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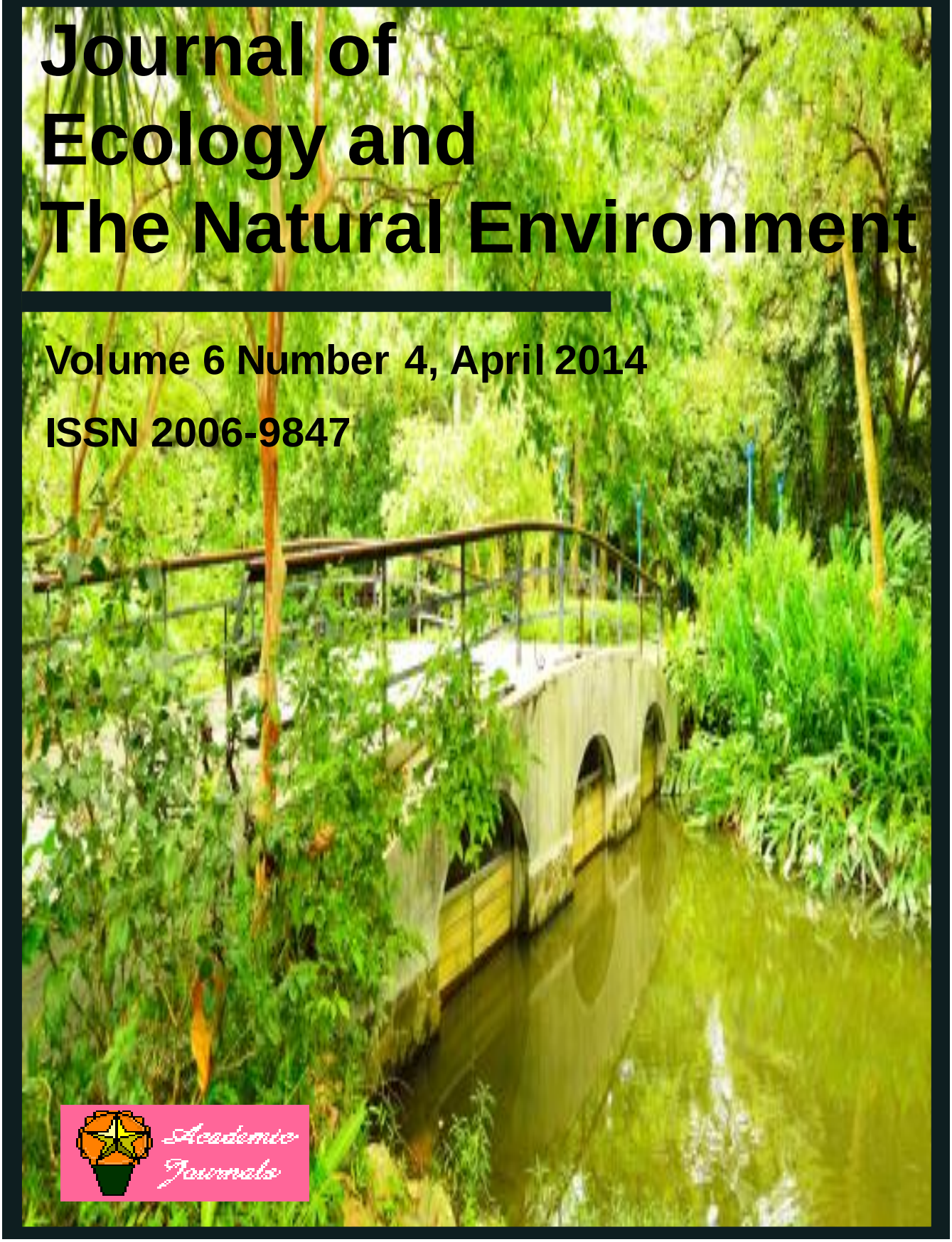

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*Review*

# Macroinvertebrates (oligochaetes) as indicators of pollution: A review

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**Macroinvertebrates formed an important constituent of an aquatic ecosystem and had functional importance in assessing the trophic status as the abundance of benthic fauna mainly depends on physical and chemical properties of the substratum and thus the benthic communities respond to changes in the quality of water and available habitat. This review discussed the occurrence, composition and distribution of macroinvertebrates of lakes and wetlands, and some environmental factors which regulated their occurrence and distribution. Also, analysis of the benthic community helped in the determination of trophic status of lakes because of their sensitivity to pollution and is, therefore, an important criterion in the ecological classification of lakes.**

**Key words:** Macroinvertebrates, substratum, lake, wetlands, trophic status, habitat.

## INTRODUCTION

The benthic macroinvertebrates are associated with bottom or any solid liquid interface, those that are retained by a sieve or mesh with pore size of 0.2 to 0.5 mm which includes a heterogeneous assemblage of organisms belonging to various phyla like Arthropoda, Annelida, Mollusca and others. The benthos occupies an important position in the lake ecosystem, serving as a link between primary producers, decomposers and higher trophic levels (Pandit, 1980). They also play an important role in the decomposer food chain which in turn affects the cycling of minerals (Gardner et al., 1981). Macroinvertebrates are used as indicators of pollution as invertebrate community change in response to changes in physicochemical factors and available habitats (Sharma and Chowdhary, 2011). The importance of macroinvertebrates as bioassessment tools is widely recognised because of their limited mobility, comparatively long life cycles and differential sensitivity to pollution of various types and they reflect the impact of cultural eutrophication on aquatic habitats quite satisfactorily. According to Jumppanen (1976) the first signs of eutrophication and pollution in a lake are reflected in the benthic flora and fauna as the suspended waste immediately sink to the bottom to decompose and thus cause a change in the benthic organisms. The lakes and wetlands having soft bottom sediments are characterised by annelids either as dominant group or an important contributor to the macrobenthic fauna. Of the fresh water annelids, the oligochaetes display the greatest diversity and have the greatest indicator value.

