			
R squared	0,5543			

Bartlett's test for equal variances				
Bartlett's statistic (corrected)	14,95			
P value	0,0019			
P value summary	**			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	37560000	3	12520000	
Residual (within columns)	30200000	44	686300	
Total	67750000	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-22,17	0,06554	P > 0.05	-845.4 to 801.0
North Khobar vs Anfoushy	1368	4,045	P < 0.01	544.9 to 2191
North Khobar vs Abu Qir	2024	5,985	P < 0.01	1201 to 2847

d) Total Alkalinity

The Total Alkalinity values of marine water are given in (Tables 13 & 16 ; Histograms 7 & 8) for pan marine habitats in different seasons of the year. Low Total Alkalinity in water during 2009 was found in Abu Qir during the months of March – November (169-133mg/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and South Khobar -225 at P > 0.05 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -136,2 at P > 0.05.

The highest value of Total Alkalinity was observed during July in North Khobar (494mg/l) in 2012 and (579mg/l) in South Khobar during October 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 185,3 at P > 0.05 during 2009 & 2012 and 69,42at P < 0.01during 2010 & 2013.

Tables 13 & 14 : Seasonal variation of water total alkalinity (mg/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	310	434	312	242
February	370	420	232	267
March	429	475	165	169
April	433	387	132	155
May	354	388	152	175
June	382	462	232	201
July	494	430	183	210
August	420	442	265	197
September	340	430	186	270
October	376	455	134	171
November	383	559.0	175	133
December	332	2441	232	278

Table 14 Analyzed				
total alkalinity 1				
One-way analysis of variance				
P value	0,0035			
P value summary	**			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	5,247			
R squared	0,2635			

Bartlett's test for equal variances				
Bartlett's statistic (corrected)	95,87			
P value	P<0.0001			
P value summary	***			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1348000	3	449200	
Residual (within columns)	3767000	44	85610	
Total	5115000	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-225	1,884	P > 0.05	-515.7 to 65.74
North Khobar vs Anfoushy	185,3	1,551	P > 0.05	-105.5 to 476.0
North Khobar vs Abu Qir	179,6	1,503	P > 0.05	-111.2 to 470.3

Tables 15 & 16 : Seasonal variation of water total alkalinity (mg/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	374	506	280	340
February	310	537	320	333
March	391	499	349	304
April	362	482	260	265
May	373	489	330	253
June	382	479	275	283
July	360	497	298	320
August	383	488	300	319
September	399	474	280	330
October	364	579	299	297
November	393	495.0	296	340
December	320	520	291	310

Table 16 Analyzed				
total Alkalinity 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	140			
R squared	0,9052			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	0,3564			
P value	0,9491			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			

ANOVA Table	SS	df	MS	
Treatment (between columns)	323000	3	107700	
Residual (within columns)	33850	44	769,2	
Total	356900	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-136,2	12,03	P < 0.01	-163.7 to -108.6
North Khobar vs Anfoushy	69,42	6,131	P < 0.01	41.86 to 96.98
North Khobar vs Abu Qir	59,75	5,277	P < 0.01	32.19 to 87.31

e) Total Hardness

The Total Hardness values of marine water are given in (Tables 17 & 20 ; Histograms 9 & 10) for pan marine habitats in different seasons of the year. Low Total Hardness in water during 2009 was found in Anfoushy during the months of April, June – July and September (210-220 mg/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and South Khobar -297 at P > 0.05 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -422,6 at P > 0.05.

The highest value of Total Hardness was observed during March-April in South Khobar (980-985mg/l) in 2012 and (940 mg/l) during May 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 411,9 at P > 0.01 during 2009 & 2012 and 212,9 at P < 0.01during 2010 & 2013.

Tables 17 & 18 : Seasonal variation of total hardness (mg/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	630	925	295	430
February	670	945	235	499
March	635	985	220	420
April	685	980	210	470
May	620	935	240	490
June	695	955	210	400
July	676	976	220	490
August	692	982	232	440
September	682	936	210	450
October	570	910	260	470
November	630	890.0	240	490
December	580	910	250	480

Table 18 Analyzed				
total hardness 1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	940,2			
R squared	0,9846			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	3,108			
P value	0,3752			
P value summary	ns			

Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	3230000	3	1077000	
Residual (within columns)	50390	44	1145	
Total	3281000	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-297	21,5	P < 0.01	-330.6 to -263.4
North Khobar vs Anfoushy	411,9	29,81	P < 0.01	378.3 to 445.5
North Khobar vs Abu Qir	183	13,25	P < 0.01	149.4 to 216.6

Tables 19 & 20 : Seasonal variation of total hardness (mg/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	590	900	298	450
February	520	930	240	430
March	500	890	290	420
April	480	910	257	400
May	440	940	340	470
June	450	985	330	440
July	490	975	270	530
August	560	886	240	450
September	440	956	300	510
October	420	889	250	490
November	500	880	260	450
December	480	800	240	530

Table 20 Analyzed				
total hardness 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	428,1			
R squared	0,9669			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	1,748			
P value	0,6263			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	2592000	3	864000	
Residual (within columns)	88810	44	2018	
Total	2681000	47		

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-422,6	23,04	P < 0.01	-467.2 to -377.9
North Khobar vs Anfoushy	212,9	11,61	P < 0.01	168.3 to 257.6
North Khobar vs Abu Qir	25	1,363	P > 0.05	-19.64 to 69.64

f) Salinity

The Salinity values of marine water are given in (Tables 21 & 24 ; Histograms 11 & 12) for pan marine habitats in different seasons of the year. Low Salinity in water during 2009 was found in Anfoushy during the months of January and May– October (2,1-2,7 g/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and South Khobar -2,758 at P > 0.01 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -3,575 at P > 0.05.

The highest value of Salinity was observed during almost all months in South Khobar (7,3- 7,9 g/l) in 2012 and (7.9 – 9,1 mg/l) during 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 1,575 at P > 0.01 during 2009 & 2012 and 1,433 at P < 0.01during 2010 & 2013.

Tables 21 & 22 : Seasonal variation of Salinity (g/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	4,1	7,9	2,1	3,9
February	4,7	7,5	3,7	3,6
March	5,1	7,4	3,9	3,6
April	5,9	7,8	3,5	3,7
May	5,5	7,5	2,4	3,2
June	5,3	6,9	3,3	3,7
July	3,7	7,3	2,9	3,8
August	4,4	7,9	2,4	3,2
September	5,8	6,8	2,8	3,1
October	4,1	6,5	2,7	3,3
November	3,7	7.90	3,8	2,8
December	3,8	7,8	3,7	2,4

Table 22 Analyzed				
Salinity1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	126,5			
R squared	0,8961			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	5,12			
P value	0,1633			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	141,8	3	47,27	
Residual (within columns)	16,44	44	0,3736	
Total	158,3	47		

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-2,758	11,05	P < 0.01	-3.366 to -2.151
North Khobar vs Anfoushy	1,575	6,312	P < 0.01	0.9676 to 2.182
North Khobar vs Abu Qir	1,317	5,277	P < 0.01	0.7093 to 1.924

Tables 23 & 24 : Seasonal variation of Salinity (g/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	2,9	7,9	3,5	4,8
February	3,3	7,4	3,7	4,9
March	6,8	6,6	3,3	4,6
April	5,1	7,2	3,8	3,9
May	5,5	7,8	3,4	4,7
June	6,2	8,7	3,7	4,8
July	3,5	8,5	2,9	3,8
August	4,9	9,1	2,4	3,2
September	5,8	9,6	2,3	4,1
October	3,2	8,2	2,6	4,9
November	3,7	8.70	3,8	4,4
December	3,8	7,9	2,1	4,7

Table 24 Analyzed				
Salinity2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	70,59			
R squared	0,828			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	10,36			
P value	0,0157			
P value summary	*			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	166,5	3	55,51	
Residual (within columns)	34,6	44	0,7863	
Total	201,1	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-3,575	9,875	P < 0.01	-4.456 to -2.694
North Khobar vs Anfoushy	1,433	3,959	P < 0.01	0.5522 to 2.314
North Khobar vs Abu Qir	0,1583	0,4374	P > 0.05	-0.7228 to 1.039

g) Chlorides

The Chlorides values of marine water are given in (Tables 25 & 28 ; Histograms 13& 14) for pan marine habitats in different seasons of the year. Low Chlorides in water during 2009 was found in Abu Qir during the months of August - November (630-880 mg/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and South Khobar -1269 at P > 0.01 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -1379 at P > 0.05.

The highest value of Chlorides was observed during almost all months in South Khobar (2350- 2680 mg/l) in 2012 and (2310 – 2660 mg/l) during 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 16,25 at P > 0.05 during 2009 & 2012 and 21,75 at P < 0.05 during 2010 & 2013.

Tables 25 & 26 : Seasonal variation of Chlorides (mg/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	1390	2340	1060	1090
February	1370	2350	1180	1460
March	890	1758	1300	1510
April	1210	2250	1050	990
May	1350	2340	1057	1070
June	1360	2570	1590	1450
July	1780	2680	1460	1870
August	890	2030	1070	880
September	989	2860	780	1090
October	676	2740	890	660
November	857	2780.0	630	840
December	1060	2350	1560	1160

Table 26 Analyzed				
Chlorides1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	47,24			
R squared	0,7631			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	0,1926			
P value	0,9788			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	14460000	3	4821000	
Residual (within columns)	4491000	44	102100	
Total	18960000	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-1269	9,728	P < 0.01	-1586 to -951.4
North Khobar vs Anfoushy	16,25	0,1246	P > 0.05	-301.2 to 333.7
North Khobar vs Abu Qir	-20,67	0,1585	P > 0.05	-338.1 to 296.8

Tables 27 & 28 : Seasonal variation of Chlorides (mg/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	1320	2340	1020	1090
February	1310	2310	1180	1470
March	830	2770	1300	1550
April	1250	2285	1020	999
May	1370	2355	1040	1040
June	1320	2576	1580	1450
July	1710	2660	1430	1820
August	896	2020	1060	893
September	910	2870	770	1048
October	645	2786	840	650
November	880	2715.0	670	860
December	1010	2310	1280	1140

Table 28 Analyzed				
Chlorides 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	64,92			
R squared	0,8157			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	0,8398			
P value	0,8399			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	16940000	3	5645000	
Residual (within columns)	3826000	44	86960	
Total	20760000	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-1379	11,45	P < 0.01	-1672 to -1086
North Khobar vs Anfoushy	21,75	0,1807	P > 0.05	-271.3 to 314.8
North Khobar vs Abu Qir	-46,58	0,3869	P > 0.05	-339.6 to 246.4

h) Total Nitrogen

The Total Nitrogen values of marine water are given in (Tables 29 & 32 ; Histograms 15& 16) for pan marine habitats in different seasons of the year. Low Total Nitrogen in water during 2009 was found in Anfoushy during almost all months (1,50-1,8 mg/l). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and South Khobar -2,1 at P > 0.01 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -2,617 at P > 0.01.

The highest value of Total Nitrogen was observed during almost all months in South Khobar (3,9- 5,4 mg/l) in 2012 and (4,4 – 5,7 mg/l) during 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs Anfoushy 0,65 at P

> 0.05 during 2009 & 2012 and 0,8833 at P < 0.01 during 2010 & 2013.

Tables 29 & 30 : Seasonal variation of total Nitrogen (mg/l) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	3,7	4,2	1,7	2,8
February	1,4	4,5	1,5	1,9
March	2,7	4,3	1,6	2,7
April	1,8	4,2	1,7	2,5
May	2,6	3,7	1,6	1,6
June	2,5	3,9	1,5	3,7
July	2,7	4,6	1,8	3,4
August	1,4	5,4	1,5	1,5
September	1,3	4,8	1,8	1,7
October	2,6	4,2	1,5	2,8
November	2,4	4.50	1,8	2,9
December	2,3	4,3	1,6	2,8

Table 30 Analyzed				
total Nitrogen 1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	55,83			
R squared	0,792			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	25,7			
P value	P<0.0001			
P value summary	***			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	50,11	3	16,7	
Residual (within columns)	13,16	44	0,2991	
Total	63,27	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-2,1	9,405	P < 0.01	-2.643 to -1.557
North Khobar vs Anfoushy	0,65	2,911	P < 0.05	0.1065 to 1.193
North Khobar vs Abu Qir	-0,2417	1,082	P > 0.05	-0.7852 to 0.3018

Tables 31& 32 : Seasonal variation of total Nitrogen (mg/l) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	3,9	5,6	1,4	3,6
February	2,8	4,7	1,5	3,7

March	3,7	5,6	1,6	3,9
April	3,8	5,5	1,4	3,7
May	2,5	4,7	1,8	2,8
June	1,4	4,8	1,9	2,4
July	1,6	5,4	1,6	2,6
August	1,4	5,3	1,4	1,8
September	1,3	5,2	2,2	2,9
October	2,3	4,4	1,4	2,8
November	3,1	4.60	1,6	2,4
December	2,3	5,7	1,7	2,8

Table 32 Analyzed				
total Nitrogen 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	65,56			
R squared	0,8172			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	18,06			
P value	0,0004			
P value summary	***			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	79,68	3	26,56	
Residual (within columns)	17,82	44	0,4051	
Total	97,5	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-2,617	10,07	P < 0.01	-3.249 to -1.984
North Khobar vs Anfoushy	0,8833	3,4	P < 0.01	0.2509 to 1.516
North Khobar vs Abu Qir	-0,4417	1,7	P > 0.05	-1.074 to 0.1908

i) The Tributyl tin (TBT)

The Tributyl tin TBT values of marine water are given in (Tables 33& 36 ; Histograms 17& 18) for pan marine habitats in different seasons of the year. Low Tributyl tin TBT in water during 2009 was found in North Khobar during almost March, April, June, October-November (290-380 µgg-1). Dunnett's Multiple Comparison Test showed the loSouthern Mean Dif between North Khobar and Abu Qir -191,3 at P > 0.01 during 2012. During 2013 the loSouthern Mean Dif between North Khobar and South Khobar -223,3 at P > 0.01.

The highest value of Tributyl tin TBT was observed during almost all months in Abu Qir (500-680 µgg-1) in 2012 and (550 – 670 mg/l) during 2013. Dunnett's Multiple Comparison Test showed the highest Mean Dif between North Khobar vs South Khobar -87,5 at P > 0.01 during 2012 and -101,7 at P < 0.01 during 2013.

Tables 33 & 34 : Seasonal variation of Tributyl tin TBT (µgg-1) in the four marine localities during January to December 2009 Egypt and during January to December Saudi Arabia 2012

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	430	520	510	610
February	390	490	500	520
March	320	440	490	490
April	380	505	520	570
May	410	480	490	680
June	380	490	520	500
July	410	430	450	530
August	405	550	590	650
September	380	410	480	510
October	320	430	450	530
November	290	380	400	680
December	460	500	510	600

Table 34 Analyzed				
Tributyl tin 1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	24,77			
R squared	0,6281			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	2,334			
P value	0,506			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	223000	3	74340	
Residual (within columns)	132100	44	3001	
Total	355100	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-87,5	3,912	P < 0.01	-141.9 to -33.06
North Khobar vs Anfoushy	-111,3	4,974	P < 0.01	-165.7 to -56.81
North Khobar vs Abu Qir	-191,3	8,551	P < 0.01	-245.7 to -136.8

Tables 35 & 36 : Seasonal variation of Tributyl tin TBT (µgg-1) in the four marine localities during January to December 2010 Egypt and during January to December Saudi Arabia 2013

Months	North Khobar	South Khobar	Anfoushy	Abu Qir
January	410	560	530	660
February	340	480	510	500
March	370	450	500	470

April	330	520	510	590
May	460	470	500	660
June	340	480	530	590
July	420	460	470	580
August	450	560	520	670
September	330	420	450	550
October	310	420	440	590
November	300	390	410	670
December	440	510	550	650

Table 36 Analyzed				
Tributyl tin 2				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	32,08			
R squared	0,6863			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	2,288			
P value	0,5148			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	301000	3	100300	
Residual (within columns)	137600	44	3127	
Total	438600	47		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
North Khobar vs South Khobar	-101,7	4,453	P < 0.01	-157.2 to -46.10
North Khobar vs Anfoushy	-118,3	5,183	P < 0.01	-173.9 to -62.76
North Khobar vs Abu Qir	-223,3	9,782	P < 0.01	-278.9 to -167.8

Qualitative analysis of zooplankton in Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt &Northern and Southern estruarine water of the Arabian Gulf – Saudi Arabia.

In a previous study, the second author of this work (Saad, 2015) collected zooplankton from four marine ecosystems namely the North Sea (Helgoland - Germany), Banyuls-sur-Mer (Mediterranean sea - France), Abu Qir Bay (Mediterranean Sea - Egypt) and the northern estruarine harbour of the Arabian Gulf (Saudi Arabia). Collection tools used involved primarily the filtration of water by net, collecting the water in bottles / water samplers or by pumps. Collected zooplaktons were prepared for both macroscopic or / and scanning electron microscopy.All zooplankton were stained with Evans stain or Nile blue or Borax carmine to observe their internal structures since they are mostly transparent. Others were dissected with micro-needles and incised to ease their identification. Marine Species Identification Portal has been applied:

http://species-identification.org/index.php//.Six species of Bryozoa were identified namely *Bugula neritina* (Linnaeus, 1758) and its barrel shaped larva, *Electra crustulenta* (Pallas, 1766) , *Bowerbankia gracilis* (Leidy, 1855) and its coronated larva, *Hippaliosina depressa* (Busk, 1854), *Nolella dilatata* (Marcus, 1940) and *Reptadeonella violacea* (Johnston, 1847). Two hydrozoan cnidarians were identified namely *Obelia geniculata* (Linnaeus, 1758) and *Pennaria disticha* (Goldfuss,1820). Planula larva of Hydrozoa and the

anthozoan *Actinodendron* sp. were collected from the Mediterranean sea. Two rotifers were identified namely *Paraseison annulatus* (Claus, 1876) and *Seison nebaliae* (Grube, 1861). The nematode *Anisakis simplex* and its third stage larva were extracted from the branchial chambers of ascidians whereas free nematode toothless larval stage has been collected from nekton.

Four polychaetes were identified namely *Harmothoe* sp.,(scale worm), *Pomatocerous triqueter* (Linnaeus, 1758), *Nemidia lawrencii* (McIntosh, 1874) with synoneme *Nemidia torelli* and *Notomastus latericeus* (Sars, 1851). The copepod *Megacyclops viridis* (Jurine, 1820) and the gammarid *Gammaropsis* sp. with Naupli, zoaea and megalopods were found in the nekton. The the isopod *Caecocassidias patagonica* (Kussakin, 1967) has been collected from the benthos. The scaphopod *Dentalium vulgare* (da Costa, 1778) and the bivalve *Microgloma turnerae* (Sanders & Allen,1973) were found in the benthos. Veliger and glochidia larvae were collected from the nekton. Two species of brittle star namely *Amphiura* sp and *Ophiomastix annulosa* were collected from the benthos. Nine ascidian larvae were identified namely larvae of *Styela plicata* (Lesuaer, 1823), *Phallusia mammilata* (Cüvier 1815), *Corella parallelogramma* (Müller,1776), *Diplosoma migrans* (Menker und Ax. 1970), *Halocynthia roretzi* (Drasche), *Microcosmus claudicans* (Savigny,1816), *Molgula manhattensis*(Dekay, 1843), *Ascidiella aspersa*(Müller, 1776), and *Cnemidocarpa mollis* (Stimpson,1852).The abundance and distribution of all plankton studied varied considerably according to seasons and habitats.The findings of this work, the density of each genus or / and species in Anfoushy and Abu Qir Bay(Mediterranean Sea – Egypt) and the Northern and Southern Khobar estruarine beaches of the Arabian Gulf (Saudi Arabia), and the presence or absence of a certain zooplankton in the different seasons of the year (faunal composition) will be statistically analyzed in this study. This study also tried to analyze the abiotic factors of the four marine habitats studied and correlated them to zooplankton faunal compositions.

The present study provides information about the seasonal abundance of major groups of zooplankton namely, Bryozoa,Cnidaria, Rotifera, Nematoda, Annelida, Amphipoda, Copepoda, Isopoda, crustacean larvae, Scaphopoda, Bivalvia, molluscan larvae, andascidian larvae. The relative densities of different genera have been commented.

Table 37 & 38 showed monthly densities distribution of zooplankton (per sixty litre samples) collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 and 2010 &Northern and Southern estruarine water of the Arabian Gulf – Saudi Arabia during 2012 and 2013. To survive in a habitat successfully, zooplankton have to had an opportunity in the past to be dispersed into the area; it must successfully compete within already existing communities; and survive in or adapt to changing physical and chemical condition.

j) Bryozoa

During 2009, *Bugula neritina* and *Nolella dilatata* were not found during winter and spring in Anfoushy,sparse in summer season and fall , not found in Abu Qirduring winter, highly abundantin summer season in Abu Qir,totally not found in Northern and Southern Khobar. *Electra crustulenta* was many during winter and spring in Anfoushy, maximal density was found in Abu Qir. Density of *Bowerbankia gracilis* was highly intensively dense in Anfoushy. Equal densities of *Hippaliosina depressa* were found in Anfoushy and Abu Qir. *Reptadeonella violacea* dominated Anfoushy in summer season. The general densities of Bryozoa declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38). These sea mats generally were collected during all seasons of the year from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010.Sparse or many zooecia were collected from Northern Khobar. This study did not found any sea mats in SouthernKhobar. Sea mats were highly abundant and dense in summer season andAbu Qir Bay, Anfoushy contained the maximal density of sea mats. Newman-Keuls Multiple Comparison Test showed considerable difference in faunal composition of sea mats in Anfoushy vs Abu Qir, Mean Diff. -3,75 at $P > 0.05$, (see tables 39-40 & Histograms 19-20).

Table 39 : Clarifying Bryozoa Densities in the Four Study Marine Localities During 2009 & 2012

Table Analyzed Table 39			
Bryozoa density 1 during 2009 & 2012			
One-way analysis of variance			
P value	0,0001		
P value summary	***		
Are means signif. different? ($P < 0.05$)	Yes		
Number of groups	4		

F	11,69		
R squared	0,6369		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)			
P value			
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	1122	3	374
Residual (within columns)	639,6	20	31,98
Total	1761	23	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
South Khobar vs Abu Qir	-16,25	7,039	P < 0.001
South Khobar vs Anfoushy	-12,5	5,414	P < 0.01
South Khobar vs Northern Khobar	-2,083	0,9024	P > 0.05
Northern Khobar vs Abu Qir	-14,17	6,136	P < 0.001
Northern Khobar vs Anfoushy	-10,42	4,512	P < 0.01
Anfoushy vs Abu Qir	-3,75	1,624	P > 0.05

Table 40 : clarifying Bryozoa densities in the four study marine localities during 2010 & 2013

Table Analyzed Table 40			
Bryozoa density 2 during 2010 & 2013			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	16,83		
R squared	0,7162		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)			
P value			
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	639,6	3	213,2
Residual (within columns)	253,4	20	12,67
Total	893	23	
Newman-Keuls Multiple Comparison Test	Mean	q	P value

	Diff.		
Southern Khobar vs Abu Qir	-12,5	8,602	P < 0.001
Southern Khobar vs Anfoushy	-9,167	6,308	P < 0.001
Southern Khobar vs Northern Khobar	-1,708	1,176	P > 0.05
Northern Khobar vs Abu Qir	-10,79	7,427	P < 0.001
Northern Khobar vs Anfoushy	-7,458	5,133	P < 0.01
Anfoushy vs Abu Qir	-3,333	2,294	P > 0.05

k) Cnidaria

During 2009 and 2012, in summer seasonequal distribution densities of *Obelia geniculata*in Anfoushy and Abu Qir, many polyps were collected from Northern Khobar and sparse from Southern Khobar.*Pennaria disticha* and *Actinodendron* sp.were highly dense in Anfoushy and dense in Abu Qir, many in Northern Khobar and sparse in SouthernKhobar. Cnidarian polyps have been collected from the four marine localities studied. Planula larvae were collected only from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010.The general densities of Cnidaria declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38). The minimal distribution of polyps were found in Arabian Gulf. Maximal density of polyps were collected during the summer season from Anfoushy. Cnidarian density in Abu Qir Bay was highly dense. Newman-Keuls Multiple Comparison Test showed Mean Diff. -3,333 at P > 0.05 between Abu Qir vs Anfoushy during 2009 and -5,833 at P < 0.01 during 2010. There were signif. means different at P < 0.05 between Mediterranean Sea and Arabian Gulf faunal composition of Cnidaria (see tables 41-42 and Histograms 21-22)

Table 41 : Clarifying Cnidaria Densities in the Four Study Marine Localities during 2009 & 2012

Table Analyzed Table 41			
Cnidaria density 1 during 2009 & 2012			
One-way analysis of variance			
P value	0,0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	27,94		
R squared	0,9129		
ANOVA Table	SS	df	MS
Treatment (between columns)	916,7	3	305,6
Residual (within columns)	87,5	8	10,94
Total	1004	11	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Anfoushy	-21,67	11,35	P < 0.001
Southern Khobar vs Abu Qir	-18,33	9,602	P < 0.001
Southern Khobar vs Northern Khobar	-6,667	3,491	P < 0.05
Northern Khobar vs Anfoushy	-15	7,856	P < 0.01
Northern Khobar vs Abu Qir	-11,67	6,11	P < 0.01
Abu Qir vs Anfoushy	-3,333	1,746	P > 0.05

Table 42 : Clarifying Cnidaria Densities in the Four Study Marine Localities during 2010 & 2013

Table Analyzed Table 42			
Cnidaria density 2 during 2010 & 2013			

One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	49,94		
R squared	0,9493		
ANOVA Table	SS	df	MS
Treatment (between columns)	468,2	3	156,1
Residual (within columns)	25	8	3,125
Total	493,2	11	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Anfoushy	-16,67	16,33	P < 0.001
Southern Khobar vs Abu Qir	-10,83	10,61	P < 0.001
Southern Khobar vs Northern Khobar	-5	4,899	P < 0.01
Northern Khobar vs Anfoushy	-11,67	11,43	P < 0.001
Northern Khobar vs Abu Qir	-5,833	5,715	P < 0.01
Abu Qir vs Anfoushy	-5,833	5,715	P < 0.01

l) Rotifera

During 2009& 2012, *Paraseison annulatus* and *Seison nebaliae*dominated Anfoushy in summer season and were highly dense in Abu Qir, sparse in Northern Khobar and not found in Southern Khobar.The general densities of rotifers declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38).Rotifers were collected mainly from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010. Maximal density of rotifers were found in Anfoushy in summer season 2009 & 2010 while rotifers were highly abundant in Abu Qir Bay in summer season 2009 and many were found in summer season 2010. Sparse amount were collected from Northern Khobar only in summer season of 2012 & 2013. This study did not found any rotifers in Southern Khobar.The minimal distribution of rotifers were found in Northern Khobar of the Arabian Gulf. Maximal density of Rotifers were collected during the summer season from Abu Qir compared with Anfoushy. Newman-Keuls Multiple Comparison Test showed Mean Diff. -3,333 at P > 0.05 between Anfoushy vs Abu Qir during 2009 and -1,25 at P > 0.05 during 2010. There were signif. means different at P < 0.05 between Northern Khobar vs Abu Qir faunal composition of rotifers (see tables 43-44 and Histograms 23-24).