## MACROINVERTEBRATES AS BIOINDICATORS

Liebmann (1942) claims the microscopic benthic organisms being the most useful as true indicators of pollution and investigators like Richardson (1921, 1929), Gaufin and Tarzwell (1952, 1956), Heut (1949), Brinkhurst (1966) and Wilhm and Dorris (1966), among many others have relied almost entirely on them though their suitability and again diminished because of their rapid rate of reproduction and the difficulty of sampling

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and correctly identifying the species (Liebmann, 1951). Except in case of macroinvertebrates which are generally regarded as most suitable indicators of pollution, the presence of a particular species does indicate the suitability of the environment for its growth and development but the absence of any species does not necessarily indicate the unsuitability of the environment, instead the absence of an entire group of species with same ecological needs indicate adverse environmental conditions (Kaul and Pandit, 1981). According to Jumppanen (1976) also the first signs of eutrophication and pollution in some Finnish lakes are usually seen in the benthic fauna as the suspended wastes immediately sink to the bottom to decompose and thus causing a change in the benthic organisms. Thus, certain species of sponges, for example, respond to various types of poisonous pollutants even in very mild cases, while others (tubificids, sludge worms, maggots and chironomids) can tolerate even the most gross organic pollution and high levels of toxic pollution. Hence, the species analysis of benthic community enables the determination of trophic type of lakes and is, therefore, an important criterion in the ecological classification of lakes (Thut, 1965; Seather and McLean, 1972; Bazzanti, 1975). As the emergence of species like *Tubifex* sp. and *Chironomus* sp. in Nilnag lake indicated the eutrophic status of the lake (Yaqoob et al., 2007). Benthoses of the Shallabugh wetland were represented by Arthopoda (10), Annelida (7) and Mollusca (6). The abundance of some specific pollution indicator species, especially annelids such as *Limnodrilus* sp, *Tubifex tubifex* and *Branchiura sowerbyii*, is depictive of transition in trophic status of the wetland from meso- to eutrophy (Siraj et al., 2010). Dar et al. (2010) reported a few species of annelids like *Tubifex tubifex*, *Limnodrilus* sp. and *Erpobdella octoculata* to be dominant in terms of taxa and abundance. However, Mollusca were poorly represented and Insecta although represented by one taxa namely *Chironomus* sp. was abundant throughout the study period revealing the eutrophic status of Hokera wetland as the organisms recorded mostly occur in eutrophic waters. Lang (1985) studied the eutrophication of lake Geneva and recorded species like *Potamothrix hammoniu*, *P. Heuscheri* and *Tubifex tubifex* to be numerically dominant ones as compared to *P. veidovskyi* (mesotrophic), *Stylodrillus heringianus* (oligotrophic) in the community structure indicating a meso- eutrophic status of lake. Awal and Svozil (2010) identified 481 to 629 organisms in three constructed wetlands in South East metropolitan Melbourne comprising of 16 taxa. There was no significant differences between the wetlands on the basis of one way analysis of variance (ANOVA) for species richness (P> 0.05, F= 0.19) and Shannon-weiner index (P. 0.05, F=2.54) but the data collected was compared with the earlier published data which depicted differences in species richness and diversity. Hence, macroinvertebrates were used as a universal measure of wetland ecosystem integrity and consequently the management and conservation of constructed wetlands. Kaul and Pandit (1982) while describing the biotic factors and food chain structure in different wetlands of Kashmir observed the macrozoobenthos to be limited in number of species. They also observed summer predominance of annelids and molluscan predominance in winter. *Tubifex tubifex* and *Glossiphonia weberi* exhibited highest energy content during summer, where as *Chironomus plumosus* and *Viviparus bengalensis* revealed highest values during winter (Gupta and Pant, 1983b). The diversity of benthic macroinvertebrates was much lower in Lake Carl Blackwell. Nineteen genera of benthic macroinvertebrates were found, but nine genera were the maximum being found at one time and station with density ranging from 2310 ind./$m^2$ in fall to 1625 ind./$m^2$ in spring of which greatest density ( 91.5%) was contributed by *Chaoborus* of the assemblage (Howick and Wilhm, 1984). The faunal diversity was minimum at Perumathura where the substratum was highly unstable, but the density was maximum at Murukampuzha where the substratum was relatively stable. There was also the varying pattern of regional and seasonal variations in different groups of benthic organisms (Nair et al., 1984). Singh and Ahmad (1989) compared the benthic fauna of lotic and lentic water bodies and observed oligochaetes/insects to be the chief component of lentic waters while as the polychaetes were the major contributors of lotic system. Oligochaetes were the groups with higher similarity whereas the polychaetes were altogether absent in lentic systems.

## FACTORS AFFECTING LAKE MACROINVERTEBRATES

The environmental factors that affect the structure of macrozoobenthic community should be considered while scaling the ecological status (Trayanova et al., 2007). Pearson et al. (1986) described long term changes in the benthic community of two areas Loch Linn and Loch Eil, Scotland. They held the view that the changes in population of benthic community over a period of twenty years were related to and dependent upon changing organic inputs which in turn determined the carrying capacity of sedimentary benthos, while as the species composition was dependent upon climatic fluctuations like long term temperature changes. A low species diversity index was observed at thermal effluent site due to deteriorated water quality at that site and hence, the community structure at the effluent site was reported to be under stress (Singh, 1988). The nature of the sediment influenced the population dynamics of the oligochaetes of the lake as dominant oligochaetes of Dal lake, Kashmir includes *Limnodrilus hoffmeister*, *Tubifex tubifex*, *Branchura sowerbyii*, *Aelosoma* sp. and *Nais* sp. which thrive in sediments rich in organic nutrients (Mir and Yousuf, 2003). Among 24 taxa of benthic macroinverebrates in Lake Uluabat, Bursa, Turkey, Insecta and Oligochaeta were the

most abundant groups, dominated by species characteristic to nutrient rich waters, including *Pristina aequiseta*, *Nais communis*, *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, *Potamothrix hammoniensis* and *Tanypus punctipennis*. Most of the variance (63.5%) in relationships between species and environmental variables as explained by the first two axes of a canonical correspondence analysis (CCA) and placed most Oligochaeta and Chironomidae near the vectors of high nutrients and chlorophyll-a concentrations, while the sensitive Crustacea and some Oligochaeta (Lumbricidae) species on sectors of the plot with the smallest weight of those variables (Celik et al., 2010). Eighteen taxa of macrozoobenthic organisms, belonging to Annelida, Mollusca and Arthropoda, were recorded during the course of a yearlong study in the Dal lake, Kashmir and marked variations were found in the spatial distribution of various taxa, which was influenced by the texture of the sediment as well as by the macrophytic community structure (Mir and Yousuf, 2002). The macrozoobenthic community was found to be influenced by the type of substrate, the organic matter, the abundance of macrophytes as well as the concentration of calcium (Qadri and Yousuf, 2004). During the survey, 5 macrozoobenthic taxa belonging to Annelida, Mollusca and Arthropoda were recorded among which Annelida formed the most dominant group being represented by two oligochaetes that is, *Tubifex tubifex* and *Branchiura sowerbyii*, which have been designated as an indicators of pollution (Oliver, 1971; Milbrink, 1980; Bazzanti, 1983). The water quality, sediment characteristics and general ecology indicated the association of distribution, diversity and population density with habitat ecology, substratum diversity, altitude and climatic conditions of the concerned area (Roy and Nandi, 2008). Gong et al. (2000) made comparative studies on macrozoobenthos in two shallow mesotrophic lakes (Biandantang and Houhu) and found macrozoobenthos more diverse in Lake Biandantang where macrophytes were abundant than in Lake Houhu where macrophytes were scarce. In shallow lakes, submerged macrophytes are essential for the maintenance of biodiversity of macrozoobenthos because the macrophytes increase habit heterogeneity and availability of suitable food, and may also decrease predation by fish on the macrozoobenthos (Gong et al., 2000). The epiphytic oligochaetes were more diverse and more abundant in the *naiko* than those in littoral Lake Biwa, probably because of higher temperatures, denser aquatic vegetation, and higher primary production (Othaka and Nishino, 2006). Organic matter, ammonium and phosphates were positively correlated with the mean oligochaete abundance, but not with the granulometry. The canonical correspondence analysis (41.2% cumulative variance) indicated that the oligochaetes distributed along both an eutrophication-pollution gradient and a turbidity-conductivity gradient (Armendariz et al., 2011). Increasing temperatures due to climate change were found to influence abundance and timing of species in numerous ways (Burgmer et al., 2007). Whereas many studies have investigated climate-induced effects on the phenology and abundance of single species, less is known about climate-driven shifts in the diversity and composition of entire community. They analysed time series of entire community of macrozoobenthos in lakes and streams in Northern Europe but, no direct linear effects of temperature and climate indices (North Atlantic Oscillation index) on species composition and diversity was found. However, multivariate statistics showed that trends in average temperature have had profound impacts on species composition in lakes and future climate shifts may thus induce strong variance in community composition (Burgmer et al., 2007). Amakye (2001) while monitoring the seasonal as well as depth wise distribution of macroinvertebrates in the sediments of lake Volta at Yeji area observed the highest density of macroinvertebrates between the shore and depths of 8-10 m and their abundance in July. It was also found that Chironominae were abundant while Orthocladinae and Ephemeroptera were scare in the sediments compared to the formative years of the lake. The observed changes in the composition and diversity of benthos were attributed to increasing anthropogenic influences on the lake which was depicted by the changing chemistry of the lake water. In temporary or permanent wetlands, the total macroinvertebrate biomass and densities were positively related to coarse particulate organic matter abundance (living and nonliving plant matter; CPOM) and negatively related to turbidity. Density of ecologically sensitive EOT (Ephemeroptera, Odonata and Trichoptera) taxa was also positively related to CPOM and negatively related to turbidity. Total taxa richness was negatively related to turbidity, and percent of total macroinvertebrate density consisting of EOT (% EOT) was positively related to CPOM (Stewart and Downing, 2008). Stepwise multiple regression analysis demonstrated that the water depth, conductivity and chlorophyll *"a"* were the key factors affecting macrozoobenthic abundance in the lakes (Yongde and Hongzhu, 2007). The diversity and distribution patterns of certain species were clearly related to water quality (Latha and Thanga (2010). Leech community composition was best described by an ordination incorporating alkalinity, primary productivity and lake area. In general, highest species richness occurred in small eutrophic lakes where as lowest richness was recorded in medium to large lakes with low productivity. Contrary to results for some other taxa, lake pH was not a dominant variable describing only a small amount of variance in the species-environment relationship (Grantham and Hann, 1994). The oligochaete community of the acidified lakes was poorer compared to the neutral ones. Taxa richness, total biomass of the oligochaetes, their relative density and relative biomass in macroinvertebrate communities were lower in the strongly acidified lakes. Changes of major taxa proportions in the total density and biomass of the oligochaetes were recorded with lowering of pH (Ilyashuk, 1999). The

oligochaetes were separated into three functional feeding groups, as gatherers (S) that are selectively ingested mainly on the sediment surface and other substrates, gatherers (T) that are selectively ingested mainly in the sediments, and predators. Total density of gatherers (T) as well as their relative density in the oligochaete assemblage and macroinvertebrate community was lower in the acidified lakes (Ilyashuk, 1999). In an eutrophic subarctic lake, the largest populations of animals were found in the deepest part of the lake. However, in the anoxic part of the lake, species were in low number due to the low oxygen levels in water and high organic content of the sediments of the lake (Moore, 1981). Further, the anoxic zone of Nainital lake was found to be devoid of macrobenthos (Gupta and Pant, 1983a). Efitre et al. (2001) quantified the spatial and temporal distribution of macroinvertebrates in Nabugabo lake, Uganda with a focus on habitat associations and they found the total absence of bivalves and crustaceans and less abundance (1.8%) of gastropods. The dominant taxa, however, were ephemeropterans (77.7%), dipterans (11.1%) and smaller contributions were made by annelids (5.4%), odonates (2.8%) and tricopterans (1.3%) to the benthic assemblage. Further, the study revealed that abundance of macroinvertebrates was due to the habitat effects as water lily habitat reflected low level of oxygen near the sediments. Gong and Xie (2001) reported 33 taxa belonging to Mollusca, Oligochaeta and Arthropoda in lake Donghu-China and observed low species diversity in highly eutrophic areas measured in terms of species number, diversity index and k-dominant curves. Abundance of *Limnodrilus hoffmeisteri* was positively correlated to the degree of eutrophication due to its ability to tolerate low dissolved oxygen as the worms exhibit very marked physiological tolerance for oxygen depletion related to excess decomposable organic matter present in the environment, but they do decrease in number when condition are at their worst. Few other organisms can survive under these circumstances, so that worms, which have a very efficient oxygen uptake mechanism, may take up the entire benthic community (Zajic, 1971).

## CONCLUSION

From the preceding review, it is evident that macroinvertebrates occupies an important position in the lake ecosystem serving as a link between primary producers and higher trophic levels. They also play an important role in the decomposer food chain which in turn affects the cycling of minerals macroinvertebrate community change in response to changes in physicochemical factors and available habitats and hence, are used as bioassessment tools because of their limited mobility, comparatively long life cycle, differential sensitivity to various types of pollution and reflectance of cultural eutrophication and health status of aquatic habitats, thus can be used as robust bioindicators. In addition, it may be said that the occurrence, composition and distribution of macroinvertebrates in lakes and wetlands is governed by numerous environmental factors that affect the structure of macrozoobenthic community and their distribution pattern should be considered while evaluating the ecological status.

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# Molecular diversity of arbuscular mycorrhizal fungi (AMF) in Lake Victoria Basin of Kenya

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**Arbuscular mycorrhizal fungi (AMF) play a key role in land reclamation, sustaining soil fertility and cycling of nutrients, which in turn increases plant vigour and productivity. AMF differ in both structural characteristics and global distribution, which is strongly correlated with the respective functional role. This study investigated the molecular diversity of arbuscular mycorrhizal fungi (AMF) in selected representative farmlands across Lake Victoria Basin and wheat farms in Njoro District of Kenya. Native AMF genera were identified by morphological techniques and their molecular diversity assessed by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) techniques and genetic distance analysis. In all five field sites, three AMF genera were identified with varying relative abundances, namely, *Glomus* (50%), *Scutellospora* (30%) and *Gigaspora* (16%). Lambwe fields had the highest spore densities (13 spores per gram dry weight) and evenness (0.84) while Kibos and Njoro had least spore count (4 - ditto) and evenness (0.32), respectively. The AMF population from Njoro wheat farms had highest heterozygosity (He = 0.257) and hence was the most genetically diverse compared to other populations.**

**Key words:** *Glomus* spp., *Gigaspora* spp., *Scutellospora* spp., molecular diversity.

## INTRODUCTION

Productivity of agricultural land among small-holder peasant communities is diminishing due to depleted soil fertility and destabilized nutrient acquisition by plants. Major factors that constrain tropical soil fertility and sustainable agriculture are low nutrient capital, moisture stress, erosion, increased phosphorus fixation, high acidity with aluminium toxicity, and low soil biodiversity. The fragility of many tropical soils limits food production in annual cropping systems (Bonfante and Perotto, 2000; Ashman and Puri, 2002; Muchovej, 2004).