Table 43 : Clarifying Rotifera Densities in the Four Study Marine Localities during 2009 & 2012

Table Analyzed Table 43			
Rotifer density 1 during 2009 & 2012			
One-way analysis of variance			
P value	0,0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	11,69		
R squared	0,6369		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)			

P value			
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	1122	3	374
Residual (within columns)	639,6	20	31,98
Total	1761	23	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Abu Qir	-16,25	7,039	P < 0.001
Southern Khobar vs Anfoushy	-12,5	5,414	P < 0.01
Southern Khobar vs Northern Khobar	-2,083	0,9024	P > 0.05
Northern Khobar vs Abu Qir	-14,17	6,136	P < 0.001
Northern Khobar vs Anfoushy	-10,42	4,512	P < 0.01
Anfoushy vs Abu Qir	-3,75	1,624	P > 0.05

Table 44 : Clarifying Rotifera Densities in the Four Study Marine Localities During 2010 & 2013

Table Analyzed Table 44			
Rotifera density 2 during 2010 & 2013			
One-way analysis of variance			
P value	0,0005		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	78,33		
R squared	0,9833		
ANOVA Table	SS	df	MS
Treatment (between columns)	183,6	3	61,2
Residual (within columns)	3,125	4	0,7813
Total	186,7	7	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Abu Qir	-11,25	18	P < 0.001
Southern Khobar vs Anfoushy	-10	16	P < 0.001
Southern Khobar vs Northern Khobar	-2,5	4	P < 0.05
Northern Khobar vs Abu Qir	-8,75	14	P < 0.01
Northern Khobar vs Anfoushy	-7,5	12	P < 0.01
Anfoushy vs Abu Qir	-1,25	2	P > 0.05

m) Nematoda

During 2009 & 2012, in summer season*Anisakis simplex*was highly abundant in Anfoushy, and Abu Qir, many inNorthern Khobar and Southern Khobar.

Nematoda were collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern Khobar andSouthern Khobar during 2012 & 2013. The general densities of nematodsfluctuated in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38).Nematodes were generally highly abundant in Abu Qir Bay and Anfoushy in summer season 2009 & 2010 while many nematodes

were collected from Northern Khobar and Southern Khobar in summer season of 2012 & 2013. Newman-Keuls Multiple Comparison Test showed Mean Diff. -5 at P > 0.05 between Anfoushy vs Abu Qir during 2009 and -2,5 at P > 0.05 during 2010. There were signif. means different at P < 0.05 between Southern Khobar vs Abu Qir faunal composition of nematodes (see tables 45-46 and Histograms 25-26).

Table 45 : Clarifying Nematoda Densities in the Four Study Marine Localities During 2009 & 2012

Table Analyzed Table 45			
Nematoda density 1during 2009 & 2012			
One-way analysis of variance			
P value	0,0017		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	13,4		
R squared	0,834		
ANOVA Table	SS	df	MS
Treatment (between columns)	314,1	3	104,7
Residual (within columns)	62,5	8	7,813
Total	376,6	11	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Anfoushy	-12,5	7,746	P < 0.01
Southern Khobar vs Abu Qir	-7,5	4,648	P < 0.05
Southern Khobar vs Northern Khobar	-0,8333	0,5164	P > 0.05
Northern Khobar vs Anfoushy	-11,67	7,23	P < 0.01
Northern Khobar vs Abu Qir	-6,667	4,131	P < 0.05
Abu Qir vs Anfoushy	-5	3,098	P > 0.05

Table 46 : Clarifying Nematoda Densities in the Four Study Marine Localities During 2010 & 2013

Table Analyzed Table 46			
Nematoda density 2 during 2010 & 2013			
One-way analysis of variance			
P value	0,2421		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	4		
F	1,708		
R squared	0,3905		
ANOVA Table	SS	df	MS
Treatment (between columns)	64,06	3	21,35
Residual (within columns)	100	8	12,5
Total	164,1	11	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value

Northern Khobar vs Anfoushy	-5	2,449	P > 0.05
Northern Khobar vs Abu Qir	-4,167	---	P > 0.05
Northern Khobar vs Southern Khobar	0	---	P > 0.05
Southern Khobar vs Anfoushy	-5	---	P > 0.05
Southern Khobar vs Abu Qir	-4,167	---	P > 0.05
Abu Qir vs Anfoushy	-0,8333	---	P > 0.05

n) Annelida

During 2009 & 2012, *Harmothoe* sp. , *Pomatocerous triqueter*, *Nemidia lawrencii* and *Notomastus latericeus* were highly intensive dense in Anfoushy, dense in Abu Qir and Southern Khobar , many inNorthern Khobar. The general densities of annelids declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38). Generally, annelida were collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern Khobar andSouthern Khobar during 2012 & 2013. Annelida were highly abundant in Abu Qir Bay in summer season and the maximal density was found in Anfoushy in summer season 2009 & 2010 while many annelids were collected from Northern Khobar and Southern Khobar in summer season of 2012 & 2013. Newman-Keuls Multiple Comparison Test showed Mean Diff. -2,5 at P > 0.05 between Anfoushy vs Abu Qir during 2009 and -2,5 at P > 0.05 during 2010. There were signif. means different at P < 0.05 between Southern Khobar vs Abu Qir faunal composition of annelids (see tables 47-48 and Histograms 27-28).

Table 47 : Clarifying Annelida Densities in the Four Study Marine Localities During 2009 & 2012

Table Analyzed Table 47			
Annelida density 1 during 2009 & 2012			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	32,41		
R squared	0,8902		
ANOVA Table	SS	df	MS
Treatment (between columns)	1469	3	489,6
Residual (within columns)	181,3	12	15,1
Total	1650	15	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Northern Khobar vs Abu Qir	-22,5	11,58	P < 0.001
Northern Khobar vs Anfoushy	-20	10,29	P < 0.001
Northern Khobar vs Southern Khobar	-5	2,573	P > 0.05
Southern Khobar vs Abu Qir	-17,5	9,006	P < 0.001
Southern Khobar vs Anfoushy	-15	7,719	P < 0.001
Anfoushy vs Abu Qir	-2,5	1,287	P > 0.05

Table 48 : Clarifying Annelida Densities in the Four Study Marine Localities During 2010 & 2013

Table Analyzed Table 48			
Annelida density 2 during 2010 & 2013			
One-way analysis of variance			
P value	0,0002		

P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	15,05		
R squared	0,79		
ANOVA Table	SS	df	MS
Treatment (between columns)	476,2	3	158,7
Residual (within columns)	126,6	12	10,55
Total	602,7	15	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Northern Khobar vs Abu Qir	-13,75	8,468	P < 0.001
Northern Khobar vs Anfoushy	-11,25	6,928	P < 0.001
Northern Khobar vs Southern Khobar	-4,375	2,694	P > 0.05
Southern Khobar vs Abu Qir	-9,375	5,774	P < 0.01
Southern Khobar vs Anfoushy	-6,875	4,234	P < 0.05
Anfoushy vs Abu Qir	-2,5	1,54	P > 0.05

o) Crustacea

During 2009 & 2012, in summer season amphipods *Gammaropsis* sp.&*Monocorophium acherisicum* ; copepods *Megacyclops viridis*; the isopodCaecocassidias patagonica and crustacean larvae naupluis, zoaea and megalopod were intensively dense in Anfoushy and dense in Abu Qir, highly abundant in Northern Khobar and sparse in Southern Khobar.The general densities of Crustacea declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38).Generally, Crustacea were collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern Khobar andSouthern Khobar during 2012 & 2013. Crustacea were intensive dense in Abu Qir Bay andhighly intensive abundant in Anfoushy in summer season 2009 & 2010 while highly abundant Crustacea were collected from Northern Khobar and sparse in Southern Khobar in summer season of 2012 & 2013. Newman-Keuls Multiple Comparison Test showed Mean Diff-0,9091at P > 0.05 between Abu Qir vs Anfoushy during 2009 and -1,042 at P > 0.05 during 2010. There were signif. means different at P < 0.05 between Southern Khobar vs Anfoushy faunal composition of Crustacea (see tables 49-50 and Histograms 29-30).

Table 49 : Clarifying Crustacea Densities in the Four Study Marine Localities During 2009 & 2012

Table Analyzed Table 49			
Crustacea density 1 during 2009 & 2012			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	47,21		
R squared	0,7798		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	5,093		
P value	0,1651		
P value summary	ns		

Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	2217	3	739
Residual (within columns)	626,1	40	15,65
Total	2843	43	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Anfoushy	-15,68	13,15	P < 0.001
Southern Khobar vs Abu Qir	-14,77	12,38	P < 0.001
Southern Khobar vs Northern Khobar	-2,273	1,905	P > 0.05
Northern Khobar vs Anfoushy	-13,41	11,24	P < 0.001
Northern Khobar vs Abu Qir	-12,5	10,48	P < 0.001
Abu Qir vs Anfoushy	-0,9091	0,7621	P > 0.05

Table 50 : Clarifying Crustacea Densities in the Four Study Marine Localities During 2010 & 2013

Table Analyzed Table 50			
Crustacea density 2 during 2010 & 2013			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	24		
R squared	0,6207		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	1,583		
P value	0,6633		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	826,8	3	275,6
Residual (within columns)	505,2	44	11,48
Total	1332	47	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Anfoushy	-9,375	9,585	P < 0.001
Southern Khobar vs Abu Qir	-8,333	8,52	P < 0.001
Southern Khobar vs Northern Khobar	-1,271	1,299	P > 0.05
Northern Khobar vs Anfoushy	-8,104	8,285	P < 0.001
Northern Khobar vs Abu Qir	-7,063	7,22	P < 0.001
Abu Qir vs Anfoushy	-1,042	1,065	P > 0.05

p) Mollusca

During 2009 & 2012, in summer seasonthe bivalve *Microgloma tumidula* and molluscan larvae trochophore, veliger and glochidia were intensively dense in Northern Khobar, dense in Southern Khobar and Anfoushy and highly abundant in Abu Qir. The scaphopod*Dentalium vulgare* wasintensively densein Northern Khobar and dense in Southern Khobar ; totally absent in Abu Qir Bay and Anfoushy. Generally, Mollusca were collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern Khobar andSouthern Khobar during 2012 & 2013. Bivalvia were dense in Abu Qir Bay andhighly abundant in Anfoushy in summer season 2009 & 2010 while intensive dense Scaphopoda and Bivalvia were collected from Northern Khobar and highly abundant in Southern Khobar in summer season of 2012 & 2013. The general densities of Mollusca declined in 2010 in both Anfoushy and Abu Qir (see Tables 37 & 38).Newman-Keuls Multiple Comparison Test showed Mean Diff-5 at $P > 0.05$ between Southern Khobar vs Northern Khobar during 2012 and -3,5 at $P > 0.05$ during 2010. There were signif. means different at $P < 0.05$ between Anfoushy vs Northern Khobar faunal composition of Mollusca (see tables 51-52 and Histograms 31-32).

Table 51 : Clarifying Mollusca Densities in the Four Study Marine Localities During 2009 & 2012

Table Analyzed Table 51			
Mollusca density 1 during 2009 & 2012			
One-way analysis of variance			
P value	0,3523		
P value summary	ns		
Are means signif. different? ($P < 0.05$)	No		
Number of groups	4		
F	1,169		
R squared	0,1798		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	2,087		
P value	0,5545		
P value summary	ns		
Do the variances differ signif. ($P < 0.05$)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	236,3	3	78,75
Residual (within columns)	1078	16	67,34
Total	1314	19	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Anfoushy vs Northern Khobar	-9,5	2,589	$P > 0.05$
Anfoushy vs Southern Khobar	-4,5	---	$P > 0.05$
Anfoushy vs Abu Qir	-3	---	$P > 0.05$
Abu Qir vs Northern Khobar	-6,5	---	$P > 0.05$
Abu Qir vs Southern Khobar	-1,5	---	$P > 0.05$
Southern Khobar vs Northern Khobar	-5	---	$P > 0.05$

Table 52 : Clarifying Mollusca Densities in the Four Study Marine Localities During 2010 & 2013

Table Analyzed Table 52			
´Mollusca density 2 during 2010 & 2013			
One-way analysis of variance			

P value	0,4929		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	4		
F	0,8375		
R squared	0,1357		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	1,568		
P value	0,6667		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	156,3	3	52,08
Residual (within columns)	995	16	62,19
Total	1151	19	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Anfoushy vs Northern Khobar	-7	1,985	P > 0.05
Anfoushy vs Southern Khobar	-3,5	---	P > 0.05
Anfoushy vs Abu Qir	-0,5	---	P > 0.05
Abu Qir vs Northern Khobar	-6,5	---	P > 0.05
Abu Qir vs Southern Khobar	-3	---	P > 0.05
Southern Khobar vs Northern Khobar	-3,5	---	P > 0.05

q) Ascidian larvae

Anfoushy contained the maximal density of ascidian larvae in all study periods. Ascidian larvae were collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 & 2010 and Northern Khobar and Southern Khobar during 2012 & 2013. Ascidian larvae were dense in Abu Qir Bay and highly intensive dense in Anfoushy in summer season 2009 & 2010 and Northern Khobar 2012 while many ascidian larvae were collected from Southern Khobar in summer season of 2012 & 2013. Newman-Keuls Multiple Comparison Test showed Mean Diff-2,5 at P > 0.05 between Anfoushy vs Abu Qir during 2009 and -5 at P > 0.05 during 2010. There were signif. means different at P < 0.05 between SouthernKhobar vs Abu Qir faunal composition of ascidian larvae (see tables 53-54 and Histograms 33-34).

Table 53 : Clarifying Ascidian Larvae Densities in the Four Study Marine During 2009 & 2012

Table Analyzed Table 53			
Ascidian larvae density 1 during 2009 & 2012			
One-way analysis of variance			
P value	0,0706		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	4		
F	3,039		
R squared	0,4318		

ANOVA Table	SS	df	MS
Treatment (between columns)	968,8	3	322,9
Residual (within columns)	1275	12	106,3
Total	2244	15	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Abu Qir	-20	3,881	P > 0.05
Southern Khobar vs Anfoushy	-17,5	---	P > 0.05
Southern Khobar vs Northern Khobar	-10	---	P > 0.05
Northern Khobar vs Abu Qir	-10	---	P > 0.05
Northern Khobar vs Anfoushy	-7,5	---	P > 0.05
Anfoushy vs Abu Qir	-2,5	---	P > 0.05

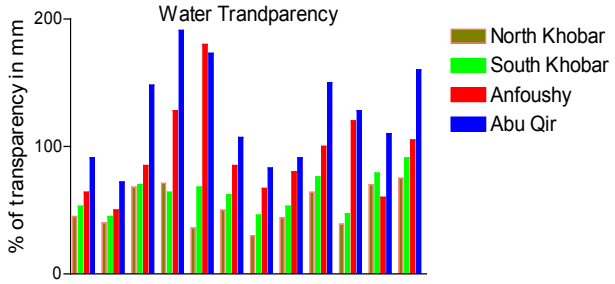
Table 54 : Clarifying Ascidian Larvae Densities in the Four Study Marine During 2010 & 2013

Table Analyzed Table 54			
Ascidian larvae density 2 during 2010 & 2013			
One-way analysis of variance			
P value	0,0946		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	4		
F	2,673		
R squared	0,4005		
ANOVA Table	SS	df	MS
Treatment (between columns)	918,8	3	306,3
Residual (within columns)	1375	12	114,6
Total	2294	15	
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	P value
Southern Khobar vs Abu Qir	-20	3,737	P > 0.05
Southern Khobar vs Anfoushy	-15	---	P > 0.05
Southern Khobar vs Northern Khobar	-7,5	---	P > 0.05
Northern Khobar vs Abu Qir	-12,5	---	P > 0.05
Northern Khobar vs Anfoushy	-7,5	---	P > 0.05
Anfoushy vs Abu Qir	-5	---	P > 0.05

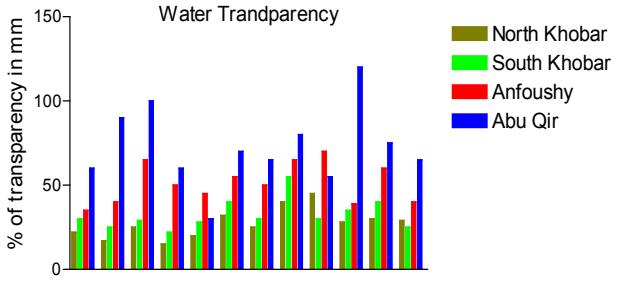
Histograms 1 – 18 clarifying variations in the abiotic factors in the four study marine localities during the different months of the year.

Histograms 19 – 34 clarifyingmonthly density distribution of zooplankton (per sixty litre samples) collected from Anfoushy and Abu Qir Bay (Mediterranean Sea) Egypt during 2009 and 2010 & Northern and Southern Khobar of the Arabian Gulf – Saudi Arabia during 2012 and 2013.

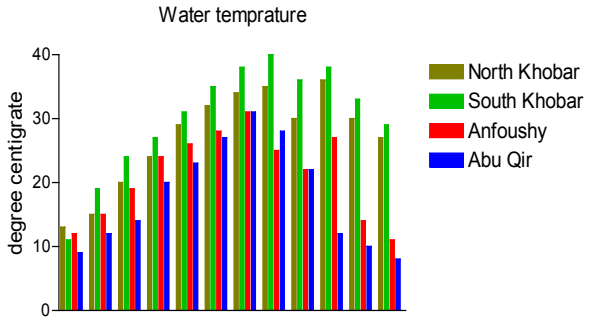
Histogram 1 (Egypt 2009 & Saudi Arabia 2012)



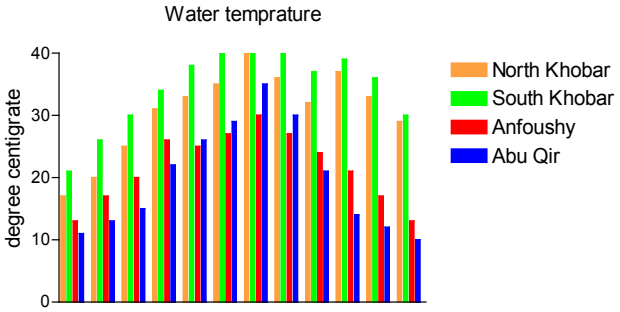
Histogram 2 (Egypt 2010 & Saudi Arabia 2013)



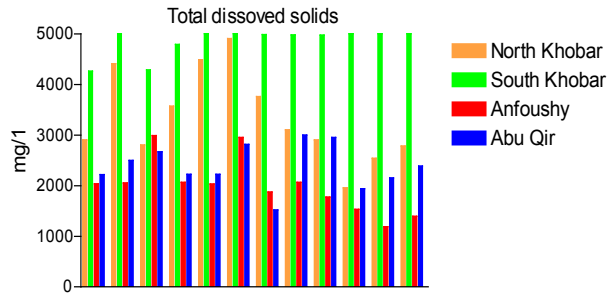
Histogram 3 (Egypt 2009 & Saudi Arabia 2012)



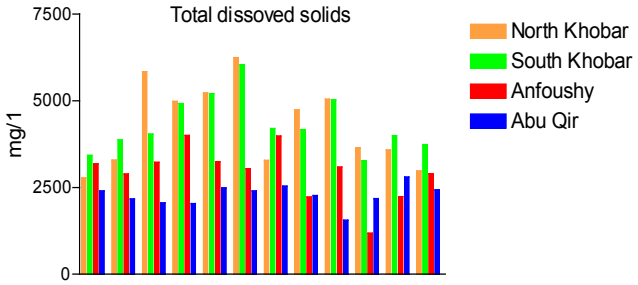
Histogram 4 (Egypt 2010 & Saudi Arabia 2013)



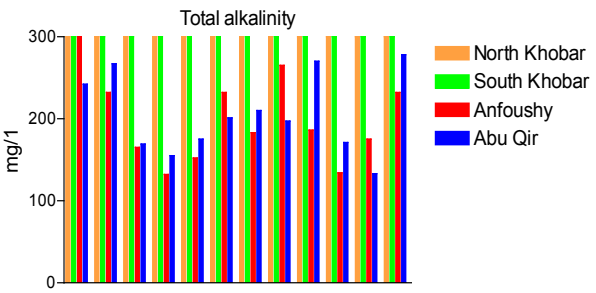
Histogram 5 (Egypt 2009 & Saudi Arabia 2012)



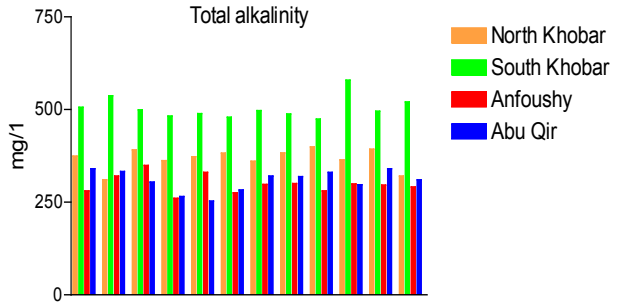
Histogram 6 (Egypt 2010 & Saudi Arabia 2013)



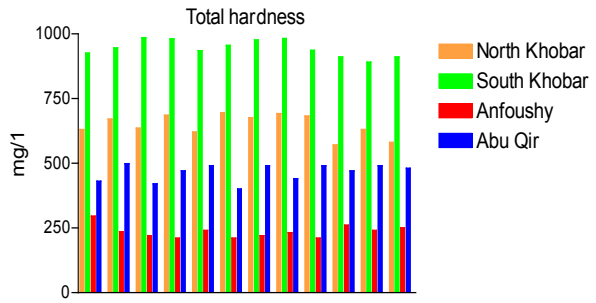
Histogram 7 (Egypt 2009 & Saudi Arabia 2012)



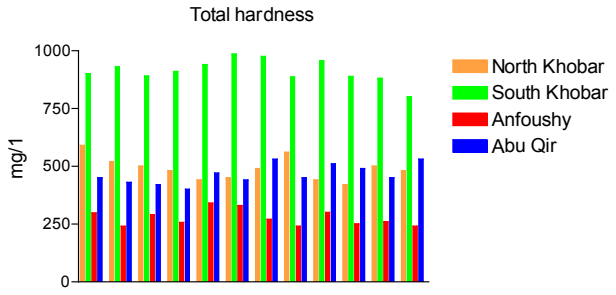
Histogram 8 (Egypt 2010 & Saudi Arabia 2013)



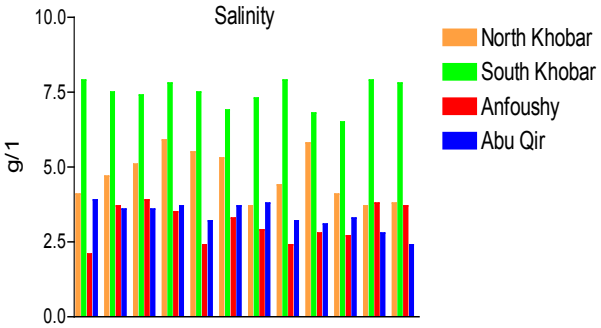
Histogram 9 (Egypt 2009 & Saudi Arabia 2012)



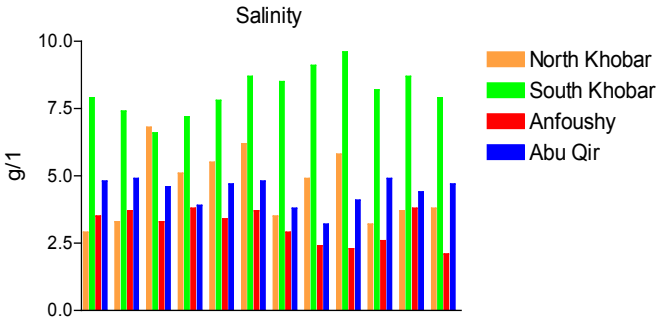
Histogram 10 (Egypt 2010 & Saudi Arabia 2013)



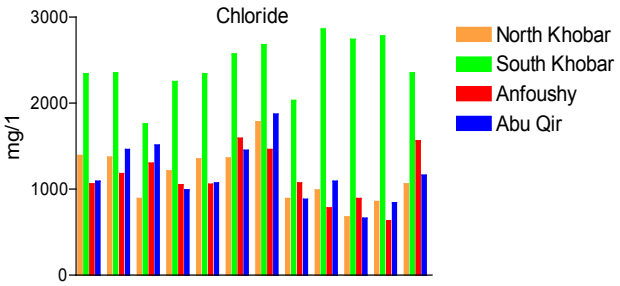
Histogram 11 (Egypt 2009 & Saudi Arabia 2012)



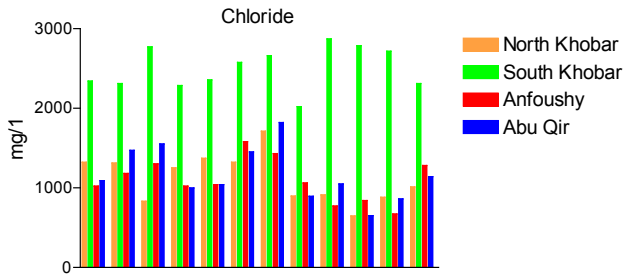
Histogram 12 (Egypt 2010 & Saudi Arabia 2013)



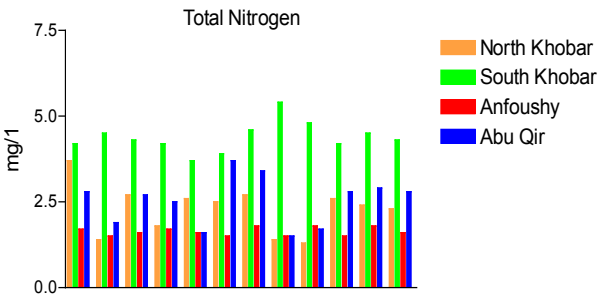
Histogram 13 (Egypt 2009 & Saudi Arabia 2012)



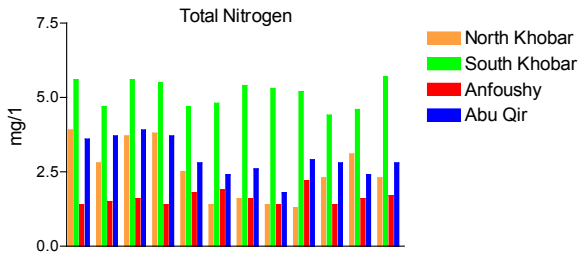
Histogram 14 (Egypt 2010 & Saudi Arabia 2013)



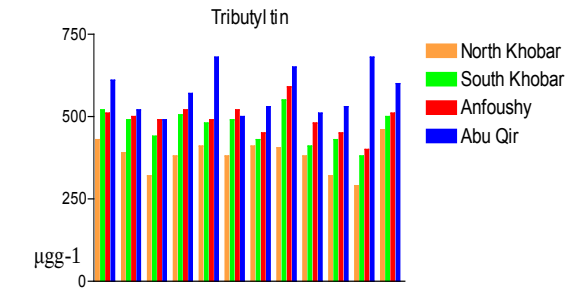
Histogram 15 (Egypt 2009 & Saudi Arabia 2012)



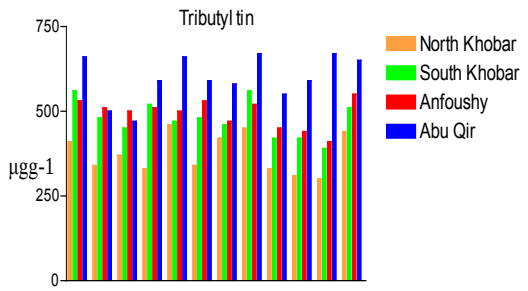
Histogram 16 (Egypt 2010 & Saudi Arabia 2013)



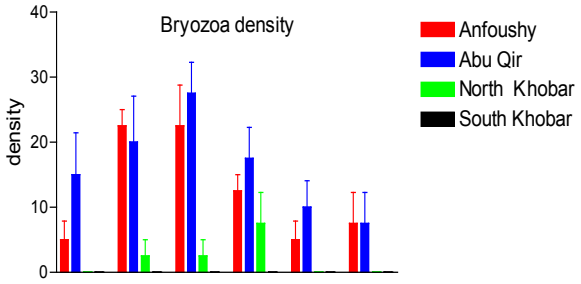
Histogram 17 (Egypt 2009 & Saudi Arabia 2012)



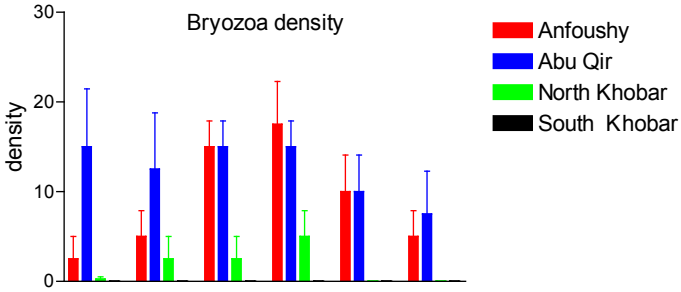
Histogram 18 (Egypt 2010 & Saudi Arabia 2013)



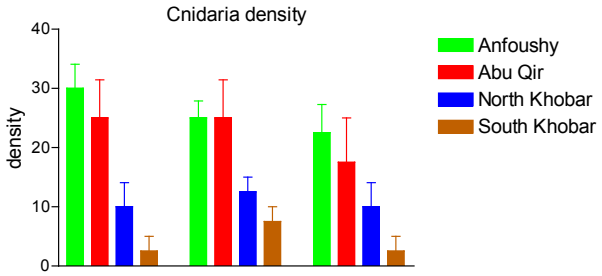
Histogram 19 (Egypt 2009 & Saudi Arabia 2012)



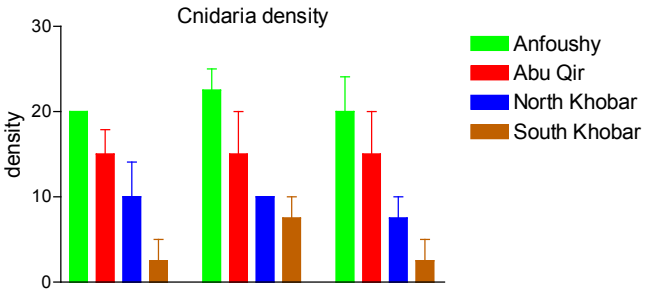
Histogram 20 (Egypt 2010 & Saudi Arabia 2013)



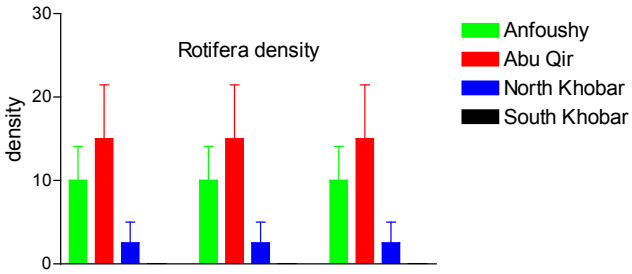
Histogram 21 (Egypt 2009 & Saudi Arabia 2012)



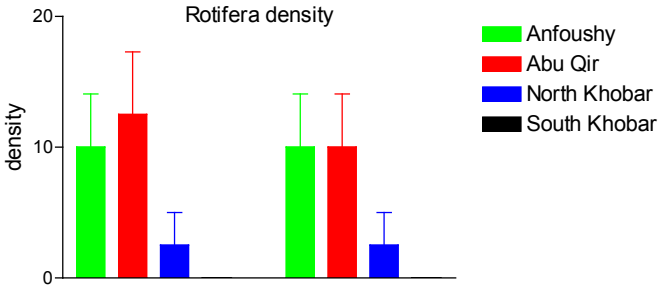
Histogram 22 (Egypt 2010 & Saudi Arabia 2013)



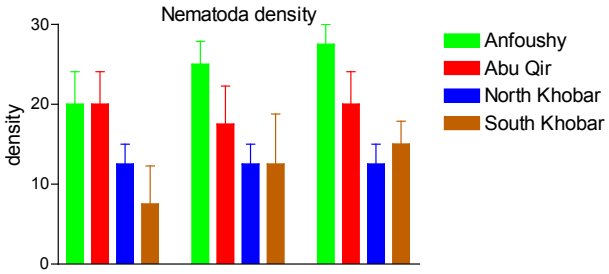
Histogram 23 (Egypt 2009 & Saudi Arabia 2012)



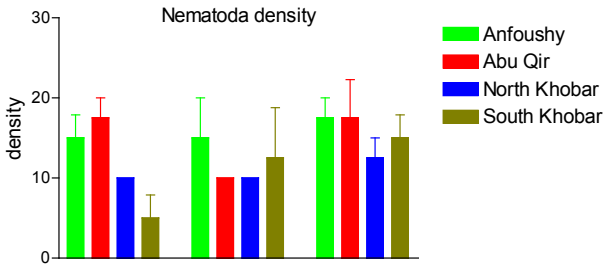
Histogram 24 (Egypt 2010 & Saudi Arabia 2013)



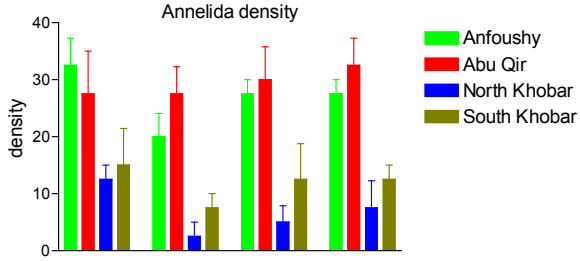
Histogram 25 (Egypt 2009 & Saudi Arabia 2012)



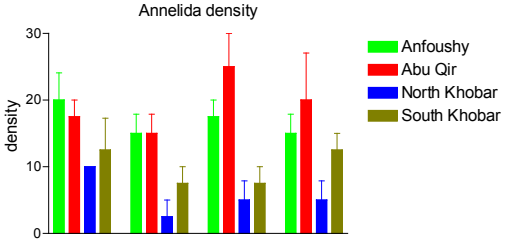
Histogram 26 (Egypt 2010 & Saudi Arabia 2013)



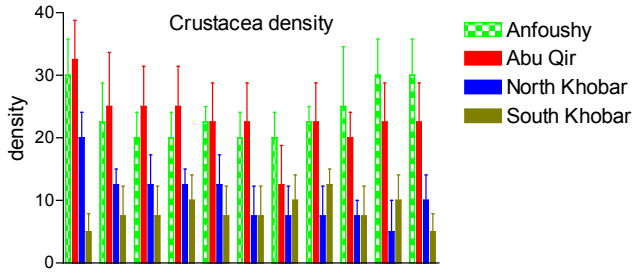
Histogram 27 (Egypt 2009 & Saudi Arabia 2012)



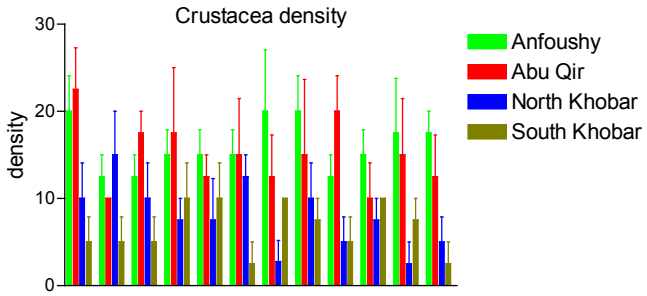
Histogram 28 (Egypt 2010 & Saudi Arabia 2013)



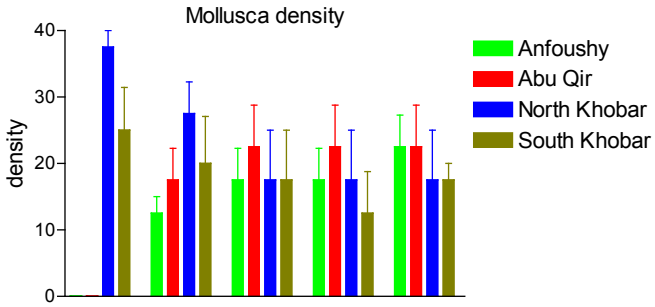
Histogram 29 (Egypt 2009 & Saudi Arabia 2012)



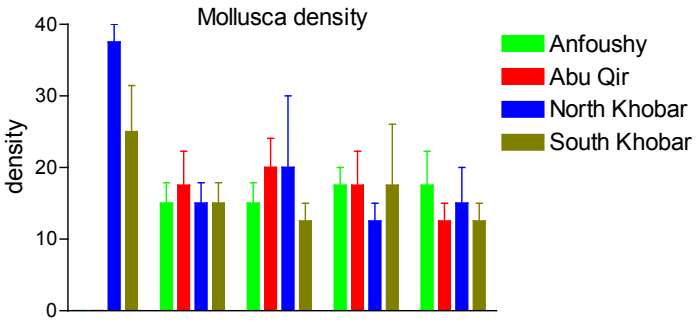
Histogram 30 (Egypt 2010 & Saudi Arabia 2013)



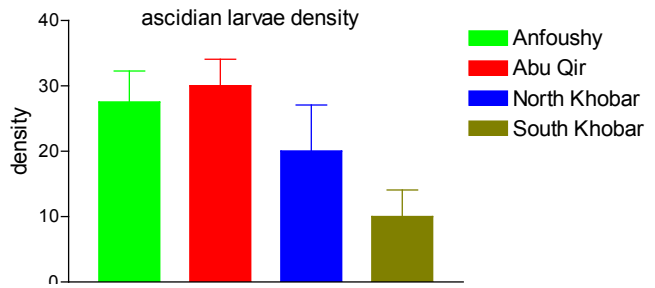
Histogram 31 (Egypt 2009 & Saudi Arabia 2012)



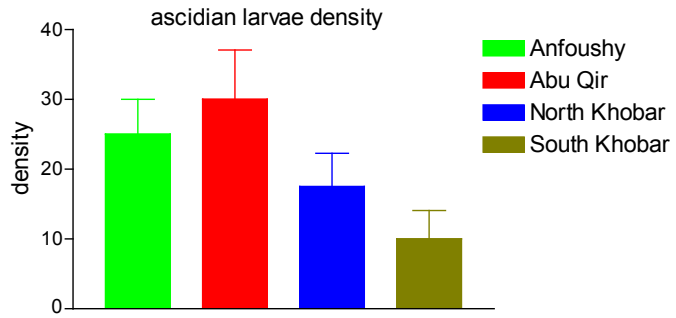
Histogram 32 (Egypt 2010 & Saudi Arabia 2013)



Histogram 33 (Egypt 2009 & Saudi Arabia 2012)



Histogram 34 (Egypt 2010 & Saudi Arabia 2013)



IV. Discussion

The zooplankton composition of the present study are in accordance with other similar studies (Crispim & Watanabe 2000, Simõeset al.2008, Sousaet al.2008). The dominance of rotifers in aquatic ecosystems has been well documented by several authors (Sampaioet al.2002) and is generally attributed to their high fecundity, parthenogenetic reproduction and short developmental rates (Pourriotet al.1997). Furthermore, the less specialized feeding makes rotifers an opportunistic r-strategist group (Allan 1976), which is favoured by the typically unpredictable and seasonal nature of Brazilian semi-arid aquatic systems. Among the rotifers, Brachionidae was the predominant family in terms of species richness and densities in the present study. This family is one of the most important rotifer taxa in tropical waters, with most species being planktonic (Almeida, et al.2006, 2010). Studies on seasonal variations of zooplanktonstanding stocks mainly concern marinewaters and demonstrate the importance of the fluctuationsrelated to seasonal biotic and abioticchanges. In the study zooplankton biomassesin the Meditrranean Sea Egypt are usually found in summer whereas the loSouthernvalues are observed inwinter.

Arabian Gulf contained the loSouthern quantity of zooplankton. Densities of zooplankton in this study were in order Anfoushy >Abu Qir > Northern Khobar > Southern Khobar all the year round. The maximum count of zooplankton in summer is due to high tempratures values and concentration of nutients. Results presented in this study have implications not only for the densities of zooplankton of the four marine systems in Mediterranean Sea and Arabian Gulf , but also to their abiotic environments. Since zooplankton have been regarded as an important trophic link between primary production and other consumers according to (Medeiros & Arthington 2008, 2011). Changes in their composition may have a cascading effect up and down the food web (Joneset al.1999). Furthermore, given the notion that factors affecting the structure and

composition of the zooplankton community, as well as the potential mechanisms that maintain their diversity lie at several levels of the marine watershed, decision-makers must identify the parts of the marine ecosystems that are vital to maintaining itshealth, in order to propose management and conservation policies for Mediterranean Sea and Arabian Gulf. The temperature of water is of enormous importance to marine organisms as it regulates various physico-chemical and biological activities of the organisms. Depending on temperature fluctuations the various species of zooplankton thrive and grow in waters in different months. In summer season increasing temperature enhances the rate of decomposition due to which the water becomes nutrient rich, similarly due to concentration followed by evaporation in summer season. The nutrient concentration increased and abundant food present in the form of phytoplankton and micro-organisms to zooplankton that is why high zooplankton population density during the summer season could be related to stable hydrological factors and low water level. They were resumed again in monsoon due to dilution and high water level which has been reported by Rajagopal et al.,(2010), Mulani et al., (2009), Pejaver and Gurav (2008), Jayabhaye and Madlapure (2006). The growth of rotifers occurs in summer months (Dumont, 1983), while it gives a thrust of increase to copepods and amphipods in winter months. It has been stated that the physico-chemical parameters and quantity of nutrients in water play significant role in distributional patterns and species composition of plankton (Mahar et al., 2000). Fluctuation in plankton population is a general phenomenon in the aquatic impoundments (Welch, 1952). Factors contributed to its variations are rainfall, depth, silting and other physicochemical parameters. The presence of a species depend on its environmental tolerance, but the resources available would determine their abundance. If competition or predation is reduced or food supply or suitable habitat increased, the species would become more abundant. The addition of sewage laden wastewater and the open defecation practices in the catchment are fully responsible for enriching its basin. Phosphorus and nitrogen inputs from domestic wastewaters accelerate the process of eutrophication (Rao et al , 1994). A fully eutrophicated lake with organic enrichment sustain a large number of flora and fauna as evident from the statistical analysis of this study. The copepods as a group is the index of eutrophic waters (Sladecek, 1983), and its abundance is considered as a biological indicatror of eutrophication (Nogueira, 2001, Samperio et. al, 2002). Similar observations were recorded in reservoir of Buldhana district by researchers viz. in Nalganga reservoir, Wari reservoir, Takli reservoir which have reached upto eutrophic stages similar to these findings. The present study revealed that zooplankton species richness was high in summer season compared to winter season. In summer the death and decay of macrophytes and the availability of organic matter production is much more on which zooplankton thrive best. The above factors contribute for high species diversity in that season. The increased input of sewage, siltation and high input loading in the form of wastewater are major cause of eutrophication resulting in species increase. Similar observations were recorded by Arora and Mehra (2003b& 2009) in Yamuna river. The study throws light on the rich fauna present in this small water body affected by anthropogenic activities. So from the present study it can be concluded that the four study localities harbors a bio-diverse fauna which fluctuated according to prevailing physico-chemical conditions of the marine ecosystem.

The low species richness of Copepoda during autumn and winter recorded in this study was also observed by Sousa *et al.*(2008) in Brazilian semi-arid reservoirs. Those authors explained the overall patterns of zooplankton composition in terms of trophic status (eutrophication), siltation and salinization due to evaporation. Even though such factors may be at work in the study sites, there wasno indication of eutrophication or siltation during the present study. That is inferred mostly from the low overall turbidity, relatively high rates of dissolved oxygen and low macrophyte growth at most sites.

Alternatively, this study proposed that the inadequacy of water residence time for the Copepoda in the study sites, mostly during the winter, contributed significantly to their low richness and overall paucity. In environments with periods of high flushing rates, due to the short residence time, only organisms with rapid growth and high renewal rates can increase their populations (Pourriot*et al.*1997). As exemplified by Recanto Reservoir, even larger water bodies are subject to flow during the wet Season. Similarly, the longer life cycles attributedto Copepoda (compared to Rotifera) may have been an important factor explaining their low numbers in streams during the dry season, when the rapidly contracting aquatic habitat are associated with increasingly dense fish populations (Brooks & Dodson 1965, Medeiros & Maltchik 2001a & b), more significantly so in small remnant pools in the stream bed than in the larger reservoirs. Nevertheless, low numbers of plankton have been observed in the more stable reservoirs during the dry Season, thus more information is needed on resource use and availability, and population dynamics of zooplankton as well as the dominant fish assemblages in order to fully explain this phenomenon in those water bodies.

Despite low richness, Copepoda showedhigh densities across study sites. The longer residence time of such environments is the most likely factor explaining this observation. Some groups of Copepoda have been reported as first colonizers in temporary environments

(Frisch & Green 2007). This early colonization has been associated to their ability to store sperm and capacity to survive drought. This is corroborated in the present study where large densities of juvenile stages of Copepoda were observed, indicating that early colonization may be in use as an adaptive strategy in these highly variable environments (see Cole 1966). Despite that, some studies have shown copepods to be late colonizers in other semi-arid regions (Hancock & Timms 2002). This is an indication that the local pool of species must also be taken into account in the present study, given that other groups of zooplankton have also been reported to be able to withstand dry periods and are quick colonizers (Crispim & Watanabe 2001, Hancock & Timms 2002, Frisch & Green 2007).

In order to identify patterns of occurrence of zooplankton taxa, grouping was carried out and species groups were associated with study areas (Mediterranean Sea and Arabian Gulf) and habitat type. On a larger spatial scale (study areas), grouping was not possible and the zooplankton fauna was not discriminated between the Mediterranean Sea and Arabian Gulf regions. However, within each area grouping was easily performed, separatingnot only Bay from Gulf sites but also Bay two sites and Gulf two sites between themselves. Similar results were observed by Medeiros *et al.*(2008) when characterizing the structure of the habitat in the study sites. Those authors observed that at larger spatial scales (between the Seridó and Buíque areas) the structure of the habitat could not be distinguished, despite some segregation between sites within study areas. This pattern was explained as the result of a nested hierarchy (Poff 1997) wherevarious levels of catchment-and stream-reach variables are correlated with the habitat structure. Data from the present study indicate that the composition of zooplankton may be influenced by aspects related to spatial hierarchical levels, where a commonlarge-scale pool of species is broken into more specific community traits at local scales (see Tomanova & Usseglio-Polatera 2007), the latter being likely regulated by local-scale physical and biological processes, such as competition, predation and grazing patterns. That being the case, catchment-scale processes, such as climate and geomorphology, are important to define higher levels of organization of the plankton fauna and overall species pool of the marine ecosystem. These higher levels of organization will then influence factors at a variety of lower spatial reach-scale characteristics such as morphology, flow and water variables, and consequently the local species pool, which may be particularly relevant to marine systems.

Management policies for marine systems are based on reservoir and weir construction and different degrees of flow regulation (see Maltchik &Medeiros 2006; Leal,et al. 2013). These alterations greatly modify the hydrological characteristics of the highly variable intermittent streams, which have been reported to have the extremes of flooding and drought as the driving forces organizing biotic communities (Maltchik & Florin 2002). Implications of such modifications in dry lands have been summarized by Bunn & Arthington (2002) and include changes on zooplankton assemblages structure.

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Osmotic Dehydration Kinetics of Catfish (*Clarias Garipinus*) Muscles in Sodium Chloride Solution

By Burubai, W., Etekpe, G. W. & Samson, D.J.
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Abstract- The effect of sodium chloride (solute) concentration and immersion time on weight reduction (WR), water loss (WL), solid gain (SG), mass shrinkage ratio (SR) and dehydration efficiency index (EI) on Catfish during osmotic dehydration were studied. Results show that, at 95% confidence level, all the above kinetic parameters were significantly affected by both immersion time and solute concentration levels. However, F7 which contains 70% sodium chloride and 30% water presented the best results in all kinetic parameters investigated and therefore recommended.

Keywords: *catfish, sodium chloride, osmotic dehydration, immersion, solute concentration.*

GJSFR-C Classification : *FOR Code: 270000*

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I. Introduction

Osmotic dehydration, as a preservation technique, is one of the most underutilized unit operations in post-harvest processing of biomaterials. It is a moisture removal process in which the sample is immersed in a hypertonic solution. During this process, two counter current flows dominate because of the semi-permeable nature of the plant cell structure. Thus water and other water soluble constituents flow from the tissues of the sample into the solution and a simultaneous transfer of solute from solution into the sample (Singhi et al, 2008; Taiwo et al, 2002; Park et al, 2002). Of course, the driving force for the diffusion of water from the tissue into the solution is provided by the higher osmotic pressure of the hypertonic solution. The most common hypertonic solutions applied for this complementary drying process are salts, alcohols, sorbitol and sugar solutions and the attendant beneficial effects of the osmotic dehydration are low energy cost, retention of nutritional value, eco-friendliness and stability of quality of product. However, the rate of water removal from any biomaterial, to a large extent, depends on several factors which include solute concentration, duration of dehydration, temperature, size and geometry of the material (Rastogi and Raghavarao, 2004).

These attendant advantages of osmotic dehydration over other conventional drying methods has led several scientists to investigate the osmotic dehydration of various biomaterials (Corzo and Gomez, 2004; Azoubel & Murr, 2003; Moazzam, 2012; Erle and Shubert, 2001).

However, information on the osmotic dehydration of Catfish is still lacking. Therefore, to optimize the important complementary drying process in the fishing industry, the objectives of this study are tailored to investigate the influence of solute concentration and immersion duration on weight reduction, water loss, solid gains, mass shrinkage ratio and dehydration rate, in sodium chloride solution.

II. Materials and Methods

a) Sample Preparation

Two matured market-size catfish (*Clarias garipinus*) weighing 1.8kg each were purchased from the Tombia Junction Market in Bayelsa State, Nigeria on July, 2014. The samples were immediately taken in plastic basins to the Food Processing Laboratory of the Niger Delta University, Bayelsa State and eviscerated. 32 samples weighing 5g each were cut out and five samples used to determine the initial moisture content using the oven method at 105^0C and the remainder for the experimentation proper.

b) Apparatus Used

The basic apparatus used for the experimentation were: a convective oven (Venticell, MMM, Medcener, Germany) which was used to determine the initial moisture content at 105^0C; a Camry digital balance for weight determination, 250ml beakers, conical flasks, 200ml measuring cylinder, chopping board and a kitchen knife were used.

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Nine (9) different concentration levels of sodium chloride (NaCl) solution were obtained in the following ratios:

Sample Code	Solution	
F_1	90% water	10% NaCl
F_2	80% water	20% NaCl
F_3	70% water	30% NaCl
F_4	60% water	40% NaCl
F_5	50% water	50% NaCl
F_6	40% water	60% NaCl
F_7	30% water	70% NaCl
F_8	20% water	80% NaCl
F_9	10% water	90% NaCl

The 5g samples were then immersed in the different osmotic solutions (%NaCl + % water) at a room temperature of 25^{0}C for a period of 5 hrs. A sample to solution ratio of 1:10 was used to avoid significant dilution of solute and the beaker occasionally agitated to ensure uniform mixture of absorbed water from sample. After 5 hrs, samples were withdrawn from the solution and the dehydrated samples bottled with absorbent paper to remove excess solution. Of course, at different time intervals within the 5 hrs, samples were withdrawn and weighed until the expiration of the 5 hrs. This procedure was replicated thrice and the average weights of samples recorded in accordance with AOAC (2000).

The desired response variables which included weight reduction (WR), water loss (WL), Solid gain (SG), mass shrinkage ratio (SR) and dehydration efficiency index (EI) were then calculated with the following equations.

$$WR\ (\%) = \frac{(w_i - w_f)}{w_i} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$WL\ (\%) = \frac{(w_i x_i - w_f x_f)}{w_i} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$SG\ (\%) = \left(wf\ \left(1 - \frac{x_f}{100}\right) - w_i\left(1 - \frac{x_i}{100}\right)\right) \times 100 \ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where w_i and w_f are the initial and final sample weights, in grams respectively: x_i and x_f are the initial and final sample moisture contents, respectively in percentage. Similarly, mass shrinkage ratio was determined as

$$SR = \frac{W_t}{W_o} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where W_t is weight at any time (t) (g) and W_o weight at time = 0 (g).