Arbuscular mycorrhizal fungi (AMF) play a key role in land reclamation, sustaining soil fertility and cycling of nutrients, which in turn increases plant vigour and productivity. Over 80% of plant species are associated with mycorrhizal fungi, 67% of which is AMF. AMF differ in both structural characteristics and global distribution, which is strongly correlated with the respective functional role. Soils typically contain several species of AMF, a combination of which is needed to function as an adequate plant-soil interface (Wardle and Van der Putten, 2002). Mycorrhizal fungi also play a significant role in the regulation of soil biological activity because of their abundance throughout the uppermost soil layer. The external mycelium of AMF acts as an extension of host plant roots and serves as a direct link between roots and soil nutrient reserves. The mycorrhizal fungal hyphae are involved with the scavenging and retention of nutrient ions, and with the creation of an aggregate system that

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acts as a control point for accrual and mineralization of organic matter in the soil, which creates a system that reduces erosion and leaching loss of nutrients (Gianinazzi and Gianinazzi-Pearson, 1986; Finlay and Soderstrom, 1992).

Such mycorrhizal associations also helps in maintenance and improvement of soil structure, the uptake of relatively immobile elements, both macronutrients and micro-nutrients, the alleviation of aluminium and manganese toxicity, the interactions with other beneficial soil organisms, and improved protection against pathogens (Harley and Smith, 1983; Xavier and Germida, 1998; Buking and Sachar-Hill, 2005; Cardoso and Kuyper, 2006). The mutual symbiosis between AMF and plants is known to increase plant vigour and productivity, especially under unfavourable conditions (Lendzemo and Kuyper, 2001). This could become a useful biotic interaction for effective and sustainable soil management and reclamation systems for improved productivity. The aim of this study was to investigate the molecular diversity of AMF in selected sorghum farmlands in Lake Victoria Basin and wheat farms in Njoro District of Kenya.

## MATERIALS AND METHODS

### Physicochemical characteristics of mycorrhizal soils

Five sampling sites were selected namely, Alupe, Kibos, Lambwe, and Oyugis in Western Kenya, and Njoro wheat fields in the Rift Valley region of Kenya. Soil samples were taken from homogeneous areas in terms of landscape and crop age at each site, by collecting 10 single samples consisting of 0.5 L of soil and roots of each plant. Each single sample was collected within the plant root area and 5 to 10 cm deep. The single samples per field site were pooled to make one compound sample and analyzed for physicochemical characteristics: pH, soil type, soil texture, soil drainage, at Egerton University. The sampling was done in the dry seasons (January and February) for two consecutive years.

### Isolation of arbuscular mycorrhizal fungi (AMF)

Pure isolates of AMF: *Glomus etunicatum, Scutellospora fulgida*, and *Gigaspora margarita*, were obtained from cultures kept and Mycorhiza research laboratory at National Museums of Kenya. AMF spores were isolated by wet-sieving and decanting density-gradient centrifugation method as described by Schenk and Perez (1990). One hundred grams of soil sample was placed in a 2.0 L container and vigorously mixed with 1.5 L of water to free spores from soil and roots. The suspension was left to settle for 45 min, decanted and the supernatant sieved using a 25 µm mesh sieve over a 45 µm mesh sieve. The sievings were transferred to 50 mL centrifuge tubes with a fine stream of water from wash bottle and centrifuged at 1300 × g in a swinging bucket rotor for 3 min. The supernatant and adhering organic debris were removed carefully and the soil pellet suspended in chilled 1.7M sucrose then centrifuged at 1300 × g for 1.5 min. The supernatant was poured through the 25 µm mesh sieve and rinsed with tap water. The spores were washed into a Petri dish and sorted into morphotypes and counted using a dissecting microscope at a magnification of × 50.

Samples (0.5 g) of plant roots collected from the field were placed in perforated plastic holders (OmniSet tissue cassette; Fischer Scientific, Pittsburg, PA.) and stored in cold water until they were processed. Samples were covered with 200 mL of 1.8 M KOH in a beaker and heated to 80°C in a fume hood for 30 min, then rinsed with water and briefly with dilute hydrochloric acid solution (5.0 mL conc. HCl in 200 mL $H_2O$), stirred and drained. Lactoglycerol trypan blue stain was dispensed into a beaker and heated to 80°C. Samples were placed in the stain for at least 30 min, then destained with lactoglycerol followed by two changes of tap water. The cleared and stained roots were spread in a scribed 10 cm diameter Petri dish and observed under dissecting microscope for root colonization (Phillips and Hayman, 1970).

### Morphological Identification of AMF Isolates

Several spores from the same morphological spore type were observed under a dissecting microscope at a magnification of × 50. Spores were put in a watch glass or a small Petri dish and their shape, size, colour, hyphal attachment, auxiliary cell, sporocarp, germination shield, and surface ornamentation observed following Morton and Redecker (2001), and identified to genus. AMF spores from each 100 g soil sample were counted and data expressed as mean spore density (numbers per 100 g sample). Relative abundance of each species in each field site was calculated as:

$$\text{Relative abundance} = (n_i/N_j) \times 100$$

Where, $n_i$ = number of spores that belong to species $i$ and $N_j$ = total number of spores in the site. The mean of six replicates was expressed as percent relative abundance. Significant differences were separated by Fisher's LSD test at $p<0.05$ confidence level. Mycorrhizal fungal diversity was calculated by using the Shannon index (H'), which combines two components of diversity, species richness and evenness of individuals among the species (Vestberg, 1999).

$$H' = -\Sigma P_i \ln P_i \text{ and; } E = H' / H_{max}$$

Where, H' = Shannon index, $P_i$ = proportion of the ith species, ln = natural logarithm, E = evenness, $H_{max}$ = Diversity maximum when all species are equally abundant.