The dehydration efficiency index,EI was also obtained as follows

$$EI = \frac{WL}{SG} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

c) *Statistical Analysis*

A descriptive statistical analysis was performed to compare the means and evaluate significant differences using one-way analysis of variance (Anova) at 95% confidence level.

III. Results and Discussions

a) *Weight Reduction, WR (%)*

Figure 1 provides an overview of the average values of percent weight reduction of catfish muscles as influenced by concentration of solute and dehydration time. Generally, WR (%) increased from 5 minutes to 5 hrs of immersion time for all concentration levels. At F9, 8.0% of weight reduction was noticed after 5 minutes of dehydration. This value increased to 32.0% after 5 hrs. For F1, no weight reduction was observed in the first 25 minutes of dehydration, but a weight reduction of 4.0% was recorded after 5 hrs. Beneficially, F7 showed the best results as it had the highest values for WR. These results show that both solute concentration and immersion time had significant effects on percentage weight reduction of catfish muscle during osmotic dehydration. To prove this observation, a statistical analysis (Anova) was performed at 95% confidence level and the results presented in Table 1 indicated that the effect of solute concentration and immersion was very significant .Similar results had been reported by

Collignan et al (2001) on the osmotic treatment of fish and meat

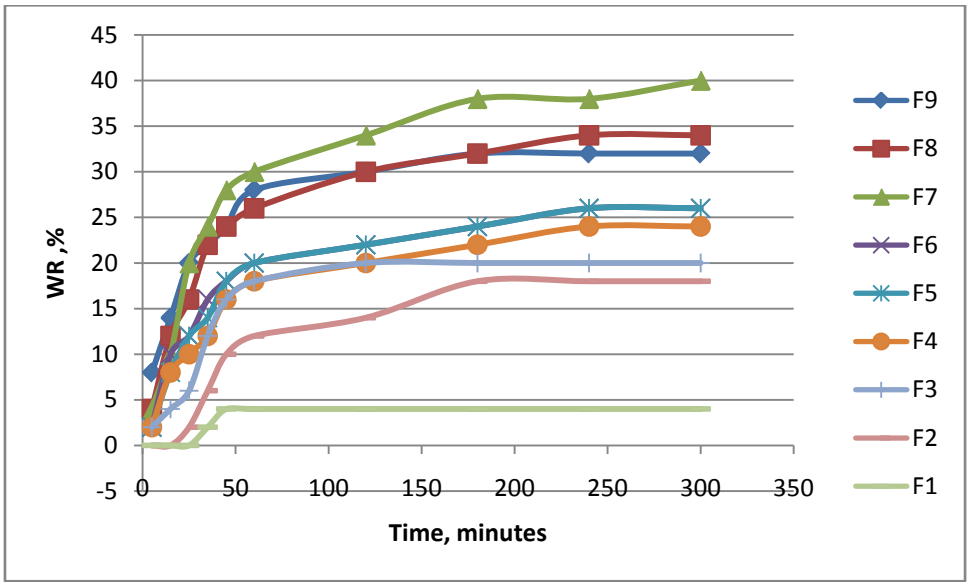

Figure 1 : Response of weight reduction WR (%) to solute concentration and immersion time

Table 1 : One-Way Anova on effect of NaCl concentration and immersion time on WR

Source	SS	DF	MS	Prob	F- ratio	F-critical
Between group	1980.250	1	1980.250	0.0001	32.867	4.6001
Within group	843.5000	14	60.25			
Total	2823.75	15				

b) Water Loss, (WL) (%)

Graphical judgment of average percent water loss values are shown in Figure 2. It is clear, that in all cases, water loss increased with increase in both solute concentration and immersion time. Evidentially, at F9, WL value of 13.7% was recorded within 5 minutes of immersion, but increased to 47.36% after 5 hrs.

It was however different at F1, where no water loss was noticed within the first 25 minutes of immersion but increased later to 7.04% after 5hrs. This initial delay in WL at F1 is, perhaps, due to the low osmotic pressure exhibited in the 10% NaCl and 90% water concentration. Here also, F7 displayed the best NaCl concentration level with the highest water loss value. These results point to the fact that both solute concentration level and immersion time had important roles to play in the osmotic dehydration of catfish muscles. These findings agree with those of Milica et al (2013) on *carassius carassius* and Azoubel and Murr (2003) on cashew. An Anova shows that these variables are significant to water loss (p= 0.0000) as indicated in Table 2.

Figure 2 : Effect of NaCl conc and immersion time on water loss

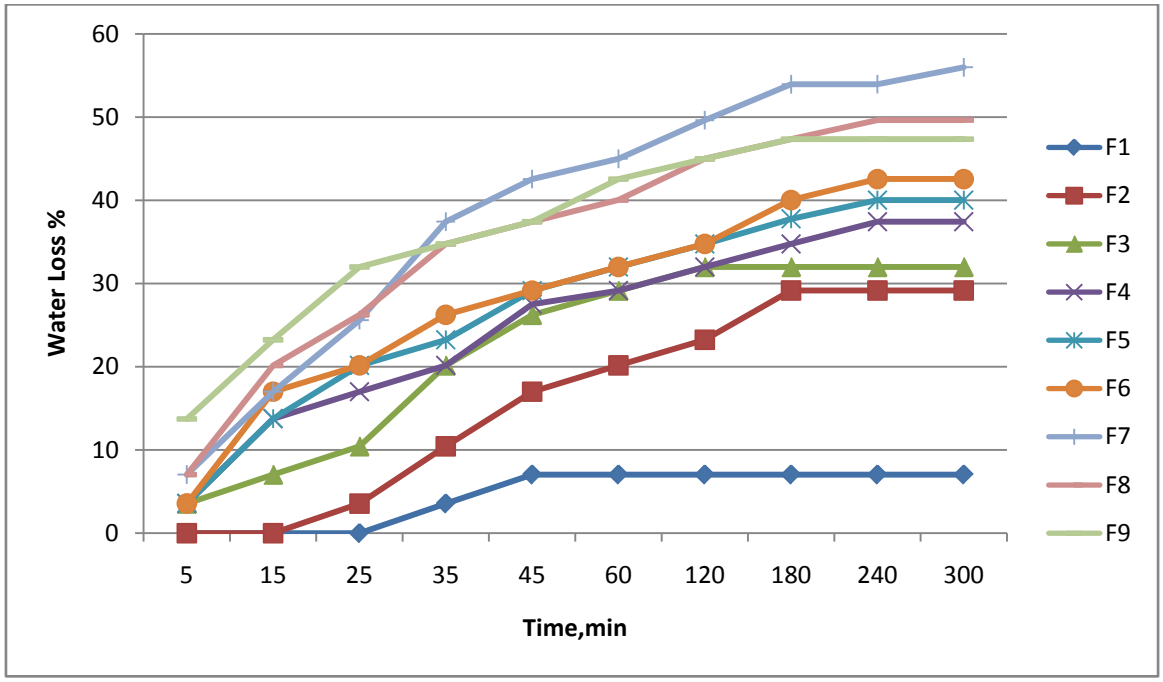

Table 2 : One-Way Anova on effect of NaCl concentration and immersion time on WL

Source	SS	DF	MS	Prob	F-ratio	F-critical
Between group	4407.6321	1	4407.6321	0.0000	38.707	4.6001
Within group	1594.1886	14	113.8706			
Total	6001.8207	15				

c) Solid Gain, SG (%)

Solid gain which is the transfer of solute molecules from the solution into the catfish muscles was investigated as a function of solute concentration and immersion duration. Figure 3, therefore shows the graphical view of the effect NaCl concentration and immersion time on solid gain. Results show that, generally, solid gain increases positively with increase in solute concentration and immersion duration. At F9, 11.03% of solid gain was recorded in 5minutes of immersion, but increased to 25.83% in 3 hrs and then remained constant for the rest 2 hrs. This trend was noticeable in virtually all solute concentration levels, implying that osmotic pressure attained equilibrium state after 3 hrs of dehydration.

However, average values of solid gain in F1 were found to be 0.0% for the first 25 minutes of dehydration but increased to 5.92% after 45 minutes and remained constant for the rest of the experimentation. At 95% confidence interval, Anova reveals a significant difference between means of SG values (Table 3). It therefore shows that solute concentration and immersion duration play significant roles in the solid gain of catfish muscle during osmotic dehydration. Comparatively, results presented here concur both in immersion time and solute concentrations with that of Milica et al (2013) on Carassius muscles.

Figure 3 : Effect of NaCl concentration and immersion time on Solid Gain

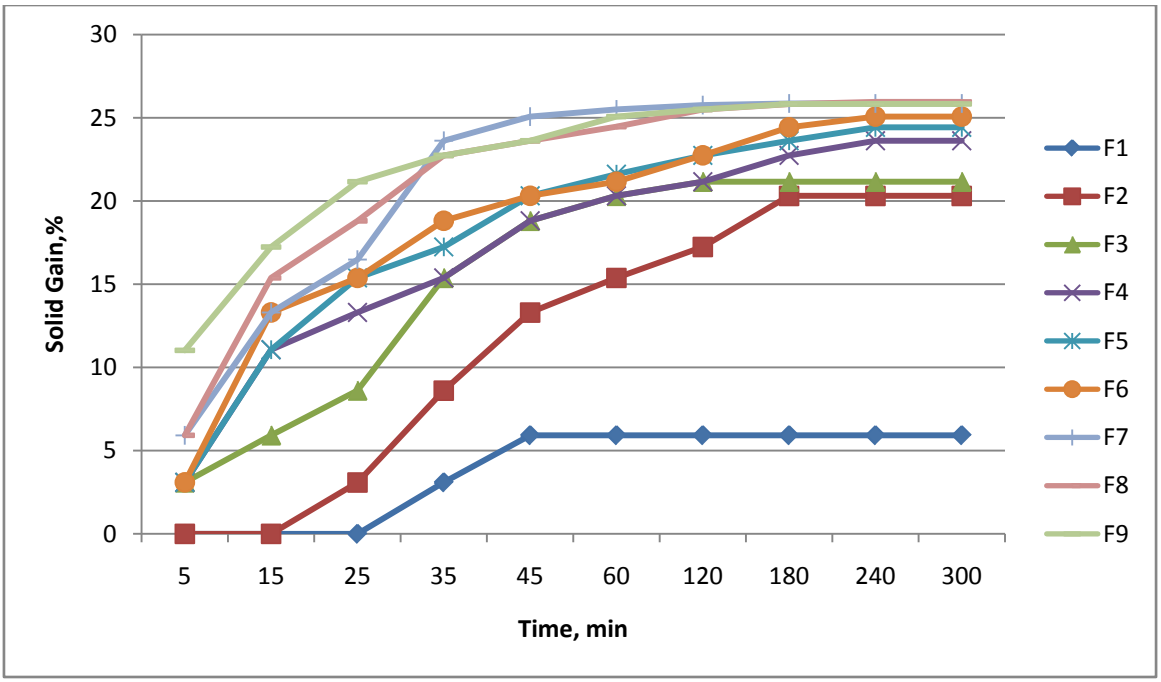

Table 3 : One-Way Anova on effect of NaCl concentration and immersion time on SG

Source	SS	DF	MS	Prob	F-ratio	F-critical
Between group	1352.9523	1	1352.9523	0.0000	54.7916	4.6001
Within group	345.6976	14	24.6927			
Total	1698.6499	15				

d) Mass Shrinkage Ratio (SR)

As dehydration progresses, structural changes occur in the sample due to weight loss. This can be accounted for by the mass shrinkage ratio as recommended by Midilli, 2011 and Shanmugama and Natarajan, 2006. Results of mass shrinkage ratio of catfish muscle as affected by sodium chloride concentration and immersion time are graphically presented in Figure 4. Generally, shrinkage ratio decreases with increase in immersion time. For F9 and at 5 min of immersion, a shrinkage ratio of 0.92 was recorded. This SR value decreased to 0.68 in 5 hrs. Also, for F1 and at 5 min of immersion, no shrinkage was noted but became 0.96 after 5 hrs. Similarly, shrinkage ratio decreased with increase in sodium chloride concentration levels. After 5 hrs of dehydration, mass shrinkage ratio values of 0.96 and 0.68 were recorded for F1 and F9 respectively. This is attributed to the fact that, as sodium chloride concentration and immersion time increases more water loss and weight reduction was observed. F7 again showed the lowest and best shrinkage ration of 0.60 after 5hrs of dehydration. Table 4 shows the result of the ANOVA on the effect of sodium chloride and immersion time on mass shrinkage ratio. These results agree positively with the findings of Saeed et al (2008) and Midilli (2001) for Roselle and Pistachio fruits respectively.

Figure 4 : Effect of NaCl concentration and immersion time on shrinkage ratio (SR)

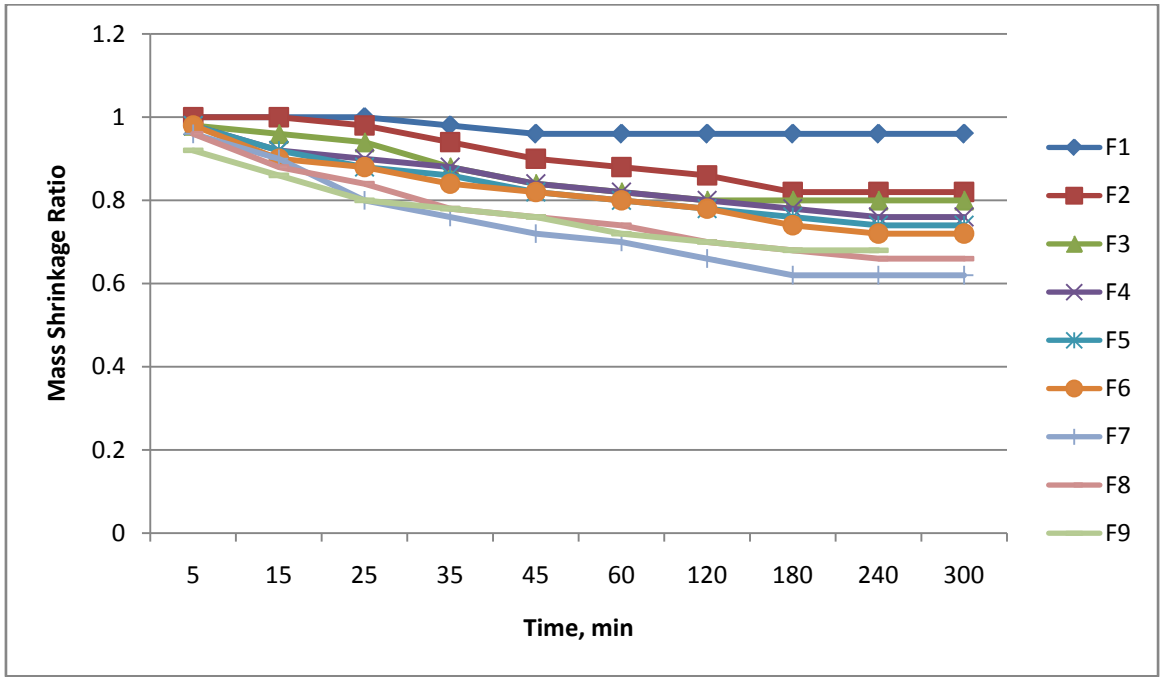

Table 4 : One-Way Anova on effect of NaCl concentration and immersion time on SR

Source	SS	DF	MS	Prob	F-ratio	F-critical
Between group	0.1980	1	0.1980	0.0001	32.867	4.6001
Within group	0.0844	14	0.0060			
Total	0.2824	15				

e) Dehydration Efficiency Index (EI)

In every osmotic dehydration regime, the effectiveness of the process is been determined by the value of the dehydration efficiency index. Thus, high efficiency index ratios indicate high water removal from the sample with minimal solid gain. Conversely, low EI ratios indicate high solid gains and minimal water removal which is undesirable in any osmotic dehydration process. For this work, EI ratios increased with increase in immersion time. At F9, EI values of 1.24 and 1.83 were recorded for immersion times of 5 min and 5 hrs respectively. For F1, no efficiency index value was recorded for the first 25 min but increased to 1.18 in 45 min of dehydration and then remained constant for the rest 4 hours. This may be as a result of low osmotic pressure characterized by low NaCl concentration in the hypertonic solution. These results points to the fact that as immersion time increases, EI increases. Similarly, EI values increases linearly with increase in solute (NaCl) concentration. However, the highest EI value of 2.18 was recorded in F7 implying that hypertonic solution concentration levels of 30% NaCl and 70% water was the best. Overall, F7 still gave the best results and the statistical analyses on Table 5 shows that EI is significantly controlled by both sodium chloride concentration and immersion time.The results here are in concordance with the work of Milica et al (2013) on Carassius muscles.

Figure 5 : Effect of NaCl concentration and immersion time on EI

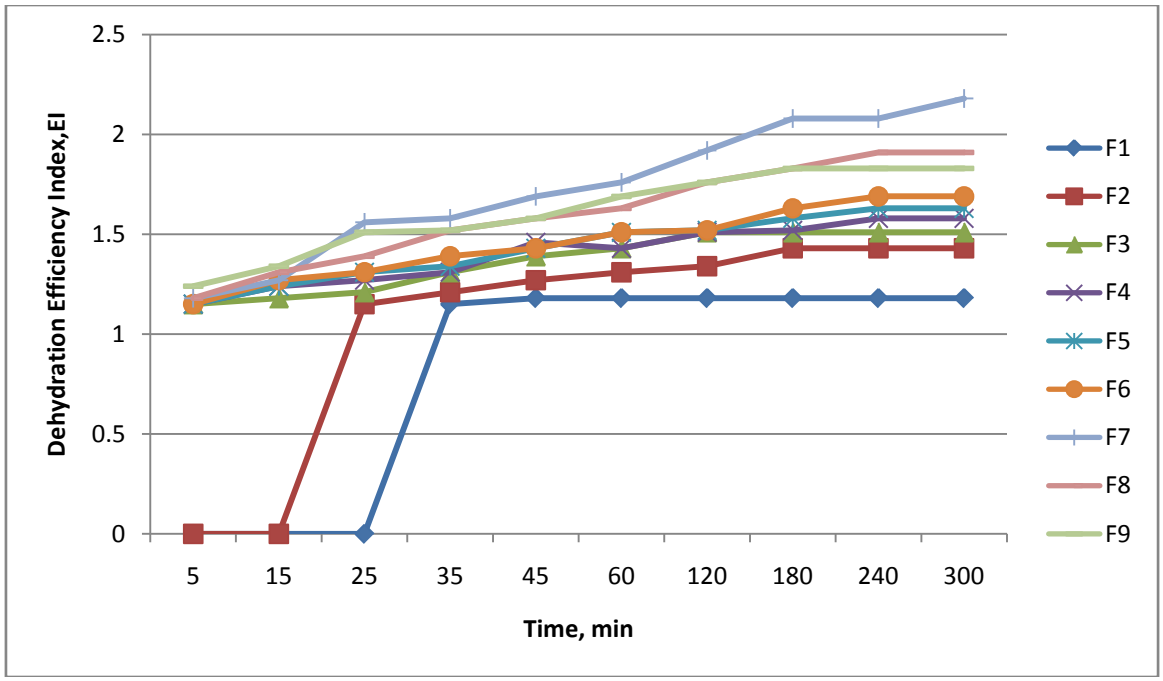

Table 5 : One-Way Anova on effect of NaCl concentration and immersion time on EI

Source	SS	DF	MS	Prob	F-ratio	F-critical
Between group	2.3639	1	2.3639	0.0034	32.867	4.6001
Within group	2.6629	14	0.1902			
Total	5.0268	15				

IV. Conclusions

Based on the findings of this experimentation, it can be concluded that solute (NaCl) concentration and immersion time both contributes significantly to osmotic dehydration of catfish muscle. The highest values of WR and WL were recorded in F7 after 5 hours of immersion in the hypertonic solution. F7 also recorded the highest dehydration efficiency index and lowest mass shrinkage ratio after 5 hours of dehydration. This makes F7 the best NaCl concentration level for dehydrating Catfish.

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Identification of the Genus *Phyllanthus* (Family Phyllanthaceae) in Southern Nigeria using Comparative Systematic Morphological and Anatomical Studies of the Vegetative Organs

By Daniel Azubuike Awomukwu, Bio Louis Nyananyo, Chiedozie Joel Uka & Clement Uwabunkeonye Okeke

Federal University, Nigeria

Abstract- Five species of *Phyllanthus* L. Family Phyllanthaceae occurring in Southern Nigeria, *P. amarus* Schum and Thonn, *P. urinaria* Linn., *P. odontadenius* Mull-Arg., *P. niruroides* Mull-Arg. and *P. muellerianus* (O. Ktze) Excel were compared using the morphology and anatomy with the view to adding to increasing the systematic lines of evidence and providing a more natural clarification than the existing one. The foliar and floral morphology of these species were described while the anatomical characteristics of the leaf, stem and root are valuable characters in delimiting the species. The results obtained from the studies showed that species of *Phyllanthus* have different attributes in their vascular characteristics that could be used together with other existing systematic evidence in clarifying the confusion in identifying these plants. Evidence from the nature of the palisade parenchyma in the mesophyll, nature of the collenchyma, sclerenchyma and vascular bundles are presented and discussed with their values in the systematic positions of these plants. A dichotomous bracketed key to the identification of the species studied is provided.

Keywords: *phyllanthus species, morphology, anatomy, taxonomic evidence.*

GJSFR-C Classification : *FOR Code: 270899*

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I. Introduction

The genus *Phyllanthus* L. is a member of pantropical family Phyllanthaceae (a segregate from Euphorbiaceae sensu lato [s.l.]) based on congruent plastid and nuclear DNA sequence data that have recovered well-resolved and strongly supported clades (Wurdack *et al*., 2004; Samuel *et al*., 2005; Kathriarachchi *et al*., 2005) that correspond to subfamilies and tribes. Estimates in the number of species in this genus vary widely from 750 (David, 2008) to 1200 (Kathariarachchi *et al*., 2005). Some of these species occur in the southern Nigeria and all tropical regions of the world from North Central and South America (Uander and Blumberg, 1991). The plants are monoecious; leaves simple, alternate or opposite, some are leathery, flowers are very small and diclinous, they cluster in cup-shaped structures, greenish, whitish or whitish-green, often with glands. The fruit is a lobed-capsule extending from the cup and commonly the long stalk pendant (Lewis and Elvin-Lewis, 1977; Wessels-Boer *et al*., 1976). The name "*Phyllanthus*" means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf. Other common names include gripe weed, stonebreaker, leaf flower etc (Cabieses, 1993). The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body. Nigeria is one country is rich in raw and useful herbs from which important drugs could be prepared or agent which serve as starting products for the potential synthesis of drugs (Sofowara, 1993). Members of the *Phyllanthus* designated as weeds are used as pot herbs. The healing powers of *Phyllanthus* as claimed by local medicinal practitioners range from headache, skin diseases to gonorrhea and syphilis (Akobundu and Agyaka, 1987, Burkill, 1994). Other aliments treated with these medicinal plants include asthma, cough, diarrhea, diabetes, malaria, eye and ear problems, indigestion and constipation, nausea and vomiting, bleeding, childcare, healing of wounds and sores and tooth extraction. Some of these medicinal plants are used as styptic and as simple laxative to cure dysentery. They are a good source of pesticides (Oliver, 1959; Burkill, 1994 and Gill, 1992). Most of these plants used for traditional medicine are equally consumed by humans in the Niger-Delta region of Nigeria, but their correct identification poses problems to the users.

Morphological characters are features of external forms or appearance. They currently provide the characters used for hypothesizing phylogenetic relationships. These features have been used for a longer time than the anatomical evidence in the beginnings of plant systematic. Morphological characters are easily observed and find practical use in keys and description. Morphological characters include

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the external features of the plant parts used, including the particulars of their size, shape and colour. Most plants are classified based on external morphological structures, such as flower and fruits. These structures are not always available on plants because they are seasonal in production.

Anatomical studies are a systematic line of evidence used in combination with other systematic lines to arrive at a good taxonomic condition (Stace, 1980). Anatomical studies apart from special references to systematic position of the taxa can also be used in noting the origin, natural distribution extent of cultivation and cultivars within species of plants (Lawson, 1967; Onwueme, 1978). The significant role of vegetative anatomy in botanical reconstruction of the origin, natural distribution, extent of cultivation and cultivars within species of plants dates back to many centuries ago. Metcalfe and Chalk (1950) dealt extensively on the relative importance of anatomical features in the systematic positioning of groups of angiosperms. In spite of this, different authors in different groups have studied the scientific importance and specific implication of anatomical features of *Phyllanthus amarus* only. They include Natural Remedies-Research Centre (2006); Edeoga *et al.*, (2007). None of such works has successfully delineated the indigenous species occurring in the southern Nigeria which includes *P. amarus* Schum and Thonn, *P. urinaria* Linn. and *P. odontadenius* Mull-Arg., *P. niruroides* Mull-Arg. and *P. muellerianus* (O. Ktze) Excel

From available literature, series of documented descriptions of the morphological characteristics and ethnobotanical uses of *Phyllanthus* are found have been reported (Burkill, 1994; Oliver, 1959; Hutchinson and Dalziel, 1963), there is confusion in recognizing individual species of these plants in Nigeria. Therefore the basic anatomical information provides data that will clarify the confusion in the identity of these taxa. The aim of this work was to provide comprehensive information on the morphological and anatomical structures that will clarify the confusion in the identification of these *Phyllanthus* species. The information provided therein will go a long way in aiding workers who may use these plants for other purposes, such as pharmaceutical, biological and other related areas of study.

II. Materials and Methods

a) Collection of plant materials

Mature plants of the five species *P. amarus, P. urinaria, P. niruroides, P. odontadenius* and *P. muellerianus.* were collected from different locations of southern Nigeria (bounding box coordinates: upper left – 6.3333, 7; lower right – 4.75, 6.8333) by various investigators as in Table 1. Only healthy, fresh and succulent parts of the plants were collected. The five specimens were identified and authenticated at the Herbaria of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State and the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. Herbarium specimens were also studied at the various institutions as well making reference to the Flora of West Tropical Africa by Hutchinson and Dalziel (1963). The accessions were deposited at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria with their sample and process identity numbers for further research.

Table 1 : Collection sites, identity numbers and collection dates of the five Phyllanthus species studied

SPECIES	SITE OF COLLECTION	CORDINATE/ ELEVATION	SAMPLE ID	PROCESS ID	DATE OF COLLECTION
P. amarus	Along the school fence, Abia State Polytechnic, Aba.	N5.42; E6.33; 25.0m	AWOM UPH PA 010	PHSN 001-14	April 15, 2014
P. urinaria	Field around National Root Crop Research Institute, Umudike.	N4.75; E6.83; 20.0m	AWOM UPH PN 050	PHSN 003-14	April 14, 2014
P. odontadenius	Road side along National Root Crop Research Institute, Umuahia.	N5.42; E7.50; 25.0m	AWOM UPH PO 040	PHSN 006-14	April 15, 2014
P. niruroides	Science Village, Nnamdi Azikiwe University, Awka.	N6.33; E7.00; 22.0m	AWOM UPH PU 030	PHSN 005-14	April 15, 2014
P. muellerianus	Near the Herbarium Building, Nnamdi Azikiwe University, Awka.	N6.33; E7.00; 23.0m	AWOM UPH PM 020	PHSN 002-14	April 15, 2014

b) Morphological studies

This involves the observation of the qualitative foliar floral characteristics of the species as well as measurement of the quantitative morphological characters of the leaves and flowers of the five species studied.

c) *Anatomical studies*

Section 22-26mm thick prepared from the leaves, stems and roots were fixed in Formalin Aldehyde Acetone (1:1:18) for 48-72hours. These were then rinsed in several alcohol series (30, 50, 70, 95 and 100%). These dehydrated materials were infiltrated with wax by passing trough different proportions of alcohol and chloroform (3:1, 1: 1, 1:3 pure chloroform v/v). As the chloroform gradually replaced the alcohol, wax was put in the bottles to slowly infiltrate the tissue with wax to make it hard enough for sectioning.

The bottles were left on a hot plate (37-40%) for 24hours before transferring to the oven (58-60%) (this procedure evaporates the chloroform). The wax at its melting point completely infiltrated the tissues in it. After a period of 2-3 days with constant addition of wax the specimens were embedded in paraffin melted wax. This was accomplished by a quick orientation of the specimens in the mould with a hot mounted needle and forceps and quick cooling in a beaker containing ice block. The resultant wax blocks were later trimmed and sectioned on a Reichert rotary microtome at 20-24mm following a slightly modified method (Culter, 1978).

The ribbons were placed on clean slides smeared with a film of Hampt`s albumen and allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate at 40°C for a few minutes to allow the ribbons to expand and were stored over night. The slides were immersed in pure xylene for 2-5 minutes in a solution of xylene and absolute alcohol with 1:1 ratio (v/v) for 5 minutes. The slides were then transferred to another solution of xylene and alcohol in the ratio of 1:3 (v/v) for a few minutes, to 95, 90, 70 and 50% alcohol. Drops of alcian blue were added to the specimens for 5 minutes, washed off with water and counter-stained with safranin for 2 minutes, then dehydrated in a series of alcohol 50, 70, 80, 90% and pure xylene at intervals of a few seconds and mounted in Canada balsam. Coloured photomicrographs were taken using a Leitz Wetzler Ortholux microscope fitted with a Vivatar-V-335camera.

III. Results and Observation

a) *Morphological description*

A summary of the morphological differences and similarities observed in the five species of *Phyllanthus* is presented in Table 2 while the detailed morphological description of each of the species is presented below.

Table 2 : Summary of important features of the *Phyllanthus* species studied

CHARACTERS	TAXA				
	P. amarus	*P. urinaria*	*P. odontadenius*	*P. niruroides*	*P. muellerianus*
Habit	Herb	Herb	Herb	Herb	Shrub
Leaf size and shape	5-10mm long 2-4mm broad Elliptic-oblong	6-15m long 3-15mm broad Oblong	6-15mm long 5-7mm broad Oblong	4-10mm long 1.5-3mm broad Oblong	2-7cm long 1.5-4cm broad Ovate-elliptic
Leaf apex	Obtuse	Mucronate	Obtuse	Mucronate	Subacute
Stem colour and form	Greenish, smooth, rounded, glabrous and woody at base.	Reddish-green, smooth, pentagonal, glabrous and woody at base.	Greenish, smooth, pentagonal, glabrous and woody at base.	Greenish, smooth, pentagonal, glabrous and woody at base	Brownish-green, thorny, rounded-pentagonal, glabrous and all-woody
Fruit and flower colour	Greenish fruits and tepals	Reddish fruits and reddish-green tepals	Greenish fruits and whitish-green tepals	Spotted-greenish fruits and tepals	Berry-like red fruits and greenish tepals
Number of tepals	Pentatepalous (5)	Hexatepalous (6)	Hexatepalous (6)	Pentatepalous (5)	Pentatepalous (5)
Nature of stipules	Greenish and laterally free	Reddish and laterally free	Reddish and laterally free	Reddish and laterally free	Greenish and spiny
Plant height	Up to 75cm high	Up to 60cm high	Up to 1.0m high	Up to 75cm high	Up to 12m high


1a.i


1a.ii


1b.i


1b.ii


1c.i


1c.ii

Figure 1 : Photographs of the morphology of the genus *Phyllanthus* studied

(a.i) *P. amarus*, showing the dorsal surface of the herb and the elliptic-oblong shape of the leaves.
(a.ii) *P. amarus*, exposing the greenish fruits and the pentatepalous flowers on the ventral surface of the leaves.
(b.i) *P. urinaria*, showing the dorsal surface of the herb and the mucronate leaf apices .
(b.ii) *P. urinaria*, exposing the reddish-green friuts, stipules and buds on the ventral surface of the leaves.
(c.i) *P. odontadenius*, showing the dorsal surface of the herb and the oblong shape of the leaves.
(c.ii) *P. odontadenius*, exposing the whitish-green tepals and reddish laterally free stipules.
(d.i) *P. niruroides*, showing the dorsal surface of the herb and the mucronate leaf apices.
(d.ii) *P. niruroides*, exposing the greenish fruits and tepals and reddish laterally free stipules.
(e.i) *P. muellerianus*, showing the dorsal surface of the shrub branch and the ovate-elliptic shape of the leaves.
(e.ii) *P. muellerianus*, exposing the spiny stipules of the leaves and thorny branches.

b) Phyllanthus amarus Schum and Thonn

F.T.A. 6, 1: 717; Chev. Bot 556. S.L.: Bonthe (fl & fr. Nov) *Deighton* 2265! Kent (fl.& fr. May) *Deighton* 2657! Rokupr (fl & fr. Apr) *Jordan* 237! Njala (fi. & fr. May) *Deighton* 1945! Lik, : Monrovia *Whyte*! Since Basin *Whyte*! Iv.C.: fide Chev. I.c. G.C.: Thonning. Axin (Jan) *Chipp* 56.1 Accra *Ansell*! N.Nig.: Idah T. Vogel! S. Nig.: Lagos *Dewodu* 24! 365! *Dulx*. 1380 ! Sapohn *Kennedy* 2896 ! Awka *Thomas* 50 ! Calabar (fi. & fr. Mar.) *Brenan* 9210 ! F. PaT. *Vogel*!.

A glabrous, erect or ascending annual herb up to 75cm tall. It is much branched with small leaves on lateral branches of the stem that give the plant the appearance of having pinnate leaves reproducing from seeds. The stem is rounded, woody at the base, horizontally branched, smooth and greenish, monoecious or rarely dioecious. The leaves are alternate, elliptic on long, 5-10cm long and 2-4.5mm broad, pale beneath and with short petioles. The inflorescence is axiliary in and consists of one male flower and one female flower in each axil. Flowers are greenish and rather small up 1.5mm diameter. Female flowers usually solitary in the proximal axils. The fruit is a round capsule, brownish, 1.5-2mm across and occurs in least axils on the lower side of the lateral branches. Each capsule contains six small seeds (Akobundu and Agyaka, 1987; Hutchinson and Dalziel, 1963).

c) *Phyllanthus urinaria Linn.*

F.T.A. 6, 1 : 721. **S. L.:** Regent, on rocks (Dec.) *Sc. Elliot* 4102 ! Freetown (Dec.) *Deighton* 499 ! Moyamba (fl. & fr. Aug.) *Deighton* 2215 ! Sembehun (fl. & fr. Aug.) *Deighton* 3794 ! Rokupr (fl. & fr. Apr.) *Jordan* 238 ! **G.C.:** Benso, Tarkwa (fl. & fr. June) *Andoh* FH 5525 ! **S. Nig. :** Sapoba (fr. Sept.) *Onochie* FHI 34311 !

This is a small, slender, sub-woody, annual herb up to 60cm high/9-12inches or 2 feet high. It has numerous small leaves on lateral branches of the stem resembling mimosa tree, disposed in two ranges. The leaves are large at the tip and towards the petiole. When touched, the leaves fold in automatically. The leaves are alternate, oblong, rounded at base, shortly pointed and minutely mucronate at apex, 6-15mm long and 3-5mm broad, margins asperulate. The stem is glabrous, round, sub-woody at the base, horizontally branched, pentagonal and greenish at the top. Petiole very short and compressed, glabrous, stipules broad and articulate at the base, narrowed into a filiform point, about ¾ lin long. Flowers are solitary and subsessile, monoecious with warted ovary. The flowers are greenish-red with sepals 6, minute and appear at axiles of the leaves as well as the seed capsules. Numerous small green-red fruits, round and smooth are found along the underside of the leaves which are erect and red. The fruit is round capsule, greenish and when ripe with reddish-green sepal along a green margin. The seeds are transversely ridged with about 10 prominent transverse ribs on the back (Burkill, 1994; Hutchinson and Dalziel, 1963).

d) *Phyllanthus odontadenius Mull-Arg*

F.T.A. 6, 1 : 727. **Port. G.:** Bissau (Nov.) *Esp. Santo* 897 ! **S.L.:** Njala (fl. & fr. May) *Deighton* 639 ! Rokupr (fl. & fr. Apr.) *Jordan* 236 ! Bafodea (fl. & fr. May) *Deighton* 4480 ! **Lib.:** Kakatown *Whyte* ! **G.C.:** Aburi Hills (fl. & fr. Oct.) *Johnson* 471 ! Kumasi *Cummins* 77! **S.Nig.:** Lagos (fl. & fr. July) *Dalz.* 1361 ! Ibadan (fl. & fr. Nov.-Apr.) *Meikle* 652 ! 1306 ! 1454 ! Okomu F. R. (fl. & fr. Feb.) Brenan 9135 ! 9148 ! Calabar (fl. & fr. Mar.) *Brenan* 9209 ! **Br.Cam.:** Buea (fl. & fr. July) *Dundas* FHI 15237! **F.Po:** *Barter* ! Extends to A.-E. Sudan and Angola, also on S. Tome.

This is a glabrous, erect or ascending, sub-woody annual herb up to 1m tall. It is much branched with leaves on lateral branches of the stem giving the plant appearance of having pinnate leaves up to 90cm high reproducing from seed. The stem is brownish glabrous, round, sub woody at the base, horizontally branched, pentagonal and greenish at the top. The leaves are large at the tip and towards the petiole. The leaves are alternate, oblong, rounded at the base, obtuse at apex, 6-15mm long and 5-7mm broaded. The branchlets are flattened and winged with distinct red stipules up to 2mm long, not conspicuous. The flowers are monoecious with the inflorescence consisting of male flowers 2-3 together in lower leaf axils of branches, females solitary in upper axils and larger. Tepals are 6, oblong-linear, not completely enclosing the fruit. The ovary is very shortly stipitate. The fruit is a round capsule, greenish, and 1.7-2mm across. The flowers are whitish-green, small on short pedicels (Burkill, 1994; Hutchinson and Dalziel, 1963) .

e) *Phyllanthus muellerianus (O. Ktze.) Exell*

F.T.A. 6, 1 : 701; F.W.T.A., ed. 1, 1 : 290 ; Chev. Bot. 556 ; Aubrev. Fl. For. Soud.-Guin. 189. **Fr.Sud.:** Bamako *Waterlot* 1368. Birgo *Dubios* 210. **Port.G.:** Granja, Catio (June) *Esp. Santo* 2088 ! **Fr. G.:** Rio Nunez *Heudelot* 659 ! Kouroussa (June) *Pobeguin* 264 ! 812 ! **S.L.** Wilberforce (fr. Mar.) *Johnston* 96 ! Mofari, Scarcies R. (Jan.) *Sc. Elliot* 4402 ! Njala (fl. Mar., fr. Apr.) *Deighton* 1097 ! 1824 ! 4742 ! Batkanu (Jan.) *Deighton* 2863 ! **Lib.:** Tappita (fr. Aug.) *Baldwin* 9100 ! Flumpa, Sanokwele (fr. Sept.) *Baldwin* 9354 ! Kulo, Sinoe (fr. Mar.) *Baldwin* 11437 ! Soplima, Vonjama (Nov.) *Baldwin* 10111*a*! **Iv.C.:** Baoule, Ferkessedougou, Bobo Dioulasso and Kampti *fide* Aubrev. *l.c.* **G.C.:** Axim (Mar.) *Chinn* 395 ! Kumasi (fl & fr. Feb.) *Irvine* 119 ! Assuantsi (Jan., Mar.) *Fishlock* 6 ! *Irvine* 1561 ! **Dah.:** Djougou *Chev*. 23875. **N. Nig.:** Nupe *Barter* 1656 ! Zungeru (July) *Dalz.* 64 ! **S. Nig.:** Lagos *Rowland* ! Ibadan (Feb.) *Meikle* 1149 ! Okomu F. R. (Feb.) *Brenan* 9039 ! Old Calabar (Feb.) *Mann* 2262 ! Br.Cam.: Buea (Mar.) *Maitland* 499 ! **F.Po:** *Mann* 12 !

A glabrous shrub or woody climber up to 12m tall, sometimes arborescent, often armed recurved stipular spines, with copious inflorescences of minute greenish flowers, and small berry-like red fruit. Monoecious, branches spreading or pendulous, main branches stout, angular, reddish tinged, branchlets 15–20(–25) cm long, with several short axillary shoots; branch basis transformed into a pair of spines c. 4 mm long, purplish brown. Leaves alternate, distichous along lateral twigs, simple, glabrous; stipules lanceolate, c. 2 mm long, acuminate; petiole 3–5 mm long; blade ovate, elliptical-ovate to ovate-lanceolate, 3–9 cm × 2–4.5 cm, base cuneate to rounded, apex acute to obtuse, with 10–14 pairs of lateral veins. Inflorescence a false raceme on short axillary shoots, 2–6 cm long, solitary or several together, with flowers in clusters having 2–3 male flowers and 1 female flower in each cluster. Flowers unisexual; perianth lobes 5, elliptical, c. 1 mm long, rounded, greenish white or greenish yellow; male flowers with pedicel c. 1.5 mm long, disk glands 5, free, minutely warted, fleshy, stamens 5, free, unequal, anthers very small; female flowers with stout pedicel c. 1 mm long, disk glands 5, free or fused, knobbly, fleshy, ovary superior, ellipsoid, warty, 4–5-celled, styles 4–5, free, c. 0.5 mm long, 2-fid at apex. Fruit a fleshy, nearly globose capsule 3–4 mm in diameter, usually smooth, green, becoming red, later black, 6-seeded. Seeds angular, c. 1 mm long, with faint ridges, bright reddish brown or yellowish brown (Burkill, 1994; Hutchinson and Dalziel, 1963).

f) Phyllanthus niruroides Mull-Arg.

F.T.A. 6, 1 : 715 ; Chev. Bot. 558. **Fr.G.:** Kouria *Chev.* 14839. **S.L.:** Freetown *Welw.* 316 ! Kambia (fl. & fr. Dec.) *Sc. Elliot* 4346 ! Newton (fl. & fr. Sept.) *Deighton* 4878 ! Rokupr (fl. & fr. Apr., Aug.) *Deighton* 3019 ! *Jordan* 235 ! Bo (fl. & fr. June) *Deighton* 5102 ! **Lib.:** Gbanga (fl. & fr. Sept.) *Linder* 522 ! Monrovia (fl. & fr. June, Nov.) *Baldwin* 5871 ! *Barker* 1462 ! Mt. Barclay (fl. & fr. Apr.) *Bunting* ! **Togo:** Misahohe *Baumann* 144 (partly). **S.Nig.:** Calabar (fl. & fr. July) *Holland* 44 ! Also in French Cameroons, Gabon, Belgian Congo, Tanganyika and S. Rhodesia.

This is an annual herb much branched, semi-woody, erect up to 75cm high. The stem is brownish, glabrous and woody at the base. The leaves are oblong with mucronate apices, 4-10mm long, 1.5-3mm broad, pale beneath and with short petioles. The inflorescence consisting of male flowers 2-3 together in lower leaf axils of branches, female solitary in upper axils; disk of female flower stellate with 5 deep lobes; style very short, deeply bifid. Tepals of both sexes 5, those of the females in a single series. Ovary prominently warted; curved back of seed marked with 12-14 fine longitudinal ridges. The fruit is a round capsule, greenish and 1.7-2mm across and occurs in leaf axils on the lateral branches. The flowers are light-green, small on short pedicels (Burkill, 1994; Hutchinson and Dalziel, 1963).

Dichotomous Bracketed Key to the morphology of the species of Phyllanthus studied

1. Habit, shrub; stipules, spiny ..... ***P. muellerianus.***
1' Habit, not shrub but herb; stipules, laterally free .....**2.**

2. Leaves, elliptic-oblong; stipules, greenish; stem form, rounded..... ***P. amarus.***
2' Leaves, not elliptic-oblong but oblong; stipules, reddish; stem form, pentagonal .....**3.**

3. Tepals, pentapetalous (5); fruits often spotted ..... ***P. niruroides.***
3' Tepals, not pentapetalous but hexapetalous (6); fruits not spotted ..... **4.**

4. Leaf apices, mucronate; stem, fruit and flower, reddish-green ..... ***P. urinaria.***
4' Leaf apices, not mucronate but obtuse; stem, fruit and flower, not reddish-green but green ..... ***P. odontadenius.***

Anatomical Observation

The results are presented in Table 3 and Fig 2-4. The epidermis of the five species was one layer thick. The mesophylls are differentiated into palisade and spongy parenchyma layers but these vary from species to species. The palisade parenchyma was 1 layer thick in *P. odontadenius* (Fig. 2c) and *P. muellerianus* (Fig. 2e), but 2 layers thick in *P. amarus* (Fig. 2a), *P. urinaria* (Fig. 2b) and *P. niruroides* (Fig. 2d). Large intercellular spaces occured in the spongy paranchyma of *P. urinaria, P. odontadenius* and *P. muellerianus* while *P. amarus and P. niruroides* have small intercellular spaces. The distribution of the collenchyma cells within the midrib of the studied species varied as well. *P. amarus, P. niruroides* and *P. muellerianus* possessed collections of collenchyma cells on both the adaxial and abaxial portions of the leaf while, *P. urinaria* and *P. odontadenius* displayed none on the adaxial but posses a few layers (1-2 layers respectively) on the abaxial portion. The vascular bundles consisting of the xylem, phloem and sclerenchyma possessed no bundle sheath. The nature of the xylem cells within the midrib varied within the five species. *P. amarus* (2 layers thick), *P. niruroides* (6 layers thick) and *P. muellerianus* (7 layers thick)

contained elongated xylem cells, while *P. urinaria* (2 layers thick) and *P. odontadenius* (3 layers thick) contained clustered xylem cells. The result showed that the vascular bundles of the five species have C3 anatomy: the vascular bundles are surrounded by small cells of the mesophylls devoid of chloroplast.

The stems of the five species consist of a single layer of somewhat spherical epidermis. The transverse section of *P. urinaria, P. odontadenius* and *P. niruroides* showed the presence of ridges and furrows while *P. amarus* and *P. muellerianus* posses none. The epidermis being a single outermost layer passes over the ridges and furrows. The presence of the ridges and furrows is as result of the angular and pentagonal nature of the epidermal circumference on the stems. The hypodermis (collenchymas) lies externally, the general cortex lies in the middle and endodermis lies internally. The hypodermis ranged from 2-3 layers in *P. amarus* (Fig. 3a), 2-5 layers in *P. urinaria* (Fig. 3b), 3-4 layers in *P. niruroides* (Fig. 3d), 3-5 layers in *P. odontadenius* (Fig. 3c) and *P. muellerianus* (Fig. 3e) respectively. Also, the shapes of the collenchymas varied from oval in *P. niruroides* to rectangular in *P. amarus, P. urinaria, P. odontadenius* and *P. muellerianus* respectively. The furrows in *P. urinaria* and *P. niruroides* consisted of about 1-2 layers collenchymatous cells respectively while *P. odontadenius* consisted of about 2-3 layers. The general cortex or cortical parenchyma forms narrow zone in the middle which ranged from 4-6 layers thick in *P. amarus,* 2-5 layers thick in *P. urinaria* and *P. niruroides,* 2-3 layers thick in *P. odontadenius* and 5-7 layers in *P. muellerianus.* Also, the shapes varied from oval in *P. amarus* and *P. niruroides*, oval and polygonal in *P. urinaria* and *P. odontadenius* to oval and rectangular in *P. muellerianus*. The endodermis lies immediately outside the pericyclic sclerenchyma. The pericycle, which represents the sclerenchyma; which is thick-walled and lignified is 2-3 layers thick in *P. amarus* and *P. muellerianus,* 2-5 layers thick in *P. urinaria,* and 1-2 layers thick in *P. odontadenius* and 3-4 layers thick in *P. niruroides.* The vascular bundles which include the xylem and phloem were arranged in ring form. The phloem vessels lay over head the xylem vessels. The phloem ranged from 4-9 layers thick in *P. amarus* and *P. odontadenius,* 3-5 layers in *P. urinaria,* 2-7 layers in *P. niruroides* and 3-5 layers in *P. odontadenius* while the number of elongated cells in xylem vessels ranged from 3-6 cells in *P. amarus,* 2-5 cells in *P. urinaria* and *P. odontadenius*, 2-8 cells in *P. niruroides* and 5-13 cells in *P. muellerianus.*

The roots of all the five species investigated are composed of an outer layer piliferous layer that is one layer thick in all the species studied. This layer is followed by thin walled cork cells. The cork cells varied from species to species. The cork cell was 5-9 cells thick in *P. amarus* (Fig. 4a), 8-11 cells thick in *P. urinaria* (Fig. 4b), 6-9 cells thick in *P. odontadenius* (Fig. 4c), 2-4 cells thick in *P. niruroides* (Fig. 4d) and 3-5 cells thick in *P. muellerianus* (Fig. 4e). The cortex of the species were seen as conspicuous as rays of cells rising from the middle which are composed of layer thin-walled, colourless paranchymatous cells with vascular bundles at the centre of the root. The cortex varied from 10-15 cells thick in *P. amarus*, 7-18 cells thick in *P. urinaria* and 6-12 cells thick in *P. odontadenius* and *P. niruroides* and 5-7 cells thick in *P. muellerianus*. The innermost layer of the cortex which is the endodermis is one layer thick in the five species. The vascular bundles contained both xylem and phloem with xylem being more prominent. The xylem size varied from 10-53μm in *P. amarus*, 11-43μm in *P. urinaria*, 15-62μm in *P. odontadenius,* 12-47μm in *P. niruroides* and 12-40μm in *P. muellerianus* while the phloem varied from 4-6 cells in *P. amarus,* 4-8 cells in *P. urinaria*, 5-8 cells in *P. odontadenius,* 3-5 cell in *P. niruroides* and *P. muellerianus* respectively.