### Molecular characterization of AMF

Fungal DNA was extracted from the spores according to Lee et al. (1988) with a few modifications. Samples were vortexed for 2 min and centrifuged at 13000 rpm for 5 min and the supernatant decanted. Three freeze thaw cycles were performed with liquid nitrogen and samples were crushed in 5.0 mL of 2% Cetyltrimethylammonium bromide (CTAB) buffer with the aid of a sterile micro pestle to ensure effective lysis of cells. Proteinase K (20.0 µl, 2.0 mg/ml) was added and allowed to stand at room temperature for 15 min. Samples were incubated at 65°C for 45 min with intermittent vortexing every 15 min. Chloroform (400 µl) was added after incubation, vortexed and centrifuged at 13000 rpm for 5 min. The aqueous phase was further extracted with another 0.5 mL of phenol: chloroform: isoamyl alcohol (24:24:1 by volume). 0.45 mL of the aqueous phase were mixed with 0.045 mL of 5.0 M ammonium acetate and 0.9 mL of cold ethanol and incubated at -20°C. After 1 to 2 h, precipitated DNA was pelleted by centrifugation at 11,800 × g for 15 min at 4°C, washed with cold 70% ethanol, dissolved in 50 µl of deionized water and incubated with 5.0 µl (50 µg) of GIBCO BRL RNase T1 for 30 min at 37°C. After extraction, DNA concentration was estimated on 0.8% agarose

**Table 1.** Characteristics of soils collected from the five field sites in Kenya.

| Zone /Site | Altitude (m) | Latitude / longitude | pH | Soil characteristic |
|---|---|---|---|---|
| Alupe | 1189 | 00°29'N / 34°08'E | 6.3 | Ferro-orthic acrisols, sandy clay; well drained |
| Kibos | 1214 | 00°04'S / 34°48'E | 6.1 | Alluvial, vetroeutic planosol, sandy loam; Well drained |
| Lambwe | 1440 | 00°31'S / 34°22'E | 7.1 | Vertisols of clay texture; well drained. |
| Njoro | 2164 | 00°19'S / 35°56'E | 6.6 | Haplic, verto-luvic phaeozems; well drained |
| Oyugis | 1320 | 00°30'S / 34°44'E | 6.4 | Chromo-luvic phaeozem; well drained |

gels buffered in 1xTBE (89 mM tris-HCl (pH 8), 89 mM boric acid and 2.0 mM EDTA) in a horizontal electrophoresis apparatus. Before loading into the gels, 10.0 µl of the DNA samples were mixed with 5.0 µL of 1 × gel loading buffer III (0.25 % bromophenol blue, 0.25% xylene cyanol and 30% glycerol). Standards of 2.5, 5.0, 10.0 and 20.0 ng of uncut unmethylated λ-DNA were loaded onto the gel and the gels run at 150V for 30 min. Gels were imaged with the gel documentation system. DNA content was estimated by comparing resultant DNA bands with those of the standards. Solid bands with no streaks signified high molecular weight DNA. After the estimation of DNA, individual extractions were adjusted with TE buffer to a standard concentration of 10.0 ng/µL and stored at -20°C (Miyumi et al., 2004).

Detection of inter- and intra-specific differences among the AMF isolates was analyzed by RAPD-PCR (Random amplified polymorphic DNA polymerase chain reaction) (Lee et al., 1988). Dilutions of genomic DNA were prepared in sterile distilled water, such that the final DNA concentration was 2.5 ng/µL. These dilutions were stored at -20°C and were only thawed twice before discarding. Amplification of DNA fragments was carried out by PCR using 10-mer arbitrary primers (Operon Technologies Inc., California, USA). The PCR conditions for each of the 18 RAPD markers were optimized and PCR reactions were set up in 10.0 µL volumes in 96-well PCR plates (Perkin Elmer, Germany). The amplification reaction was carried out in a 0.2 mL tubes using a programmable thermal cycler (Corbett Research, Germany). Each PCR reaction mixture contained 1.0 µL (2 pmol) of RAPD primer, 1.0 µL of 10 × PCR buffer, 1.0 µL of 50 mM $MgCl_2$, 0.5 µL (0.2 mM) dNTPs, 0.15 µL (0.2 U) Taq polymerase, 5.85 µL SDW and 0.5 µL (1.0 ng) of DNA in a final volume of 10.0 µL.

The reaction mixture was denatured for one minute at 94°C, annealing for one minute at 36°C, and extension for 5 min at 72°C with a final extension for 2 min at 72°C. A negative control (master mix without template DNA) was incorporated in every PCR run. The reproducibility of RAPDs was tested by repeating a subset of samples across PCR runs. PCR products were resolved by electrophoresis on 1.5% agarose gel in 1 x TBE buffer by running at 150 V for 3 h. Four microlitres of 1 x loading buffer was added to the PCR products and then were analyzed by electrophoresis on agarose gel in 0.5 × TBE (tris borate EDTA) containing 0.5 µg of ethidium bromide per mL. A 100 bp DNA ladder (Invitrogen, USA) was used as a molecular weight marker. Gels were imaged with the gel documentation system (BioRad GelDoc 2000 Documentation System).

#### Genetic diversity analysis

The bands on agarose gel were scored on a spreadsheet as '1' for the presence and '0' for the absence of DNA bands for each isolate. Bands of the same size (assessed by eye) in each PCR run were assumed to be homologous. The following genetic diversity parameters: polymorphic RAPD fragment (for convenience, each was treated as an allele) frequencies, effective number of alleles, Shannon's information index, proportion of polymorphic loci and, expected heterozygosity were computed for each AMF population using GENALEX v6.41 software (Peakall and Smouse, 2006). The same software was used to calculate genetic distance and identity based on Nei's index (Nei, 1972). A dendrogram was constructed based on Nei's genetic distances using PowerMarker v3.0 software (Liu, 2005) and viewed in TreeView software.

## RESULTS AND DISCUSSION

### Physicochemical characteristics of mycorrhizal soils

The mycorrhizal soils collected from the various field sites showed different physicochemical characteristics (Table 1). All the field sites were well drained but had varying soil pH, ranging from 6.1 in Kibos to 7.1 in Lambwe. Field sites ranged from altitude 1189 m (Alupe) to 2164 m (Njoro) above sea level. All field sites were well drained but varied in soil types. Soil types determine soil pore size, which in turn influences nutrient retention capacity in soils as well as the soil physical and chemical characteristics (Ashman and Puri, 2002).