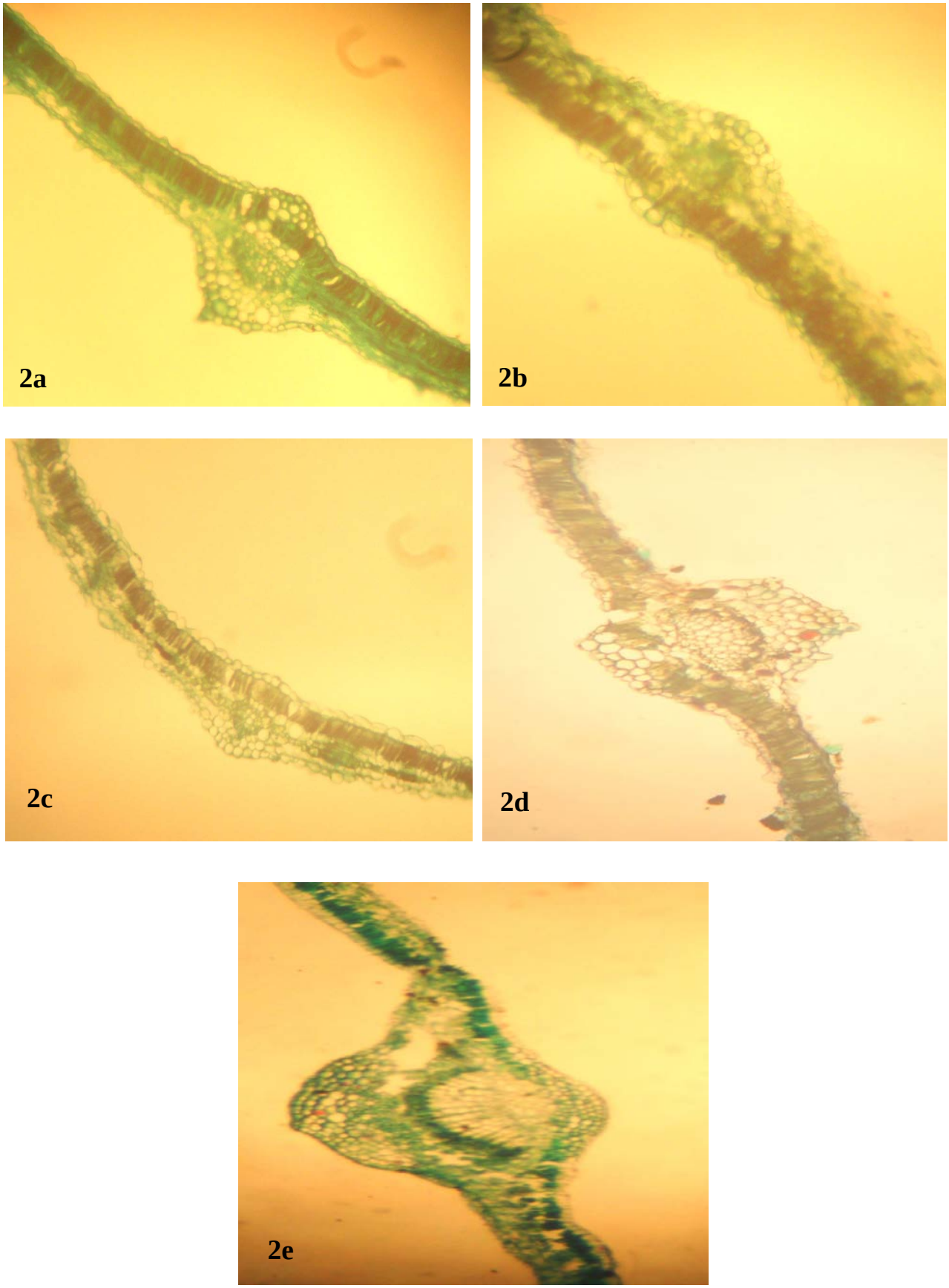


Figure 2 : Transverse section of the leaf of the genus *Phyllanthus* studied

(a) *P. amarus* – showing the tissue arrangement around the mid rib (x 200)
(b) *P. urinaria* – showing the tissue arrangement around the mid rib (x 200)
(c) *P. odontadenius* – showing the tissue arrangement around the mid rib (x 200)
(d) *P. niruroides* – showing the tissue arrangement around the mid rib (x 200)
(e) *P. muellerianus* – showing the tissue arrangement around the mid rib (x 200)

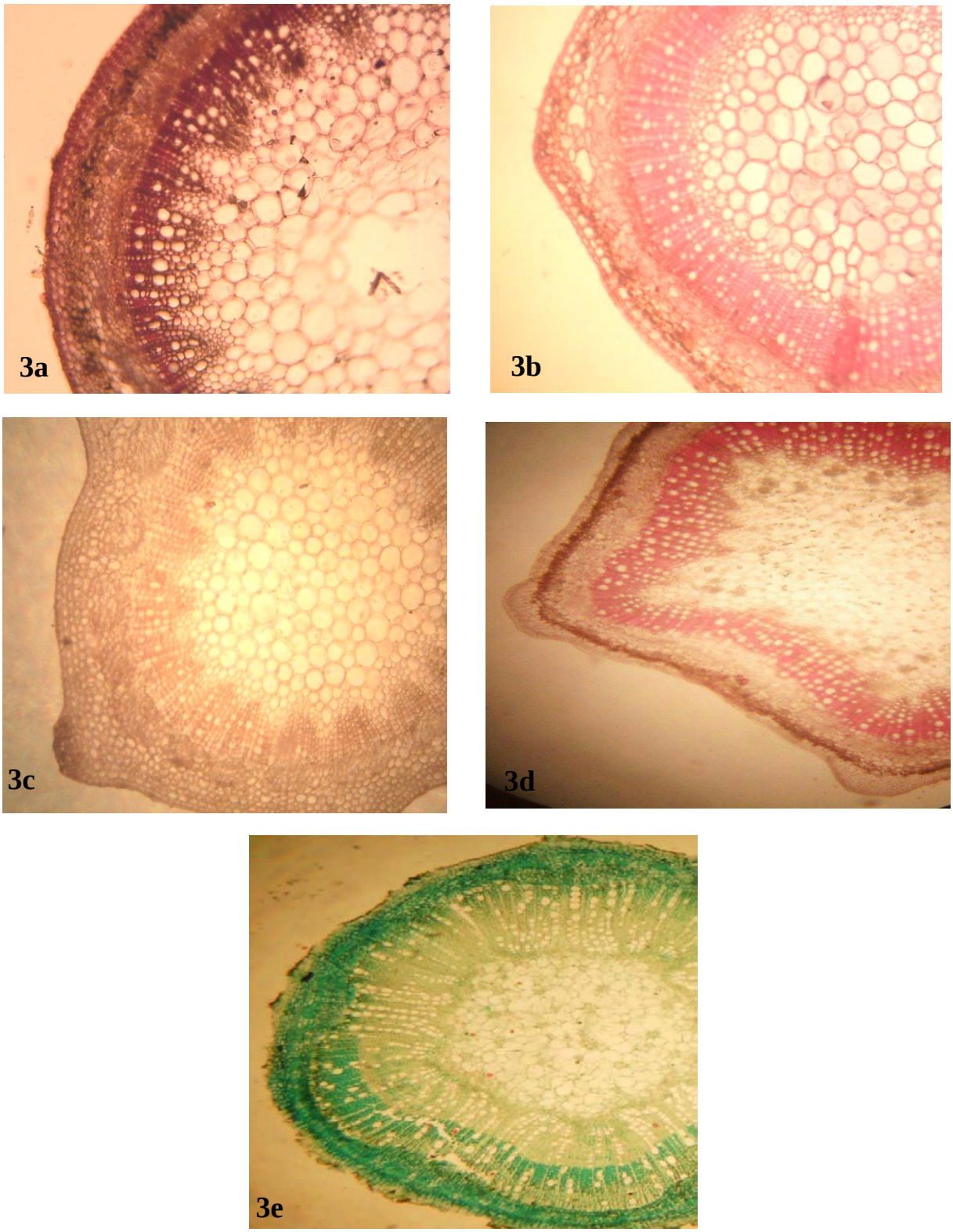
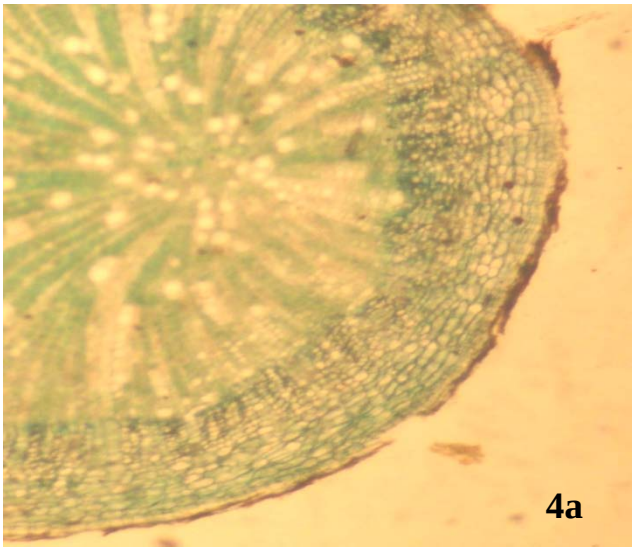
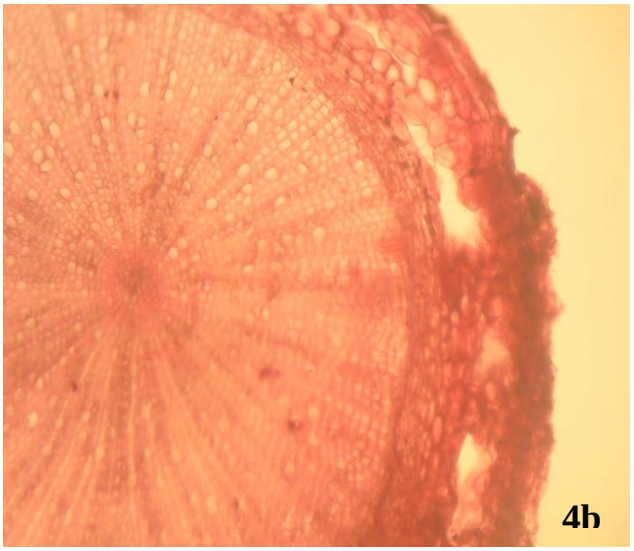
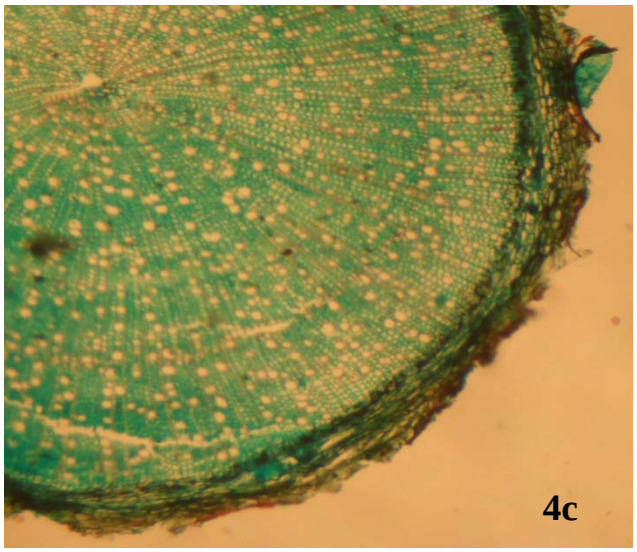


Figure 3 : Transverse section of the stem of the genus *Phyllanthus* studied

(a) *P. amarus* – showing the pith, arrangement of the cells in the cortex and rounded edges of the stem epidermis (x 100)
(b) *P. urinaria* – showing the pith, arrangement of the cortex cells and the angular edges of the stem epidermis (x 100)
(c) *P. odontadenius* – showing the pith, arrangement of the cortex cells and the angular edges of the stem epidermis (x 100)
(d) *P. niruroides* – showing the pith, arrangement of the cortex cells and the angular edges of the stem epidermis (x 100)
(e) *P. muellerianus* – showing the pith, arrangement of the cortex cells and rounded edges of the stem epidermis (x 100)







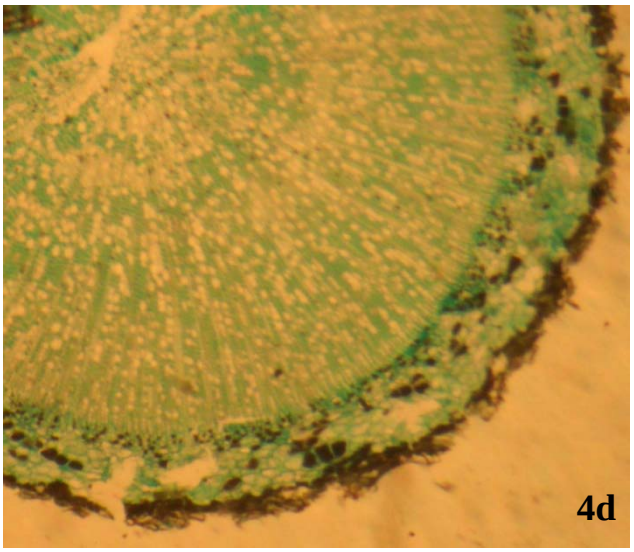


Figure 4 : Transverse section of the root of the genus *Phyllanthus* studied

(a) *P. amarus* – showing the cork cells and cortex (x 100)
(b) *P. urinaria* – showing the cork cells and cortex (x 100)
(c) *P. odontadenius* – showing the cork cells and cortex (x 100)
(d) *P. niruroides* – showing the cork cells and cortex (x 100)
(e) *P. muellerianus* – showing the cork cells and cortex (x 100)

Table 3 : Summary of the Anatomical Characters of the *Phyllanthus* species studied

CHARACTERS	*P. amarus*	*P. urinaria*	*P. odontadenius*	*P. niruroides*	*P. muellerianus*
LEAF					
Number of palisade parenchyma in the mesophyll	2 layer thick	2 layer thick	1 layers thick	2 layers thick	1 layer thick
Nature of intercellular spaces	Small	Large	Large	Small	Large
Nature of xylem cells within the mid rib	Elongated cells 2 layers thick	Clustered cells 2 layers thick	Clustered cells 3 layers thick	Elongated cells 6 layers thick	Elongated cells 7 layers thick
Distribution of the collenchyma cells within the midrib	1-2 layers on adaxial 2-4 layers on abaxial	None on adaxial 1-2 layers on abaxial	None on adaxial 1-2 layers on abaxial	1-3 layers on adaxial 2-5 layers on abaxial	1-6 layers on adaxial 2-9 layers on abaxial
STEM					
Epidermal circumference	Rounded	Angular	Angular	Angular	Rounded
Ridges and Furrows	Absent	Present	Present	Present	Absent
Hypodermis (Collenchyma)	Rectangular 2-3 layers thick	Rectangular 2-5 layers thick on ridges 1-2 layers thick on furrows	Rectangular 3-5 layers thick on ridges 2-3 layers thick on furrows	Oval 3-4 layers thick on ridges 1-2 layers thick on furrows	Rectangular 3-5 layers thick
Cortex (Parenchyma)	Oval 4-6 layers thick	Oval & polygonal 2-5 layers thick	Oval & polygonal 2-3 layers thick	Oval 2-5 layers thick	Oval & rectangular 5-7 layers

Pericycle (Sclerenchyma)	2-3 layers thick	2-4 layers thick	1-2 layers thick	3-4 layers thick	2-3 layers thick
Xylem cells	3-6 elongated cells	2-5 elongated cells	2-5 elongated cells	2-8 elongated cells	5-13 elongated cells
Phloem	4-9 layers thick	3-5 layers thick	4-9 layers thick	2-7 layers thick	3-5 layers thick
ROOT					
Cork cells	5-9 cells thick	8-11 cells thick	6-9 cells thick	2-4 cells thick	3-5 cells thick
Cortex	10-15 cells thick	7-18 cells thick	6-12 cells thick	6-12 cells thick	5-7 cells thick
Xylem	10-53µm	11-43µm	15-62µm	12-47µm	12-40µm
Phloem	4-6 cells thick	4-8 cells thick	5-8 cells thick	3-5 cells thick	3-5 cells thick

Dichotomous Bracketed Key to the leaf anatomy of the species of Phyllanthus studied.

1. Nature of intercellular spaces, small ..... 2.
1' Nature of intercellular spaces, large .....3.

2. Nature of xylem cells within the midrib, 2 layers thick..... *P. amarus.*
2' Nature of xylem cells within the midrib, 6 layers thick..... *P. niruroides.*

3. Number of palisade parenchyma in the mesophyll, 2 layers thick..... *P. urinaria.*
3' Number of palisade parenchyma in the mesophyll, 1 layer thick.....4.

4. Collenchyma cells distributed/present within the adaxial portion of the midrib; xylem cells, elongated..... *P. muellerianus.*
4' Collenchyma cells not distributed but absent within the adaxial portion of the Midrib; xylem cells, clustered..... *P. odontadenius.*

Dichotomous Bracketed Key to the stem anatomy of the species of Phyllanthus studied

1. Epidermal circumference, angular; ridges and furrows, present..... 2.
1' Epidermal circumference, rounded; ridges and furrows, absent.....3.

2. Hypodermal collenchyma, oval..... *P. niruroides.*
2' Hypodermal collenchyma, not oval but rectangular.....4.

3. Cortex parenchyma, rectangular and oval..... *P. muellarianus.*
3' Cortex parenchyma, never rectangular but oval..... *P. amarus.*

4. Phloem tissues, less abundant, 3-5 layers thick..... *P. urinaria.*
4' Phloem tissues, much abundant, 4-9 layers thick..... *P. odontadenius.*

IV. Discussion

Morphologically, the shrub habit and the presence spiny stipules in *P. muellerianus* as against the herbaceous nature and laterally free stipules of the other species are distinguishing spot characters which clearly delimits *P. muellerianus*. Other peculiar characters such as sub-acute leaf apex and ovate-elliptic leaf shape are also taxonomic attributes of the taxon. Among other species studied, *P. amarus* seems to be the only taxon that exhibited an elliptic-oblong leaf, greenish stipules and all-rounded stem form. This peculiar character presented a spot delimiting character since other herbaceous species lack the attributes. Although some overlapping characters within *P. urinaria, P. niruroides* and *P. odontadenius* in possessing reddish stipules and oblong leaf shapes, the number of tepals could be seen as a strong delimiting character in separating *P. niruroides* from *P. urinaria* and *P. odontadenius*. *P. niruroides* possessed 5 tepals while *P. urinaria* and *P. odontadenius* possessed 6 tepals respectively. Moreover, the fruits of *P. niruroides* are often spotted while other taxa are not. Also, the leaf apices and fruit colour in *P. urinaria* and *P. odontadenius* displayed a clear character spot in separating the two taxa. *P. urinaria* possessed a mucronate leaf apex and reddish fruit while *P. odontadenius* lacked the attribute but rather possessed an obtuse leaf apex and an entirely greenish fruits.

The conspicuous nature and thickness of the epidermis of the leaves among the five species of

Phyllanthus conformed to the opinion of Metcalfe and Chalk (1950) in dicotyledons. The epidermis is one layer thick and conspicuously visible in the five species investigated. On the contrary, there is a variation in the nature of intercellular spaces, which showed a line of evidence among the different groups of the taxa. *P. amarus* and *P. niruroides* possessed small intercellular spaces while *P. urinaria, P. odontadenius* and *P. muellerianus* possessed large intercellular spaces. But *P. amarus* can be separated from *P. niruroides* with the nature of xylem cells within the midrib in which *P. amarus* (Fig. 2a) is 2 layers thick while *P. niruroides* (Fig. 2d) is 6 layers thick. Among the taxa (*P. urinaria, P. odontadenius* and *P. muellerianus*) possessing large intercellular spaces in the mesophyll, the number of palisade parenchyma differed among them. This can be of taxonomic value in drawing the line among the taxa, 2 layers thick in *P. urinaria* (Fig. 2b) but 1 layer thick in *P. odontadenius* and *P. muellerianus* respectively. The distribution of the collenchyma cells within the midrib can also be of taxonomic evidence in separating *P. odontadenius* and *P. muellerianus* in which the tissues were found absent on the adaxial portion but present only on the abaxial portion in *P. odontadenius* (Fig. 2c) while *P. muellerianus* (Fig. 2e) possessed them on both the abaxial and adaxial portions. Also, the nature of the xylem cells differed between both taxa in which *P. odontadenius* possessed 3 layers of clustered xylem cells while *P. muellerianus* possessed 7 layers of elongated xylem cells. Metcalfe and Chalk (1950) used the anatomical arrangement of mesophyll layers in taxonomic conclusion of the species of *Phyllanthus*.

The variable characteristics possessed in the anatomy of the stem of the *Phyllanthus* species studied could be valuable in characterization. *P. urinaria, P. odontadenius* and *P. niruroides* possessed ridges and furrows which are passed over by a single outer layer of epidermis. The presence of the ridges and furrows were as result the angular nature of the stem circumference. This feature is absent in *P. amarus* and *P. muellerianus* due to the rounded stem circumference. With this, *P. amarus* and *P. muellerianus* can be separated from other taxa. But *P. amarus* can be further separated from *P. muellerianus* using the cortex parenchyma in which *P. amarus* (Fig. 3a) is oval while *P. muellerianus* (Fig. 3e) had a combination of oval and rectangular types within the cortex. The cortical parenchyma or the general cortex ranged from 4-6 layers thick in *P. amarus*, 2-5 layers thick in *P. urinaria* and *P. niruroides*, 2-3 layers in *P. odontadenius* and 5-7 layers thick in *P. muellerianus* respectively. The hypodermal layer of collenchymas, which differed among the species investigated, could be used in delineating the species. In this study, *P. niruroides* (Fig. 3d) can separated from other taxa in possessing oval type of collenchymas while other species displayed rectangular types. Furthermore, the possession of phloem tissues, which varied from 4-9 layers thick in *P. odontadenius* (Fig. 3c) to 3-5 layers thick in *P. urinaria* (Fig. 3b) can also be used as a good taxonomic tool to separate both taxa. It should be important to note that only young and matured stems were used for this study. Regions undergoing secondary growth were discarded from the research.

The anatomy of the roots possessed variable characters in the *Phyllanthus* species studied that could be used in their classification and characterization although they are mostly quantitative and overlapping. The possession of cork cells, which varied from 5-9 cells thick in *P. amarus,* 8-11 cells thick in *P. urinaria,* 6-9 cells thick in *P. odontadenius*, 2-4 cells thick in *P. niruroides* and 3-5 cells thick in *P. muellerianus* separates the various taxa from themselves. The cortex ranged from 10-15 cells thick in *P. amarus,* 7-18 cells thick in *P. urinaria*, 6-12cells thick in *P. odontadenius* and *P. niruroides* and 5-7cells thick in *P. muellerianus.* Another feature in the root which is of systematic value is the vascular bundles. The xylem size varied from 10-53μm in *P. amarus,* 11-43 in *P. urinaria*, 15-62μm in *P. odontadenius,* 12-47μm in *P. niruroides* and 12-40μm in *P. muellerianus.* The phloem also varied from 4-6 cells thick in *P. amarus*, 4-8cells thick in *P. urinaria*, 5-8 cells thick in *P. odontadenius,* 3-5 cells thick in *P. niruroides* and *P. muellerianus* respectively. The observation supports earlier studies of Edeoga and Okoli (1997); Edeoga *et al*. (2007) which revealed how the stellar arrangement has exhibited constancy in different plant and plant organs.

V. Conclusion

The morphological and anatomical studies of the leaves, stems and roots of the *Phyllanthus* species has provided additional evidence which may be combined with other existing lines of taxonomic evidence in arriving at a better identification and classification of *Phyllanthus* species.

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Synthesis, Characterization and Biodegradation of Some Polymeric Azo Compound

By Rehab Abdeen
Kinh Khalid university, Saudi Arabia

Abstract- A diazinum salt of Benzidine was coupled with aniline to form 4, 4\-Bis (1"-azo-4"-aminobenzene) biphenyl. The resulted azo dye was polymerized with different diacid chloride to form azopolymers. Monomer azodye and its azopolymer were determined using elemental analysis , Infrared spectroscopy and HNMR. The microbial degradation constant and rat of azopolymer were quantitatively assessed against fungi *(Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceous and Fusarium oxysporum). Pleurotus ostreatus Hngary* (HAR17) *And Ganoderma resencium* mycelial hyphae, *Candida albicans* was used as yeast model. However, *Escherichia coli* was used as bacterial organisms, using different concentration from azopolymers.

Keywords: azo dye, azo polymers, microbial degradation and azo reduction.

GJSFR-C Classification : FOR Code: 279999p

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I. Intoduction

Azo dye has received great attention due to its environmental stability, ease of preparation and its optical and electrical properties. Much work has been done on the molecular design, synthesis, and assembly of structures with desired properties [1-5]. The discovery of diazo compounds occurred around the year 1858, which parallels the beginning of what is considered the starting point of modern organic chemistry [6,7].

An area of polymer research that presents great current interest, yet has received insufficient attention, is that of the development of polymers with antimicrobial activities, generally known as polymeric biocides. In the area of health care and hygienic applications, biocidal polymers may be incorporated into fibers, or possibly extruded into fibers themselves, and used for contact disinfectants in many biomedical applications such as sterile bandages and clothing. For example, antimicrobial surgical gowns and antifungal polymeric coatings on surfaces such as shower walls and many kinds of tubing minimize the problems of biofouling and the release of pathogenic microorganisms into streams of flowing fluids [8].

To overcome problems associated with the low molecular weight antimicrobial agents, antimicrobial functional groups can be introduced into polymer molecules. The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial agents. . Also, polymeric antimicrobial agents have the advantage that they are nonvolatile and chemically stable and do not permeate through skin. Therefore, they can reduce losses associated with volatilization, photolytic decomposition, and transportation. In the field of biomedical polymers, infections associated with biomaterials represent a significant challenge to the more widespread application of medical implants [9–13]. Research concerning the development of antimicrobial polymers represents a great a challenge for both the academic world and industry [8]. Significant advances in the past three decades have been made in the synthesis and applications of polymers to prevent microbial attack and degradation for diverse end uses [15].

Basic Requirements for Antimicrobial Polymers. The ideal antimicrobial polymer should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a waterdisinfection application, (4) does not decompose to and/or emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity, and (7) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact [8]. The elucidation of degradation pathways is of special interest considering health and environmental priorities. Directly on incubation medium have been used for the first time to follow kinetics of sulfonated azo dye Orange II enzymatic degradation. Nine transformation products were identified using these complementary analyses performed ex situ without any prior treatment. Three types of cleavage are proposed for the degradation pathway: (i) a symmetrical splitting of the azo linkage that leads to the formation of 4-aminobenzenesulfonate (and 1-amino-2-naphthol, not detected); (ii) an asymmetrical cleavage on the naphthalene side that generates 1,2-naphthoquinone and 4-Diazoniumben-zenesulfonate as products, with the latter one being transformed into 4-hydroxybenzensulfonate; and (iii) a third degradation pathway that leads to 2-naphthol and 4-hydroxybenzenesulfonate [16].

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The goal of this work is to prepare and investigate the production of polyamide containing azogroup, by the reaction of Benzedine with aniline and copolymerization with different diacide chlorideby different methode and discusses the mechanism of degradation of azopolymers.

II. Experimental

a) Materials

(*Benzidine*, aniline, Terephthaldehyde, PTHFdipropionic acid and Dithiodipropionic acid) was purchased from Aldrich, USA and was used as received without further purification. Succinic acid was purchased from El-Naser pharmaceutical chemicals, Egypt and was used as received. Adipic acid was used as received from El-Gomhouria chemicals Co., Egypt. Azealic acid was purchased from Aldrich and was used as received without further purification.

b) Microorganisms

The following microorganisms were chosen to evaluate its activity towards the reduction or (degradation) of the synthesized polymers Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceous and Fusarium oxysporum were isolated from the soil and identified. Pleurotus ostreatus Hngary (HAR17), was obtained from Agricultural research center, Cairo. Egypt. *And Ganoderma resencium* mycelial hyphae were isolated and purified from their fruiting bodies, *Candida albicans* was used as yeast model. However, *Escherichia coli was used as bacterial organisms.*

c) Media

Nutrient agar and Nutrient broth medium were used for growing *Escherichia coli* as bacterial cultures. However, *Sabouraud dextrose agar was used for growing of Candida albicans.* Additionally, Malt extract medium was used for growing *Pleurotus ostreatus, Ganoderma resencium, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium oxysporum, and Aspergillus ochraceous.*

d) Evaluation of the azo polymers microbial biodegradation.

For each tested organism a series of a test tube have been prepared to obtain active vegetative microbial growth before the process of biodegradation. Stock cultures were used to inoculate 5 ml of broth medium specific for organism in number of tested tubes. After incubation for ~24 h at specific temperature for each organism, the cells were harvested by centrifugation (4000 rpm) for 10 min, suspended in 0.5 ml of PBS buffer, pH 7.2 (2mM KH2PO4, 3mM Na2HPO4. H2O, 167 mM NaCl) containing 0.125 mM benzylviologen and 6.3 mM (D.glucose monohydrate). A certain volume has been over the proper solid medium, and the colony forming unit has been determined by spread plate technique.