### Morphological identification of AMF isolates

Three AMF genera identified were: *Glomus* spp., *Scutellospora* spp., and *Gigaspora* spp. (Table 2). Significant differences in richness and relative abundances of indigenous AMF were observed in all the field sites. Lambwe site had the highest total spore count (12.59) while Kibos had the lowest (4.23). *Glomus* were dominant AMF (49.74%) in all field soils followed by *Scutellospora* (29.60%) and *Gigaspora* (15.80%). Other fungal spores were also observed but could not be conclusively identified. The high spore densities observed in Lambwe suggest that the soils have experienced less tillage over the years. This is in agreement with findings by Douds et al. (1993) who reported that AMF spore densities are generally high in low-input agricultural fields and lower at both native and high input sites (Smith and Dickson, 1997). Agricultural practices are known to affect AM fungal community structure and as such low spore densities and mycorrhization have been reported

**Table 2.** Relative abundance and spore counts of AMF genera sieved from 100 g of field soils, means and standard deviations based on 6 replicate counts of each sample.

| AMF species | Spore count | | | | | |
|---|---|---|---|---|---|---|
| | **Alupe** | **Kibos** | **Lambwe** | **Njoro** | **Oyugis** | **Mean** |
| *Glomus* | (49.18) 3.90$^{c}$ | (51.06) 2.17$^{d}$ | (44.42) 5.61$^{d}$ | (54.96) 4.54$^{d}$ | (49.06) 2.87$^{c}$ | (49.74 ± 1.33) 3.82 ± 0.68$^{a}$ |
| *Scutellospora* | (32.28) 2.56$^{b}$ | (28.71) 1.22$^{c}$ | (30.09) 3.80$^{c}$ | (28.21) 2.33$^{c}$ | (28.72) 1.68$^{b}$ | (29.60 ± 0.87) 2.32 ± 0.34$^{b}$ |
| *Gigaspora* | (12.23) 0.97$^{a}$ | (15.29) 0.65$^{b}$ | (19.32) 2.44$^{b}$ | (12.83) 1.06$^{b}$ | (19.32) 1.13$^{b}$ | (15.80 ± 0.43) 1.25 ± 0.19$^{c}$ |
| Others (unidentified) | (6.31) 0.50$^{a}$ | (4.94) 0.21$^{a}$ | (6.18) 0.78$^{a}$ | (3.99) 0.33$^{a}$ | (2.91) 0.17$^{a}$ | (4.87 ± 0.21) 0.40 ± 0.02$^{d}$ |
| **Total** | **7.93 ± 1.02** | **4.23 ± 0.08** | **12.59 ± 1.58** | **8.20 ± 1.23** | **5.85 ± 1.01** | **7.79 ± 1.41** |

Values are mean ±SE; Values in brackets are percent abundances; Letters show vertical comparisons among treatments at P = 0.05 error level; Means with the same letter are not significantly different from each other.

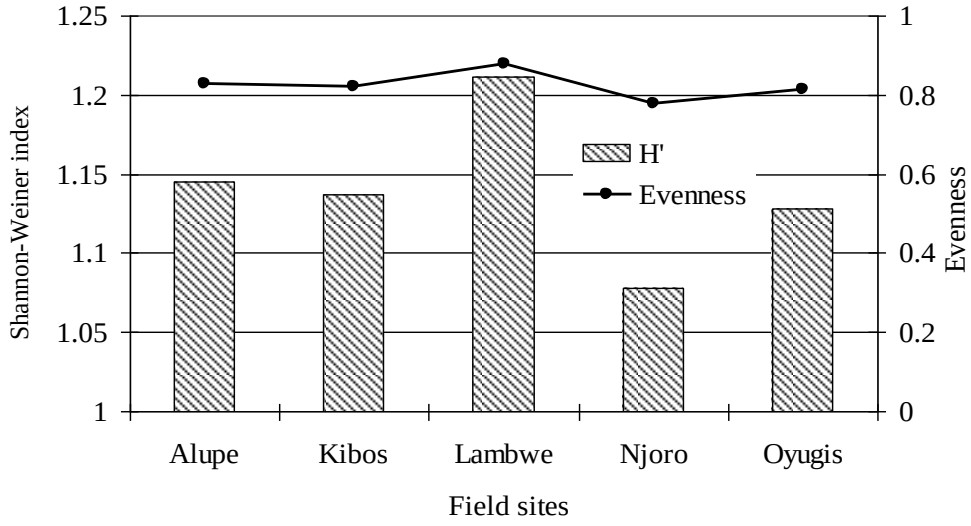


**Figure 1.** AMF species diversity as measured by the Shannon index (H').

(Douds and Millner, 1999), hence the high variability in spore density among the field sites. Furthermore, presence of non-sporulating species may not be detected through the standard technique (Schenck, 1982; Liu and Luo, 1994).

The species diversity measured by Shannon-Wiener diversity index (H) differed significantly between field sites (Figure 1). Lambwe field soils showed a higher degree of AMF diversity (H = 1.21) while Njoro had the least (H = 1.08). The possible reasons for observed biodiversity index and species abundance were the complex ecological system structure and high plant species diversity. Evenness of species population did not show significant variation across field sites except at Lambwe (E = 0.88) which was significantly high (Figure 1). This means the spatial heterogeneity of different field sites had significant influence on AMF genetic diversity. Such influence together with seasonal variations were also detected by Kwong-ma (2004) who reported that species of AMF (*Acaulospora colossica*) sporulate at the beginning of summer, remain viable as spores throughout the summer period and is only physiologically active in the cool season plant (for example, wild garlic) community. Similarly, de Oliveira and de Oliveira (2005) concluded that AMF sporulation is seasonal, dependent on soil moisture and other soil factors. There is a tendency that indigenous species of AMF though not active at one seasonal period, may become effective pending favourable environmental conditions that aids colonisation and subsequent release of spores into the soil.

## Molecular characterization of AMF Isolates

A set of reproducible bands was produced from amplification of AMF DNA fragments using a particular primer and was defined as a “pattern”. Out of the 18 arbitrar

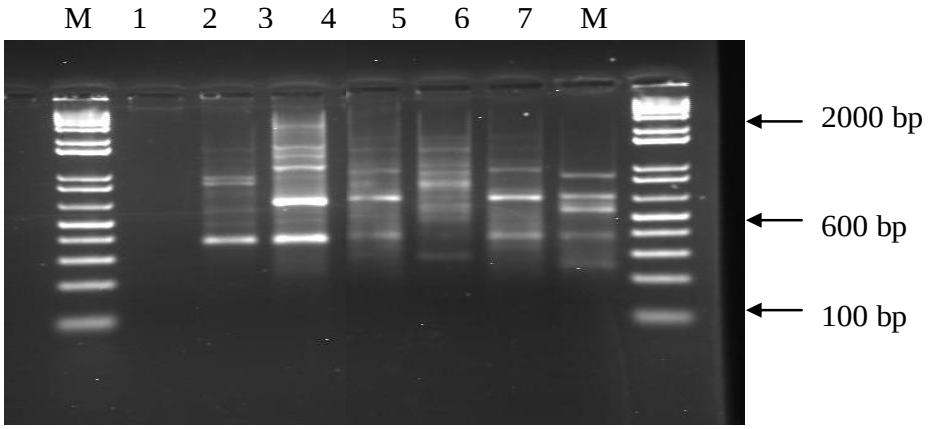


**Figure 2.** Representative Ethidium bromide stained agarose gel containing RAPD-PCR products of *Glomus* spp. isolate amplified with arbitrary primer OPB-11. Lanes M are 100-bp DNA ladder, 1-negative, 2-Alupe, 3-Kibos, 4-Lambwe, 5-Njoro, 6-Oyugis isolates, 7-positive (*Glomus etunicatum*).