Approximately 10^7 colony forming unit per ml of tested microorganisms were used to inoculate the proper medium contains 33 μM of tested azo polymers and 0.125 mM benzylviologen in test tube. Then the test tubes were closed with rubber stopper, and incubated at 37^{o}C in a horizontal shaking water bath, set at 100 rpm/min. At regular time intervals, one tube was withdrawn from the water bath, opened and 0.5 ml of 30 % trichloroacetic acid aqueous solution was added to stop the reaction, the absorbance of the clear supernatant was measured at the maximum wavelength of absorbance (λ max=332 &228 for XXI and 320 &230 for XXVII) of the tested azo polymer. A calibration graph for each azo polymer was carried out by measuring the absorbance of PBS buffer solution pH 7.2, solution containing 3% (w/w) of trichloroacetic acid and known concentration of the azo polymer. From the calibration graph, the azo polymer concentration was determined and plotted against the time. The rate of azo polymer degradation (K, the slope of the linear part of the degradation curve) was calculated as micromoles of azo polymer degraded per hour and per ml of inoculum (μ mol/h/ml).

III. Instrumentation

FTIR spectra were recorded on a Perkin- Elmer 1430 Ratio Recording Infrared Spectrophotometer apparatus from KBr pellets.

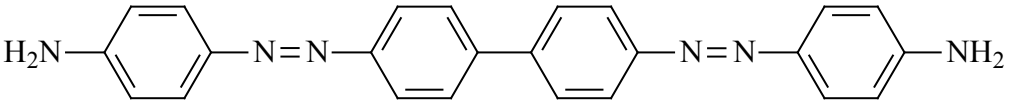
UV spectra were taken on a Shimadzu UV-2101 Dc Spectrophotometer.

^{1}H NMR spectra were recorded using a Varian 300 M, Mercury-Oxford and a Jeol JNM-PM X90 SI NMR spectroscopy.

Tetramethylsilane (TMS) was employed as the internal standard. Melting points were determined on a Gallenkamp apparatus.

IV. Preparation

a) *Synthesis of 4, 4\-Bis (1"-azo-4"-aminobenzene) biphenyl (I)*

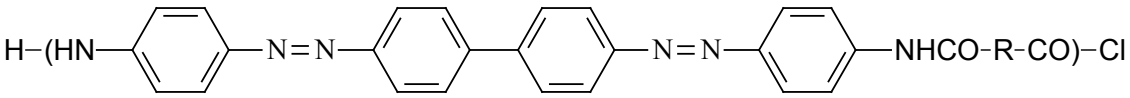


(I)

Benzedine, 1.9 g (10 mmol) was dissolved in 8 ml of conc. hydrochloric acid and 90 ml water. The solution was cooled to 2 °C, and then 5 ml of the sodium nitrite solution (1.5 g of sodium nitrite in 5 ml of water) was added dropwise, below 5 °C. The reaction mixture was stirred for another 1 h at 5 °C, and then was filtered. The filtrate was added dropwise to the aniline solution, (1.92 g, 20 mmol) aniline in 5 ml hydrochloric acid and 50 mL water. The solution was stirred for 1 h, and neutralized with a solution of sodium acetate, then kept overnight. The formed yellow azo product was filtered off, washed with water (3x), and then dried under vacuum at room temperature overnight. The product with molecular formula $C_{24}H_{20}N_6$ and molecular weight (392.46) was obtained in 90% yield. It was characterized by ^{1}H NMR, elemental analysis and IR as shown in Tables (1, 2, 3), respectively.

b) *Polymerization of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl (I) with various diacid chlorides*

Polycondensation of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl (I) with various diacid chlorides were carried out by two methods as follows:-



(II-VIII)

c) *Solution Polycondensation Method*

Polycondensation of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl (I) with various diacid chlorides was achieved by the reaction of the components in dry ethanol free chloroform. The following procedure using succinyl diacid chloride is typical.

For a cooled solution of 1.55 g (10 mmol) of the succinyl diacid chloride in 15 ml of dry ethanol free chloroform, 18 mL of TEA was added in a 100 mL round bottomed flask. The reaction mixture was stirred in an ice bath at -10 °C for 15 min, then a solution of 3.92 g (10 mmol) of compound (I), in 25 ml dry ethanol free chloroform, was added dropwise with constant stirring. The reaction mixture was further stirred at -10 °C for 30 min then for 48 h at room temperature .The chloroform layer was extracted with 0.1 M HCl (3X), 0.1 M NaOH (3X) and finally with water. The chloroform layer was dried over anhydrous $MgSO_4$ overnight at room temperature. The $MgSO_4$ was then filtered and the chloroform was evaporated on rotary evaporator and the product (II) was further dried under vacuum at room temperature overnight. The product was characterized by elemental analysis as shown in Table (2) and IR spectra as shown in Table (3).

Other solution polycondensation were carried out similarly. Scheme (2) shows the outlines of the reaction, and Table (4) show the quantities of the reactants involved. Polymers (III-VIII) were characterized by elemental analysis as shown in Table (2) and IR spectra as shown in Table (3). Polymer (VII) was also characterized by ^{1}H NMR cf. Table (1).

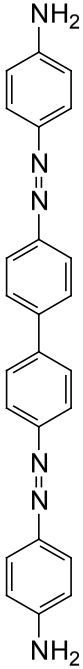
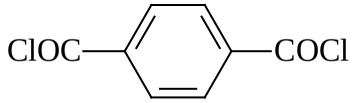
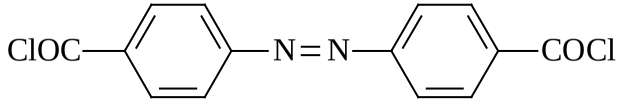
d) *Interfacial polycondensation method*

Interfacial polycondensation of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl (I) with various diacid chlorides was achieved by the reaction of the components in methylene chloride. The following procedure using succinyl diacid chloride is typical:

A solution of 3.92 g (10 mmol) of (I) in 45 mL water, 2 drops of pyridine and 13 mL dichloromethane was vigorously stirred. Then a solution of 2.31 g (10 mmol) of succinyl diacid chloride in 27 mL of dichloromethane was added with constant stirring for 10 min. Product (II) was collected by filtration using G4 sintered glass funnel, washed (3x) with dichloromethane, and dried under vacuum at room temperature overnight. Product (II) was characterized by elemental analysis as shown in Table (2) and IR spectra as shown in Table (3).

Other interfacial polycondensation were carried out similarly. Scheme (2) shows the outlines of the reaction and Table (4) shows the quantities of reactants involved. Polymers (III-VIII) were characterized by elemental analysis as shown in Table (2) and IR spectra as shown in Table (3).

Table 4 : Reactant quantities of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl with various diacid chlorides melting point

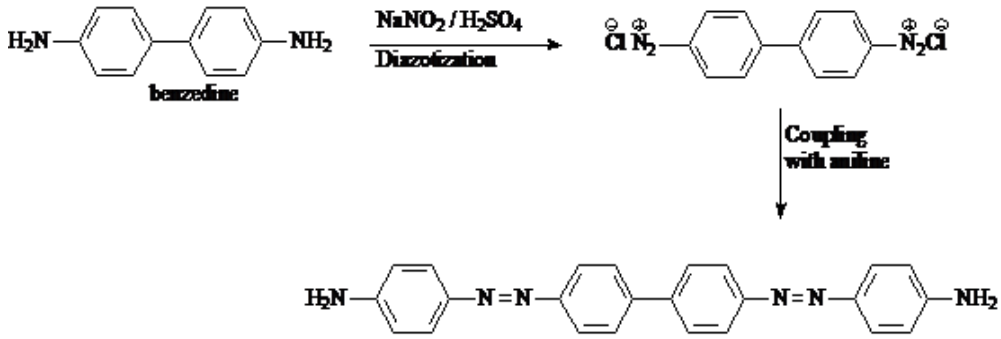
Monomer (mmol, g)	Diacid chloride COCl-R-COCl	Diamine (mmol, g)	Polymers		
			Code	m.p (° C)	Yield (%)
 (10 mmol,3.29 g)	$ClCO—(CH_2)_2—COCl$ Sucinnyl dichloride	(10 mmol, 1.55g)	**II**	175	72
	$ClCO—(CH_2)_4—COCl$ Adippyl dichloride	(10 mmol, 4.26 g)	**III**	160	75
	$ClCO(CH_2)_8COCl$ Azeil dichloride	(10 mmol, 1.8 g)	**IV**	195	77
	$ClCO—(CH_2)_3—S—S—(CH_2)_3COCl$ Dithiodipropyl dichloride	(10 mmol, 2.1 g)	**V**	___	Viscous 82
	$ClOC—(CH_2)_3—O—(CH_2CH_2CH_2CH_2O)_n—(CH_2)_3—COCl$ PTHF dipropyl dichloride	(10 mmol, 8.14 g)	**VI**	___	Viscous 80
	 Terephthaloyl dichloride	(10 mmol, 2.1g)	**VII**	Over 280	70
	 ABAC	(10 mmol, 3.71g)	**VIII**	240	74

V. Results and Discussion

a) Synthesis of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl (I)

A procedure using sodium nitrite and sulphuric acid, and coupling of the diazonium salt with aniline was done in moderately acidic medium at 0-5°C, to give a yellow dye of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl **(I)** as shown in **Scheme (1).** The azo dye **(I)** was in yield (90%), and its structure was conformed by elemental analysis. The data as given in **Table (2)**, it was in good agreement with the calculated values. IR spectrum of the dye **(I)** as in **Table (3)** showed the appearance of peak at 1517 cm^{-1}, 1453 cm^{-1} for (-N=N-), at 1614 cm^{-1} for (-NH_2) which confirmed the formation of the azo dye.

^{1}H NMR spectrum for dye **(I)** was recorded in (d_6-DMSO) and showed the following peaks: δ= 7.0-8.0 ppm (*m*, 12 H , ArH), and δ= 12.4 ppm (*s*, H, 2 NH_2) cf. **Table (1)**.



Scheme (1) : Synthetic route of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl

b) Polymerization of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl with various diacid chlorides

The polymerization 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl was carried out by two methods, the first is solution polycondensation in ethanol free chloroform, with the use of triethylamine (TEA) as an acid acceptor, and the second is the interfacial polycondensation in dichloromethane with the use of pyridine as an acid acceptor.

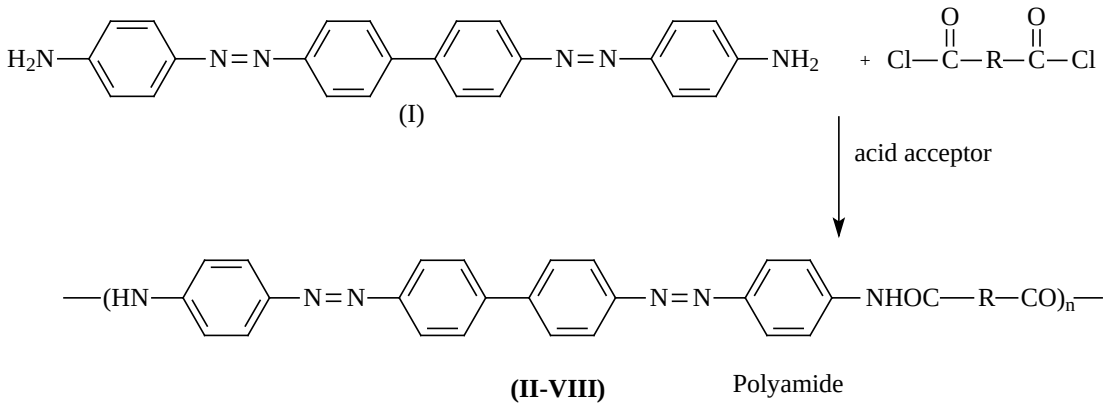
i. *Solution polycondensation*

Biodegradable azo-containing polyamides were prepared by solution polycondensation of azo monomer, 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl **(I)** with various diacid chlorides.

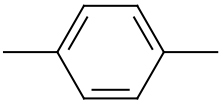
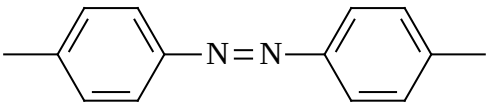
The solution polycondensation of the diacid chlorides was carried out using the quantities listed in **Table (4)**. The amount of the diacid chloride was added to OH terminated azo monomer, 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl **(I)** in the presence of dry TEA as an acid acceptor to form the corresponding azopolyester. The reactions were conducted at low temperature then at room temperature under anhydrous condition as outlined in **Scheme (3).** Both polymer products were obtained in high yields.).

The elemental analyses of the synthesized azopolyamides are in a good agreement with the calculated values as shown in **Table (2).** The IR spectra of the azopolymers as in **Table (3)** showed appearance of peaks at1520-1526 cm^{-1} and 1630-1774 cm^{-1} for (C=O) in (CONH), at 3028-3052 cm^{-1} and 3314-3426 cm^{-1} for (N-H) in (CONH), and disappearance of peak at 701 cm^{-1} for (-C-Cl). The appearance of peak at 2924-2935 cm^{-1} for $(CH)_{alph}$ in samples **(II-VII)** confirmed the formation of azopolyamides.

The ^{1}H NMR spectra for azo polyamide **(VIII)** was recorded in (d_6-DMSO) : δ= 7.0-8.0 ppm (m, 12 H, ArH), δ= 6.5 ppm (s, H, NH_2) and δ= 10.1 ppm (s, H, NH) cf. **Table (1)**.



Scheme (2) : Synthetic route of copolymer of 4, 4\-bis (1"-azo-4"-aminobenzene) biphenyl with various diacid chlorides

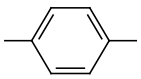
Azo polymer	Diacid chloride	-R-
II	Succinyl dichloride	—$(CH_2)_2$—
III	Adipyl dichloride	—$(CH_2)_4$—
IV	Azeil dichloride	—$(CH_2)_8$—
V	Dithiodipropyl dichloride	—$(CH_2)_3$—S—S—$(CH_3)_3$—
VI	PTHF dipropyl dichloride	—$(CH_2)_3$—O—$(CH_2CH_2CH_2CH_2O)_n$—$(CH_2)_3$—
VII	Terephthaloyl chloride	
VIII	ABAC	

ii. *Interfacial polycondensation*

Interfacial polycondensation of 4\-bis (1"-azo-4"-aminobenzene) biphenyl **(I)** with various diamines were carried out by A solution of the diacid chloride in dry methylene chloride according to the quantities of the reactants used listed in **Table (4)** was added to 4\-bis (1"-azo-4"-aminobenzene) biphenyl **(I)** in (water-methylene chloride) mixture in the presence of pyridine as acid acceptor as shown in **Scheme (2).** This method is faster than solution polycondensation, but with low yield as a result of hydrolysis apart of azobenzene diacid chloride. The quantities of the reactants used are as listed in **Table (4)** and the reaction scheme is as outlined in **Scheme (3).**

The elemental analysis and the IR spectra of the synthesized azopolyamides from interfacial polycondensation were similar to the azopolyamides synthesized from solution polycondensation.

Table 1 : ^{1}H NMR. shifts in ppm for azo dye **(I)** and polymers **(VIII)**

Groups / Compound		NH	NH_2
1	6.5-8 (m)		6.2 (s)
VIII	7-8 (m)	10.1 (s)	6.5 (s)

** s. singlet m: multiplet*

** Appear of two peaks at δ= 2.4 for DMSO and δ=3.5 for water*

Table 2 : Elemental analyses of azodyes and its copolymers

Polymer	C%		H%		N%	
	Calc.	Found	Calc.	Found	Calc.	**Found**
I	73.46	72.92	5.13	4.57	21.40	**21.30**
II	66.01	62.33	5.10	4.45	11.80	**11.10**
III	71.70	63.74	5.20	5.39	16.70	**14.44**
IV	72.66	67.92	6.05	6.35	15.40	**13.70**

V	63.56	59.98	4.20	3.95	16.09	15.11
VI	68.87	62.90	5.10	5.38	16.19	12.65
VII	75.60	70.39	5.70	4.74	10.50	8.70

Table 3 : I. R. analysis of azodyes and its copolymers

Polymer	$-NH_2$	1,4 Disubstituted benzene	$-N=N-$	$\overset{O}{\overset{\|}{C}}-NH$		$(CH)_{alph}$	N=CH
				C=O	-N-H		
I	1614	697	1453, 1517	–	—	—	—
II	—	821	1443, 1553	1630, 1520	3052, 3423	2928	—
III	—	759	1452, 1592	1664, 1520	3088, 3402	2933	—
IV	—	758	1406, 1595	1664, 1526	3040, 3314	2929	—
*V	—	808	1434, 1565	1727, 1520	3424	2924	—
*VI	–	756	1408, 1486	1732, 1519	3028, 34444	2935	—
VII	—	759	1441, 1596	1774, 1520	3421	–	—
VIII	–	761	1489, 1599	1677, 1520	3037, 3426	—	—

** measured in solution state in chloroform.*

iii. *Microbial degradation of the azo polymers*

The degradation of azopolyamide by tested fungal species as *Aspergillus*, *Candida albicans*, *Escherichia coli, Pleurotus ostreatus* and *Ganoderma resencium* vs the incubation time was study. It can be seen that, after an initial latency phase, the concentration of azo compounds decreases linearly as a function of the incubation time, indicating that the azo reduction is a zero-order phenomenon [16].

Moreover, the linearity of the degradation was confirmed by studying the influence of the dye concentration on the degradation rate (data not shown). No change of the degradation rate could be observed when the initial concentration of azo polymer was doubled.

To our knowledge, almost little or no work has been done concerning the biodegradation of azo dyes by fungi. Present study revealed that all tested microorganisms could degrade poly azo benzene diamin-*co*-PTHF dipropyl dichloride as indicated by measuring the absorbance at wave length 328 nm. The maximum biodegradation percentage was obtained by *A. fumigatus* (97%), and the minmum was achieved with *E. coli* (91%) at wave length 328 nm. However, *A. flavus* was degraded 89% of used poly azo benzene diamin-*co*-PTHF dipropyl dichloride as indicated with measuring absorbtion at 230 nm. Furthermore, *A. flavus* was also performed the highest degradation percentage for poly azo benzene diamin-*co*-Azelic dichloride (96%) at both measured wave length (332 and 228 nm). *E. coli* didn't produced any absorbance change at either at 332 nm in case of poly azo benzene diamin-*co*-PTHF dipropyl dichloride or at 228 nm in case of poly azo benzene diamin-*co*-Azelic dichloride indicating that the degradation of these polymers might with a mechanism different from that of fungi. Actually, the process of azo polymers degradation isn't easy. Bacteria or fungi are unable to oxidized azo dyes readily due to the azo linkage which doesn't occur in nature [17]. On the other hand, it has been found that aerobic bacteria can degrade aromatic amines. Therefore, non-enzymatic reduction of azo dyes to amines could facilitate further degradation by bacteria. It has been demonstrated that two *pseudomonas* strains completely degrade 6-aminonaphthalene-2-sulfonic acid [18].

Degradation of poly azo benzene diamin-*co*-PTHF dipropyl dichloride and poly azo benzene diamin-*co*-Azelic dichloride as azopolymer by microorganisms has demonstrated that *Aspergillus* fungi have produced the highest value, followed with yeast (*C. albicans*,), and then *E. coli* as bacterial organism. However, *G. resencium* and *P. ostreatus* as a model organism of basidiomycetic fungi came in the last rank.

There is extensive informations available on biodegradation of polymers by hydrolysis; although little is known about azo polymers. There is no reliable

evidence to suggest that the insoluble azopolymers degradable through azo reduction by biological systems. In spite of the ability of many bacteria and mammalin cells to cleave the azo bonds in low molecular weight azo compound and water soluble high-moleculare weight polymeric derivatives of certain azo dyes has been demonstrated.[19]

Current data indicated that the degradation of poly azo benzene diamin-*co*-PTHF dipropyl dichloride and poly azo benzene diamin-*co*-Azelic dichloride, with different tested microorganisms produced different values of degradation rate constant (Azo Reductase Activity), which varied from organism to other. The highest rate constant of poly azo benzene diamin-*co*-Azelic dichloride degradtion was obtained by *A. niger* which produced 5.5±0.115 μmol/ml/h at 230 nm, however *A. ochraceous* performed 0.84±0.12 μmol/ml/h. degradation constant increase of poly azo benzene diamin-*co*-PTHF dipropyl dichloride at 328 nm. The highest poly azo benzene diamin-*co*-Azelic dichloride degradation rate constant (μmol/ml/h) was obtained by *A. fumigatus* which preformed 5.71±0.23 μmol/ml/h at 228 nm; however *A. ochraceous* produced the lowest poly azo benzene diamond- *co*- Azelic dichloride degradation rate constant of 0.94±0.72 μmol/ml/h at 332 nm.

We anticipate that the azo polymers degradation process occurs exactly if the azo reductase was able to distinguish between the azodyes present concurrently in the medium according to their redox potential as also listed by [19]. Surprisingly, *P. ostreatus* and *G. resencium* have preformed the hightest rate constant for degradation of poly azo benzene diamin-*co*-Azelic dichloride which produced 10.43±0.21 and 8.55±0.16 μmol/ml/h at 330 nm and *Pleurotus ostreatus* (edible mushroom) was preformed a high rate of degradation constant more than *Ganoderma resencium* with two synthesized polymers as shown.

Since the discovery of the activation mechanism of sulphasalazin into 5-amino salicylic acid by the intestinal microflora. Until now, there is insufficient understanding of azo reduction mechanism and the difficulty in obtaining successful colonic delivery system to protect a drug from mouth to caecum and to afford its site- specificity are obstacles[19]. From results it appears that no relationship could be established between the structure of azopolymers and degradation rate constant value (K). The degradation process occurs exactly as if the azo reductase were able to distinguish between the azopolymers present in the medium.

We believe that adding one of the safe microorganisms used in this study to the drug coated or supplied or supplied with the azopolymer will performed hydrolysis or degradation to the polymer and hence liberate the drug.

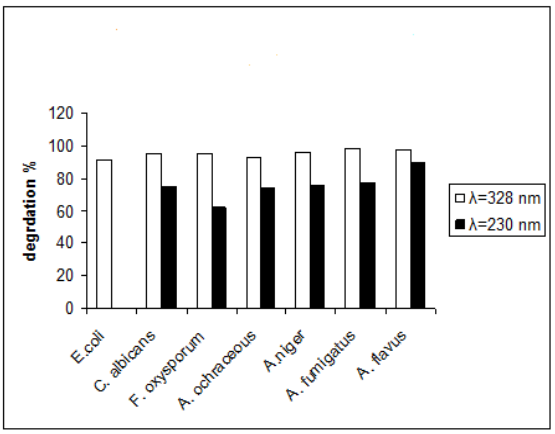


Figure 1: Degradation percent for poly azo benzene diamin-*co*-PTHF dipropyl dichloride (VI) against different microorganisms

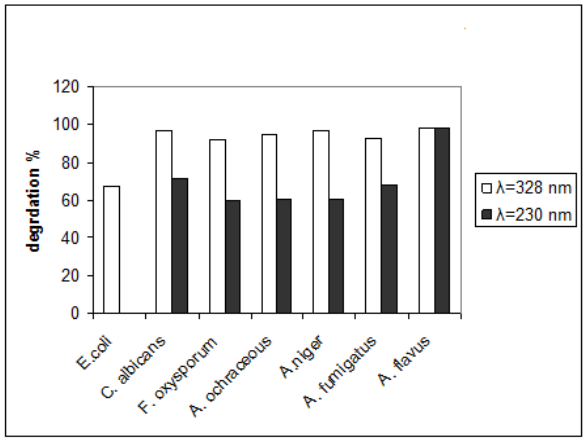


Figure 2 : Degradation percent for poly azo benzene diamin-*co*-Azelic chloride (IV) against different microorganisms

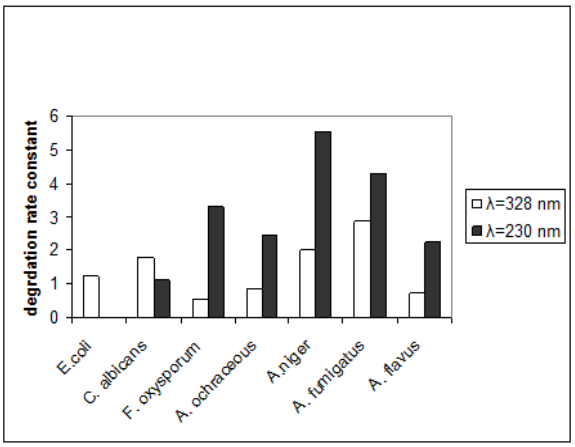


Figure 3 : Degradation rate constant for poly azo benzene diamin-*co*-PTHF dipropyl dichloride (VI) against different microorganisms

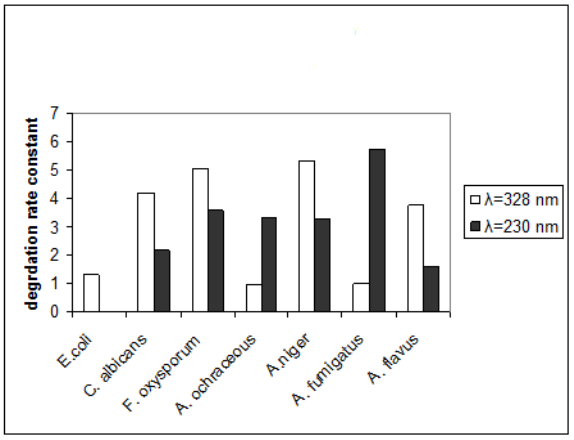


Figure 4 : Degradation rate constant for poly azo benzene diamin-*co*-Azelic chloride (IV) against different microorganisms

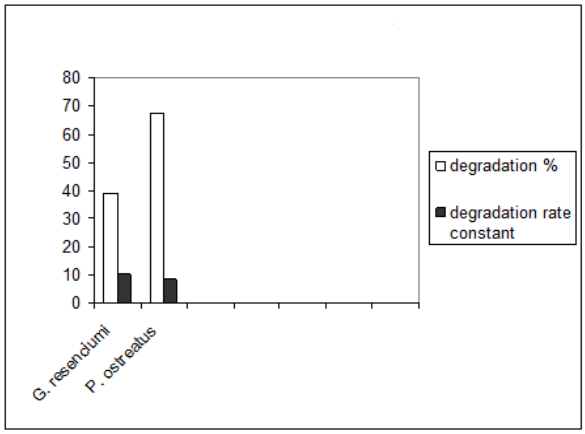


Figure 5 : poly azo benzene diamin-*co*-PTHF dipropyl dichloride (VI) against different microorganisms

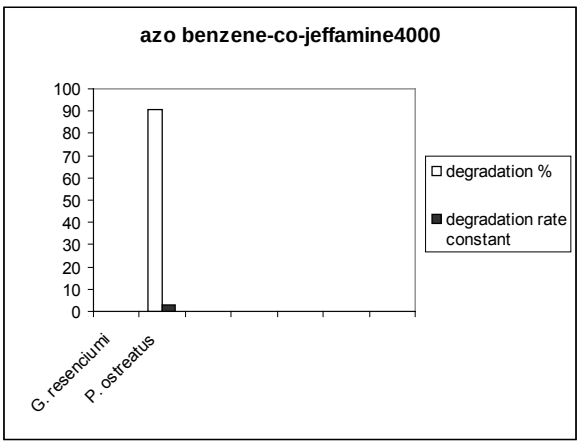


Figure 6 : poly azo benzene diamin-*co*-Azelic chloride (IV) against different microorganisms

VI. Conclusion

Azo dye polymer compounds have been synthesized from 4, 4\-Bis (1"-azo-4"-aminobenzene) biphenyl with diacid chloride. The azo dyes were investigated by elemental analysis, infrared spectroscopic, H^1NMR spectroscopic and absorption spectrum are used to prove the structure of azodye and its polymers. The UV-visible spectroscopic studies shows that the novel azo dye polymer compound has high degradation rate by treatment with different organisms.