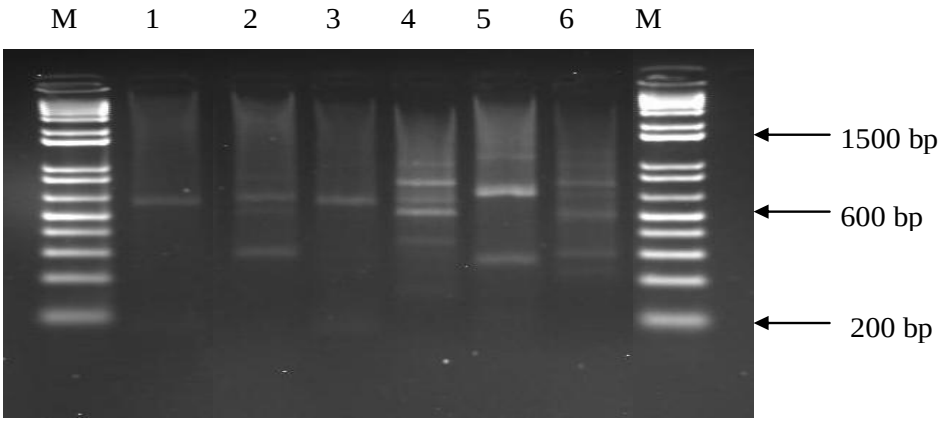


**Figure 3.** Representative Ethidium bromide-stained agarose gel containing RAPD-PCR products of *Scutellospora* spp. isolate amplified with arbitrary primer OPB-11. Lanes M are 100-bp DNA ladder, 1-Alupe, 2-Kibos, 3-Lambwe, 4-Njoro, 5-Oyugis isolates, 6-positive (*Scutellospora fulgida*).

primers screened, 4 primers namely, OPB-11 (5'-GTAGACCCGT-3'), OPE-07 (5'-AGATGCAGCC-3'), OPE-11 (5'-GAGTCTCAGG-3') and OPA-02 (5'-TGCCGAGCTG-3'), were selected because they gave clear polymorphism (Figures 2 to 4). Primer OPB-11 produced 5 amplicons and the sizes of RAPD-PCR products ranged between 200 to 1800 bp. It was noted that the majority of RAPD-PCR primers gave distinctly reproducible bands in all the five field populations. *Glomus* spp. from various field sites had common bands with 500 and 1000 bp, except Njoro population which had unique bands with 300 bp and 1200 bp. *Scutellospora* spp. had common bands with 800 bp in all samples while Alupe and Lambwe population lacked a common band with 350 bp (Figure 2). Positive control (*Scutellospora fulgida*) had 6 clear bands compared to other populations which had less (Figure 3). *Gigaspora* spp. had common bands with 800 bp except Njoro population which had unique band with 400 bp. The results obtained showed genetic differences within populations across field sites. The genetic variability observed across populations as is often seen with RAPD-PCR (Caldero et al., 2004).

## Genetic diversity analysis

The percentage of polymorphic loci was variable ranging from 38.46% in Lambwe to 61.54% in Njoro and Oyugis

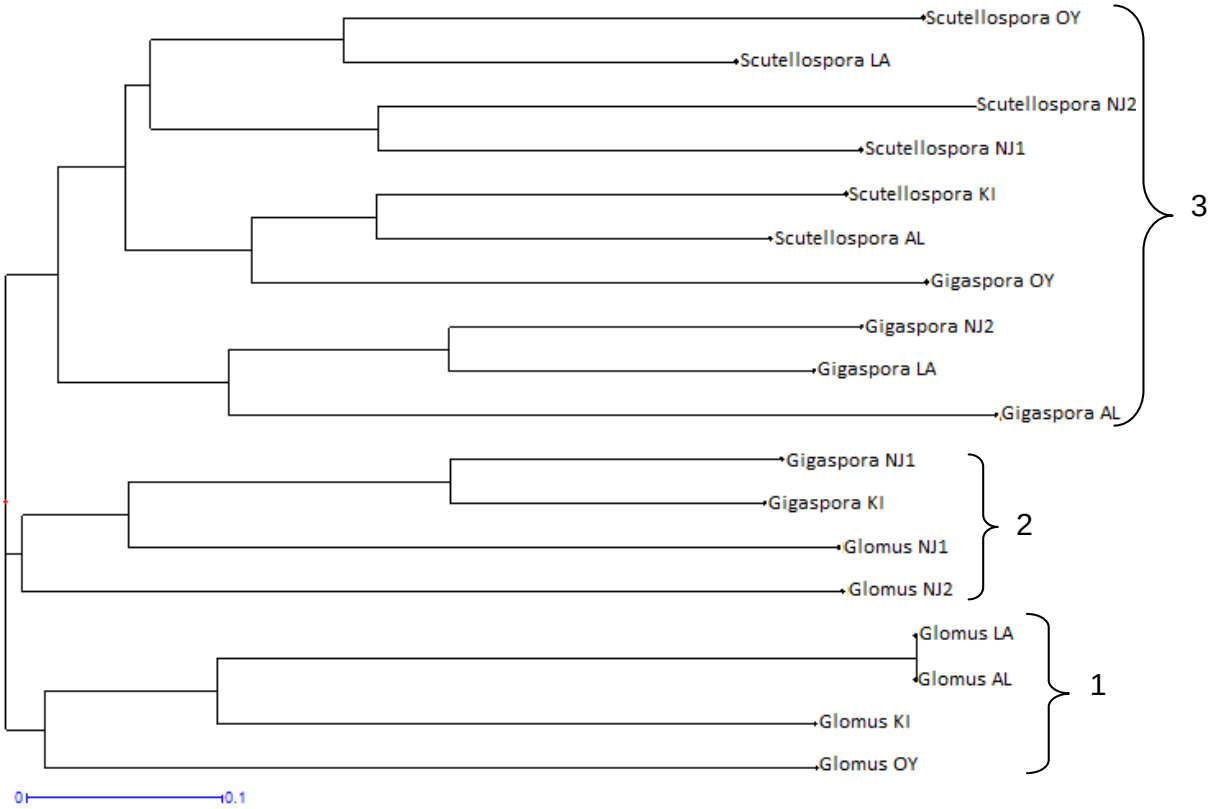


**Figure 4.** Dendrogram based on Nei's genetic distance. AMF sources: AL = Alupe, KI = Kibos, LA = Lambwe, NJ1 = Njoro, NJ2 = Positive control, OY = Oyugis.