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Nutritional Quality of Osmotically Dehydrated Catfish (*Clarias Garipinus*) and Beef as Influenced by Sodium Chloride Concentration

By Burubai, W., Samson, D. J & Etekpe, G. W.
Niger Delta University, Nigeria

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Keywords: *catfish, beef, vitamin c, osmotic dehydration, sodium chloride.*

GJSFR-C Classification : *FOR Code: 069999*

Nutritional Quality of Osmotically Dehydrated Catfish (*Clarias Garipinus*) and Beef as Influenced by Sodium Chloride Concentration

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I. Introduction

Nutritional losses have been found to accompany virtually every agricultural processing activity. These processing activities includes drying, washing, size reduction, cold storage, blanching, sterilization etc, and the effect of these activities on finished product quality, ultimately, determines the usefulness and commercial viability of that unit operation.

Drying (either oven, sun or osmotic) amongst them is one of the worst unit operations were nutritional losses are encountered and reported. It is indeed, accompanied by loss of volatiles, proteins, vitamins, aroma, texture and changes in colour. Thus, to investigate the nutritional losses during processing, scientist and food engineers have considered vitamin C as a nutrient quality index or marker during processing and storage of foods (Fennema, 1977; Tarade et al, 2007; Abioye et al, 2013).

Vitamin C, otherwise called ascorbic acid, is an important component of food with a broad biological activity relevant to human health. It is an antioxidant and the recommended daily allowance (RDA) for ascorbic acid is 75mg for women and 90mg for men. It is a white, crystalline, odourless and water-soluble compound (Andrea et al, 2006). Vitamin C supports the absorption of iron and the formation of collagen and is often added to foods not only as a nutrient to make up for processing losses, but is added to prevent the browning of fresh and canned fruits and vegetables, the acidification of fish and meat (Andrea et al, 2006; Kirby et al, 1996). This has led several scientists to investigate the degradation potentials of vitamin C in various agricultural and food products during processing and storage (Gordon and Samaniego, 1990; Vikran et al, 2005; Rao et al, 1981; Kajihausa et al, 2010; Hand et al, 2005). It is water-soluble and in water readily oxidizes first to dehydroascorbic acid, then to diketogulonic, oxalic, and threonic acids (Andrea et al, 2006). The first reaction is known to be reversible, but the subsequent ones are not. Therefore the content of vitamin C in agricultural products and foods can decrease during food preparation, preservation and storage.

Beef and catfish (*Clarias garipinus*) are the most commonly available and widely consumed sources of animal and fish proteins in developing countries like Nigeria. However, smoke-drying is the commonest means of preservation with its attendant high cost of firewoods(energy), and nutrient losses. In contemporary food technology, the focus is to conserve energy, reduce cost, improve quality and maximize retention of nutrients in both processing and storage. It is therefore appropriate to try other means of preservation such as osmotic dehydration.

Osmotic dehydration is an important complementary treatment and food preservation method in the preservation of foods. It presents some benefits which includes reduction of damage of heat to flavour, colour, decrease in energy cost, increase in shelf-life, retention of aroma etc (Alakali et al, 2006; Torres et al, 2006; Moazzam, 2012). In osmotic dehydration, the food product is immersed in osmotic solution such as salts, alcohols, sugars etc. However, there is dearth of information on the nutritional quality of osmotically dehydrated catfish and beef in sodium chloride solution. Therefore, the objective of this study is to investigate the effect of sodium chloride concentration on the nutritional quality of osmotically dehydrated catfish muscle and beef using vitamin C as quality marker.

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II. Materials and Methods

a) Sample Preparation

Three matured market-sized catfish (*Clarias garipinus*) weighing 1.5kg each and 5kg of beef were purchased from the Tombia Junction Market in Bayelsa State, Nigeria in August, 2014. They were taken immediately in plastic basins to the Food Processing Laboratory of the Niger Delta University and eviscerated and prepared for osmotic dehydration and vitamin C content experimentation. 32 samples of 5g each were cut-out from each of the specimen for replication at each sodium chloride concentration level.

b) Apparatus Used

The apparatus used were 250ml beakers, a camry digital balance, 250ml conical flasks, 200ml measuring cylinder, kitchen knife, 100ml beakers, 50ml burette with stand, glass stirring rod, mortar and pestle and a convective oven (Venticell, MMM, Medcener, Germany).

c) Chemicals and Reagents Used

The chemicals used includes; vitamin C tablets (Ascorbic acid), 5 x 10^{-2} M Potassium Iodate solution, 1.0M HCl solution, 0.6M Potassium iodide solution, starch indicator solution (Freshly prepared) and cheese cloth

d) Solute Concentrations

Nine (9) different concentration levels of the sodium chloride (NaCl) solution were obtained in the following ratios;

Sample Code	% Solution Concentration
F_1	10% NaCl + 90% H_2O
F_2	20% NaCl + 80% H_2O
F_3	30% NaCl + 70% H_2O
F_4	40% NaCl + 60% H_2O
F_5	50% NaCl + 50% H_2O
F_6	60% NaCl + 40% H_2O
F_7	70% NaCl + 30% H_2O
F_8	80% NaCl + 20% H_2O
F_9	90% NaCl + 10% H_2O

The samples were then immersed in the different osmotic solutions (% NaCl + % H_2O) at room temperature of 25^{0}C for a period of 5 hours. After 5 hrs, samples were withdrawn from the solution, blotted with absorbent paper and sent in cellophane bags for vitamin C analysis in the Chemistry Laboratory of the Niger Delta University, Bayelsa State. This was replicated thrice for each specimen at the various sodium chloride concentration levels.

e) Procedure

0.5g of vitamin C tablets were weighed and transferred into a 250ml conical flask and 150ml of distilled water added. With the aid of a glass stirring rod, the tablets were brought into solution. 5ml of 1M HCl was added to assist the dissolution process. 10ml of 0.6 KI solutions was then added, followed by 2 drops of freshly prepared starch indicator solution.

A burette was filled with 0.05M Potassium iodide (0.05M KIO_3) solution and the solution was titrated against the vitamin C solution to a permanent bluish – purple colour. The titre volume was noted.

5g of the fish/beef sample at each concentration levels were weighed and crushed in a mortar and pestled until it became properly grinded. 100ml of distilled water was then added to wash off the pestle and mortar into a 250ml beaker and boiled on the hot plate for about 15 minutes. The stock was then cooled and strained through the cheese cloth. The filtrate was transferred in a measuring cylinder and made up to 150ml with extra distilled water. The solution was then placed in a 250ml conical flask, 5ml of 1.0M HCl, 10ml of 0.6M KI solution and 4 drops of freshly prepared starch indicator added. The burette was then filled to the 'O' mark with the Potassium iodate solution. The titration was carried out in the same manner as in the case of vitamin C. This was repeated for the fresh catfish/beef and dehydrated samples from all NaCl concentrations using the formula

$$\frac{0.5g}{Vol.of\ titrant\ of\ tablet} = \frac{mg\ of\ Vitamin\ C}{Vol\ of\ titrant\ of\ fish/meat} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

III. Results and Discussions

The moisture loss information for both catfish and lean beef during the osmotic dehydration as influenced by solute (NaCl) concentration and immersion time are presented in the respective figures and tables below.

a) Catfish

Figure 1 shows the graphical behavior of moisture loss as dictated by immersion time and solute concentration. At F9, it is obvious that moisture loss ranged from 13.73% to 47.36% at 5 min and 5 hrs of immersion respectively. However, there was no moisture loss for the first 25 min for F1 but increased to 7.04% at 45 min of immersion and remained constant till the end of 5 hrs. This could be credited to the low osmotic pressure as presented by the low solute (NaCl) concentration. A closer look at the data shows that F7 is the best concentration level, as it yielded 56.0% moisture removal after 5 hrs of dehydration. The findings here agree with the works of Milica et al (2013) on fish and Anoar et al (2005) on Carica Papaya. An analysis of variance conducted on the data is presented in Table 1 and results show that, at 95% confidence level, significant difference exists between the mean values of percent moisture loss, thereby indicating that solute (NaCl) concentration and immersion time are vital to the osmotic dehydration of catfish muscle.

Figure 1 : Effect of NaCl concentration and immersion time on moisture loss

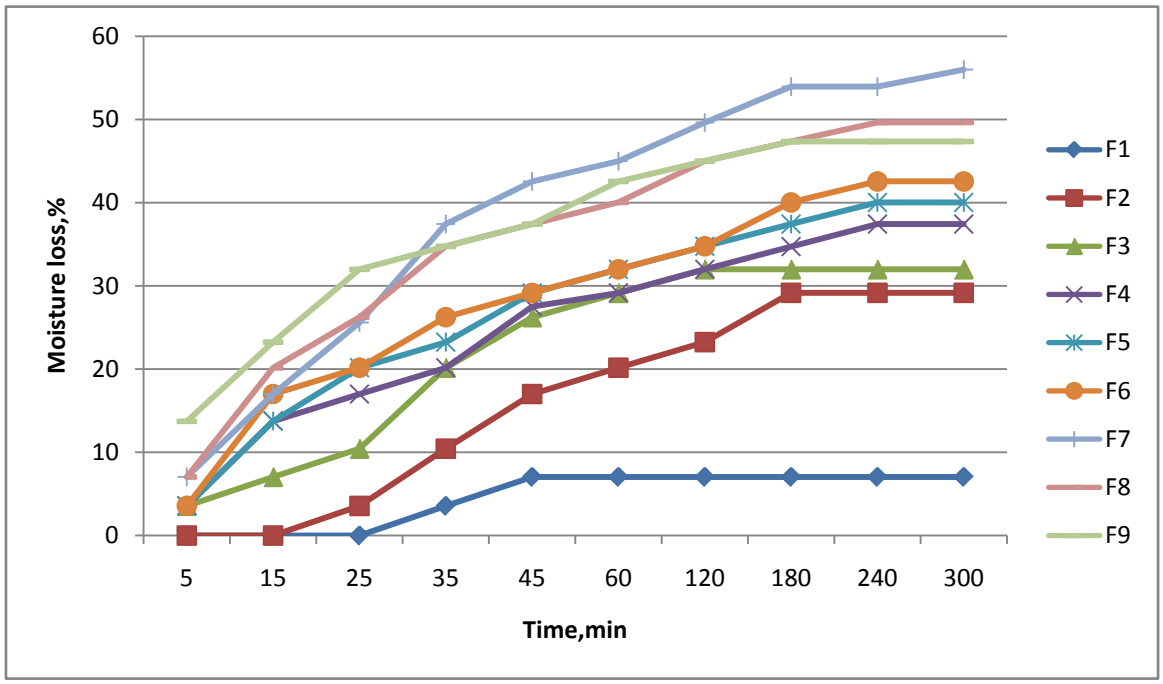


Table 1 : One-Way Anova on effect of NaCl concentration and immersion time on moisture loss

Source	SS	DF	MS	Prob	F-ratio	F- critical
Between group	4407.6321	1	4407.6321	0.0000	38.7074	4.6001
Within group	1594.1886	14	113.8706			
Total	6001.8207	15				

b) Catfish Vitamin C Analyses

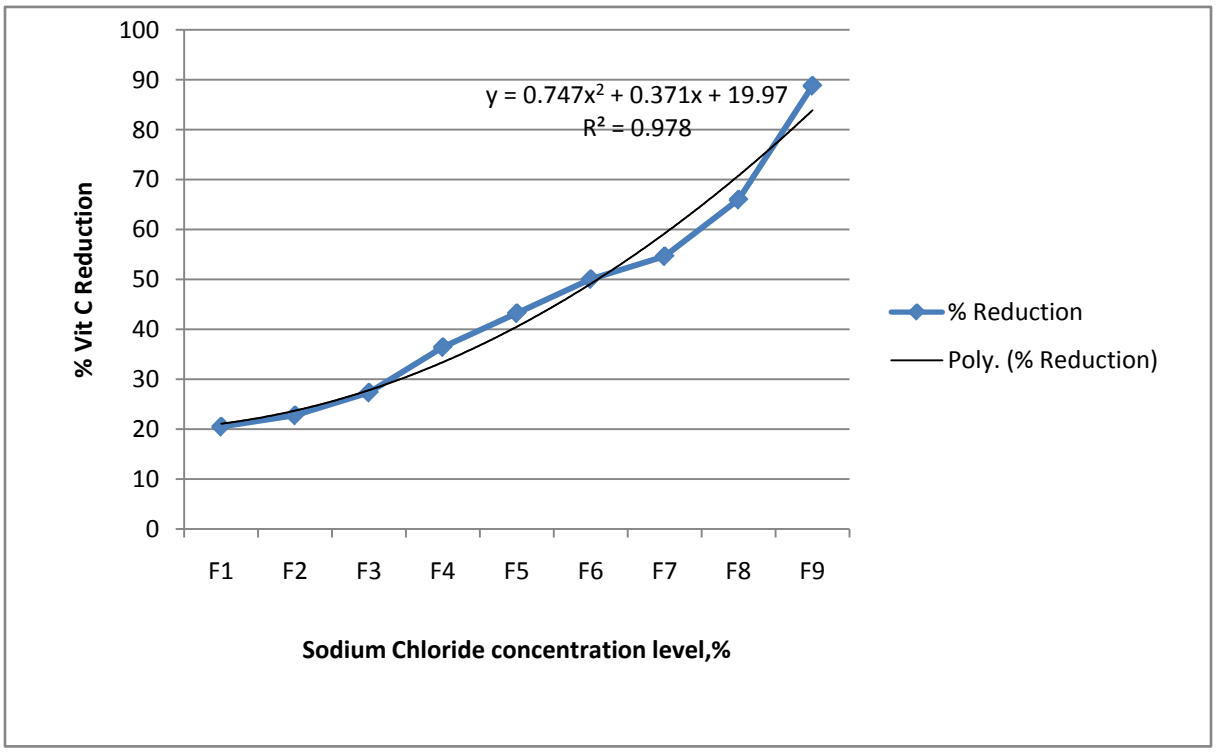
Furthermore, an analysis of vitamin C content of the fresh catfish muscle gave 97.83mg of vitamin C. This value is higher than the recommended daily allowance of 75mg for women and 90mg for men. However, the degradation of vitamin C in catfish muscle after 5 hrs of osmotic dehydration in NaCl solution is indicated in Table 2.

Table 2 : Degradation of vitamin C (mg/5g) at different NaCl concentration levels after 5 hrs of dehydration

Sample Code	% NaCl Conc.	Vitamin C (mg/5g) ± SD	% Reduction
F1	10% NaCl + 90% H_2O	77.78 ± 1.02	20.50
F2	20% NaCl + 80% H_2O	75.56 ± 0.23	22.76
F3	30% NaCl + 70% H_2O	71.10 ± 0.01	27.32
F4	40% NaCl + 60% H_2O	62.20 ± 0.05	36.42
F5	50% NaCl + 50% H_2O	55.56 ±0.35	43.21
F6	60% NaCl + 40% H_2O	48.88 ± 0.69	50.04
F7	70% NaCl + 30% H_2O	44.40 ± 0.81	54.62
F8	80% NaCl + 20% H_2O	33.30 ± 1.20	65.96
F9	90% NaCl + 10% H_2O	11.00 ± 0.07	88.76

Table 2 shows that, generally, as solute (NaCl) concentration increases, vitamin C content decreased. Specifically, vitamin C content decreased from 77.78mg to 11.00mg for a corresponding solute concentration change from F1 to F9 respectively. This quality degradation is an indication, that NaCl should never be used in making hypertonic solutions for osmotic dehydration of catfish muscles as it tempered seriously, with the nutritional value of the fish. These results agree with the findings of Saito et al (2009) and, Danijela et al (2013) on pork and Anoar et al (2006) on Papaye. It is important to note here that, if NaCl must be used as solute in the osmotic dehydration of catfish muscle, the final product must be fortified with vitamin C before consumption. This implies that solute (NaCl) concentration plays a major role in degrading the biological value of catfish in the osmotic process. Figure 2 reports the percent reduction of vitamin C as against change of solute concentration and it shows that percentage reduction of vitamin C ranged between 20.50% to 88.76% for solute (NaCl) concentration of F1 and F9 respectively.

Figure 2 : Relationship between NaCl concentration and vitamin C reduction



c) Beef moisture loss

Figure 3 shows the relationship between moisture loss and solute (NaCl) concentration and immersion time. Results reveal that, generally, moisture loss increases positively with increase in immersion time for beef also. At 5 min of immersion, no moisture loss was recorded, but moisture loss increased to 32.0% after 5hrs of experimentation in F9. For F1, instead of the beef muscle becoming dehydrated, it rather absorbed more moisture. This, perhaps, is due to the low osmotic pressure exhibited by the hypertonic solution (10% NaCl). It could also be interpreted as the beef muscle having more NaCl content than it is in the hypertonic solution, hence, the reverse osmosis. Anova (Table 3) indicated that significant difference exist between the mean values of percent moisture loss during the dehydration process at 95% level of probabilty. It is obvious; therefore, that solute (NaCl) concentration and immersion time influences the osmotic dehydration process of beef using NaCl as solute.

Figure 3 : Effect of NaCl concentration and immersion time on moisture loss

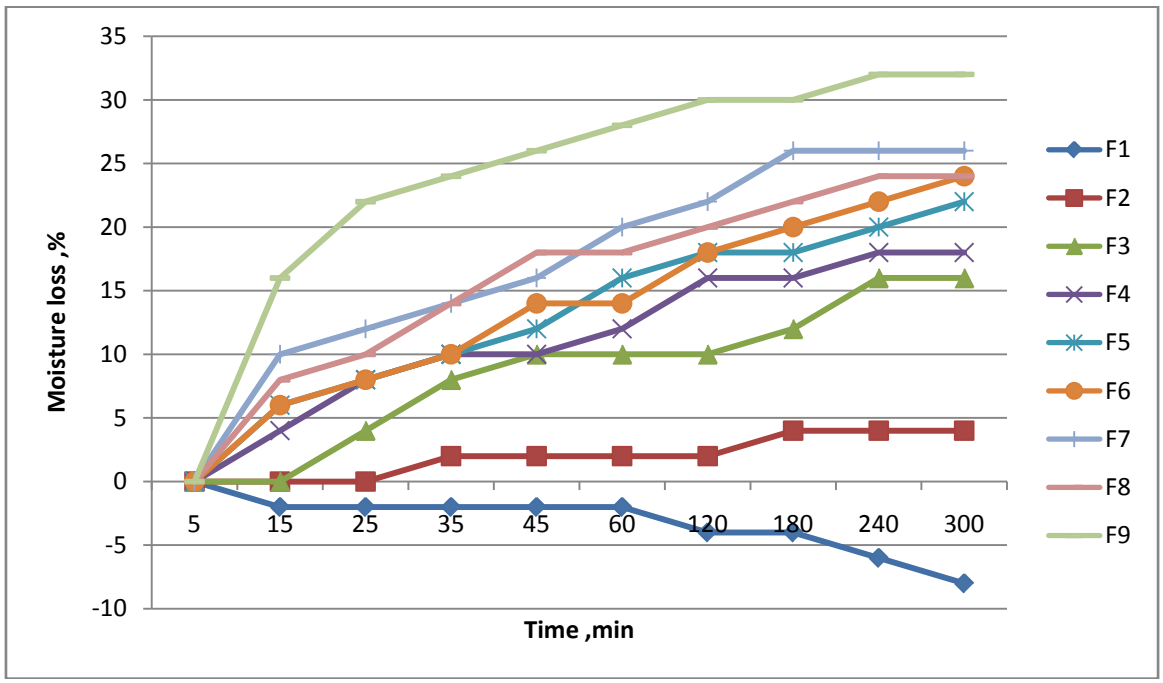


Table 3 : One-Way Anova on effect of NaCl concentration and immersion time on moisture loss

Source	SS	DF	MS	Prob	-ratio	F-critical
Between group	441.000	1	441.000	0.0498	4.6101	4.6101
Within group	1339.000	14	95.643			
Total	1780.000	15				

d) Beef Vitamin C Analysis

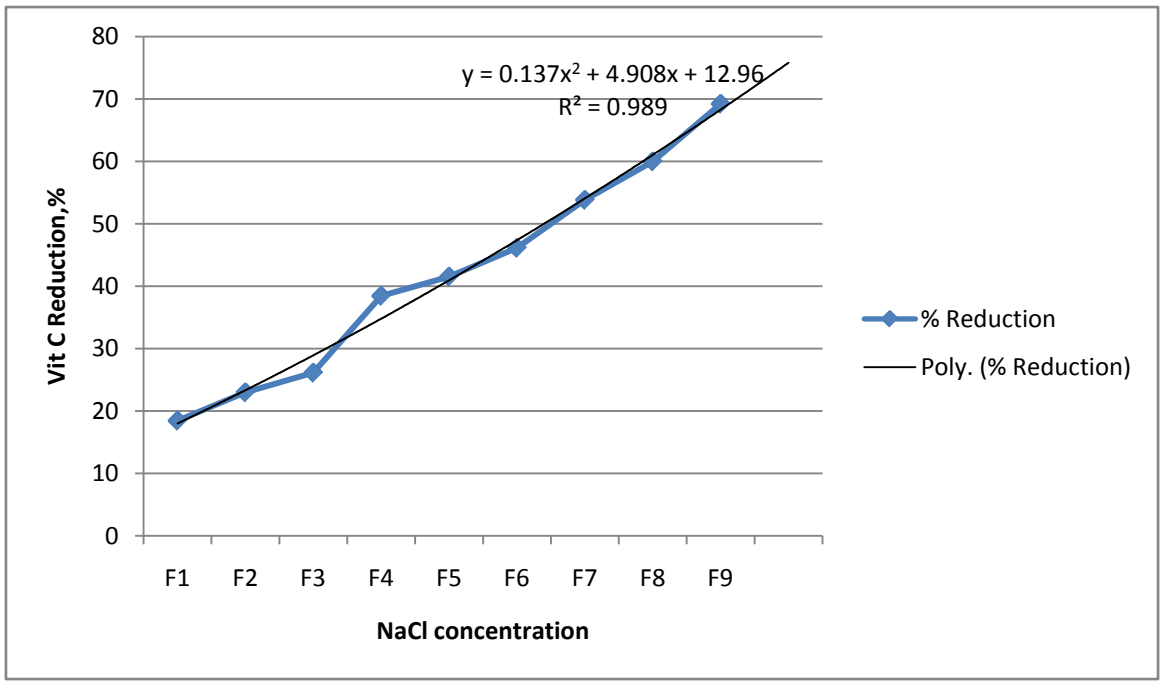
A comprehensive analysis of vitamin C content of fresh beef gave 141.30mg as against the recommended daily allowance of 90mg for men and 75mg for women. However, Table 5 shows a steady decline of vitamin C content as concentration of solute (NaCl) increases in the hypertonic solution. Vitamin C value decreased from 115.22mg to 43.48mg at corresponding increase of solute (NaCl) concentration from 10% to 90% respectively after 5hrs of immersion. It is, however, pertinent to note that, solute (NaCl) concentration levels of 10% to 30% are acceptable, as the dehydrated beef contains vitamin C levels above the recommended daily allowance (90mg – 75mg). Results presented here concur with those of Saito et al (2009) on beef and Danijela et al (2013) on pork.

Table 4 : Degradation of vitamin C (mg/5g) at different NaCl concentration levels

Sample Code	% NaCl Conc.	Average Vitamin C (mg/5g) ± SD	% Reduction
F1	10% NaCl + 90% H_2O	115.22 ±0.12	18.46
F2	20% NaCl + 80% H_2O	108.80 ±0.34	23.00
F3	30% NaCl + 70% H_2O	104.35 ±0.63	26.15
F4	40% NaCl + 60% H_2O	86.96 ± 1.04	38.46
F5	50% NaCl + 50% H_2O	82.96 ±0.87	41.54
F6	60% NaCl + 40% H_2O	76.09 ±0.72	46.15
F7	70% NaCl + 30% H_2O	65.22 ±1.08	53.84
F8	80% NaCl + 20% H_2O	56.52 ±0.39	60.00
F9	90% NaCl + 10% H_2O	43.48 ±1.20	69.23

The effect of solute (NaCl) concentration on vitamin C values as shown in Table 5 shows that significant difference exists between mean vitamin C values at various solute concentration levels. Therefore, serious attention must be paid to the solute (NaCl) concentration level when osmotic dehydration of beef is considered. Again Figure 6 shows the percent reduction of vitamin C values as solute concentration increases and yielded a polynomial relationship. At F1 and 5hrs after dehydration, 18.46% of vitamin C was degraded but increased to 69.23% at F9. These results agree with that of Filipovic et al (2012) on Pork meat and Abioye et al (2013) on baobab drink.

Figure 4 : Relationship between NaCl concentration and %Vitamin C reduction



IV. Conclusion

The effect of solute (NaCl) concentration on the vitamin C content of catfish and beef were studied. Average vitamin C values were found to decrease as solute (NaCl) concentration was increased. For catfish, percent reduction of vitamin C ranged from 20.5% to 88.76%, while for beef, percent reduction ranged from 18.46% to 69.23%. Results obtained here shows that, if nutritional quality is a priority, then NaCl should never be used as a solute in the osmotic dehydration of catfish muscle. However, for beef, 10% to 30% NaCl solution is acceptable as solute in the osmotic dehydration process. This is because the dehydrated beef from this concentration levels still retains acceptable levels of vitamin C content.

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2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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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:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- 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.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

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.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- 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

Discussion:

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."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- 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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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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