**Table 3.** Analysis of polymorphism obtained with RAPD primers in different populations of AMF spp.

| Population of AMF spp. | Percentage of polymorphic loci | Shannon information index, I | Mean Heterozygosity, He |
|---|---|---|---|
| Alupe | 53.85 ± 2.79 | 0.288 ± 0.079 | 0.190 ± 0.054 |
| Kibos | 46.15 ± 2.51 | 0.251 ± 0.081 | 0.167 ± 0.055 |
| Lambwe | 38.46 ± 1.97 | 0.215 ± 0.080 | 0.144 ± 0.055 |
| Njoro | 61.54 ± 4.01 | 0.372 ± 0.088 | 0.257 ± 0.062 |
| Oyugis | 46.15 ± 2.33 | 0.283 ± 0.090 | 0.196 ± 0.064 |
| Control | 61.54 ± 4.23 | 0.356 ± 0.085 | 0.242 ± 0.059 |
| **Mean ±SE** | **51.28 ± 3.80** | **0.294 ± 0.034** | **0.200 ± 0.023** |

Values are mean ±SE

populations and the overall mean was 51.28% (Table 3). The high percentage of polymorphic loci observed reflected noticeable levels of genetic polymorphism in the studied populations. Njoro population gave the highest Shannon information index (I = 0.372) while Lambwe population had the lowest (I = 0.215) with an overall mean of 0.294 (Table 3). The Shannon information index was clearly different from zero and reflects presence of genetic diversity. The mean expected heterozygosity among individual populations varied from 0.144 at Lambwe to 0.257 at Njoro and the overall mean was 0.200. The Njoro population was most genetically diverse (He = 0.257) than other populations (Table 3).

The genetic distance between the population ranged from 0.003 to 0.182 while the genetic identity ranged from 0.833 to 0.997 (Table 4). The highest genetic similarity

**Table 4.** Pairwise population matrix of Nei's unbiased measures of genetic distance (below diagonal) and genetic identity (above diagonal) for AMF spp.

| Population of AMF spp. | Alupe | Kibos | Lambwe | Njoro | Oyugis | Control |
|---|---|---|---|---|---|---|
| Alupe | *** | 0.959 | 0.974 | 0.935 | 0.997 | 0.908 |
| Kibos | 0.042 | *** | 0.995 | 0.932 | 0.990 | 0.865 |
| Lambwe | 0.027 | 0.005 | *** | 0.878 | 0.985 | 0.833 |
| Njoro | 0.068 | 0.075 | 0.130 | *** | 0.996 | 0.958 |
| Oyugis | 0.003 | 0.010 | 0.015 | 0.004 | *** | 0.954 |
| Control | 0.096 | 0.145 | 0.182 | 0.042 | 0.047 | *** |

was found between Oyugis and Alupe populations (0.997) while the lowest was resolute between Lambwe populations and positive control (0.833). The Nei's genetic distance was used to construct phylogenetic tree using PowerMarker software. Three major clusters labeled 1 - 3 were observed. Within each cluster were subclusters which suggest intrageneric differences across all the populations. Cluster 1 corresponded to Glomerales since they consisted of only *Glomus* spp. from different populations. Clusters 2 and 3 corresponded to Diversisporales since they contained both *Scutellospora* and *Gigaspora* spp. (Figure 4). The *Glomus* species in cluster 2 was likely *Glomus* group C which is a basal member of the Diversisporales (Redecker and Raab, 2006). The dendrogram also suggested that AMF undergoes major part of genetic variation by environmental factors. It is also possible that the lack of large differences among the various glomalean populations cover a variation in functional adaptability of identical genotypes. This would lead to differences only at an epigenetic level, for example, by regulation of gene expression (Stukenbrock and Rosendahl, 2005). The observed diversity could be evidence for genetic redundancy or an adaptive mechanism that allows symbiosis with different plants in a whole range of environments as well as fungal stress response whereby fungal ecotypes are better adapted to specific soil types.

The simple morphology of the spores apparently has concealed the large genetic variation within the polyphyletic genera. These findings also indicated that soil chemistry and geographic factors may have a stronger influence on population structuring in agriculturally used soils than previously recognized. Other soil characteristics, such as other microorganisms, might be responsible for the observed results. These microbes are likely to interact with AMF in different ways which can lead to different genetic structures of populations (Vandenkoornhuyse et al., 2001). Moreover, new primers which were not accessible for our research, have been developed that are more specific to the Glomeromycota and would provide better coverage across the Glomeromycota and also offer the possibility of a more reliable phylogenetic placement of the environmental AMF sequences (Lee et al., 2008).

## Conclusion

Three genera of AMF were identified in decreasing abundance: *Glomus, Scutellospora* and *Gigaspora* spp. *Glomus* was the dominant genus throughout all populations and could be a candidate for screening high ecological restoration strains for these areas. Lambwe populations gave highest AMF spore densities while Njoro populations were most genetically diverse. The dendrogram clustering further confirmed the molecular heterogeneity of AMF in Lake Victoria basin.

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*Full Length Research Paper*

# Residues of diazinon in Ab-bandans supplied by Babolroud, Talar and Siaroud Rivers, Iran

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**In this http://www.wessex.ac.uk/components/com_forme/uploads/ study, diazinon, as an organophosphorus pesticide, was measured in nine Abbandans (man-made wetland) in Mazandaran Province: Kharajisha, Ramenet, Esmaeelkola, Kordkola, Shrag-e-Larim, Anarmarz, Roshandan, Galeshkola and Langoor from April 2010 to March 2011. A total of 216 samples were taken from nine abbandans of the South Caspian lowland. Samples analyzed revealed that diazinon is observed frequently in aquatic ecosystems throughout the year in summer, mostly. All abbandans except Kharajisha have shown similar trend annually. Kharajisha as an aquatic system is fed by Babolroud River, which is exposed nearly three times to diazinon concentration than others in summer. In other seasons, it decreases gradually so that winter and spring have lower amount. Due to the vast expansion of agricultural fields in the study area, various contaminants such as diazinon are leached by rainfall, irrigation and drainage activities and finally are conducted to the adjacent rivers and abbandans. These findings indicate that diazinon is widespread in the environment and can possibly have adverse effects on aquatic ecosystem health. Thus, development of irrigation and drainage efficiency and some environmental observations under the small land holding condition could diminish the negative impacts and consequently serve the ecosystem balance in this region.**

**Key words:** Diazinon, Babolroud, Talar, Siaroud, Ab-bandan.

## INTRODUCTION

One of the most important types of wetland in the South Caspian lowlands is the “Abbandan”, a small, man-made reservoir or flooded rice paddy with a luxuriant growth of underwater vegetation. These shallow wetlands, that vary in size from 3 ha to 1,000 ha and with mean depth of 1m, provide excellent feeding and roosting areas for large numbers of migratory waterfowl. Most were originally built as temporary water storage areas to provide water for irrigation during the dry summer months. They also provide habitat for edible fish such as silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*). A total of 506 abbandans were estimated in Mazandarn Province, having 14811 ha area. They are distributed through 635 villages and so benefit about 50000 families. The study area, located in the Southern Coast of the Caspian Sea along the Mazandaran Province of Northern Iran, is one of the most productive agricultural regions in the Middle East. Within the Mazandaran Province, a large quantity of pesticides is used to protect crops from pests; in 2006, almost 30,000 tons of pesticides were used in the agricultural areas (Ahmadi et al., 2011). Most areas of plain regions in Mazandaran Province are planted with rice, and different pesticides and fertilizers are used at high

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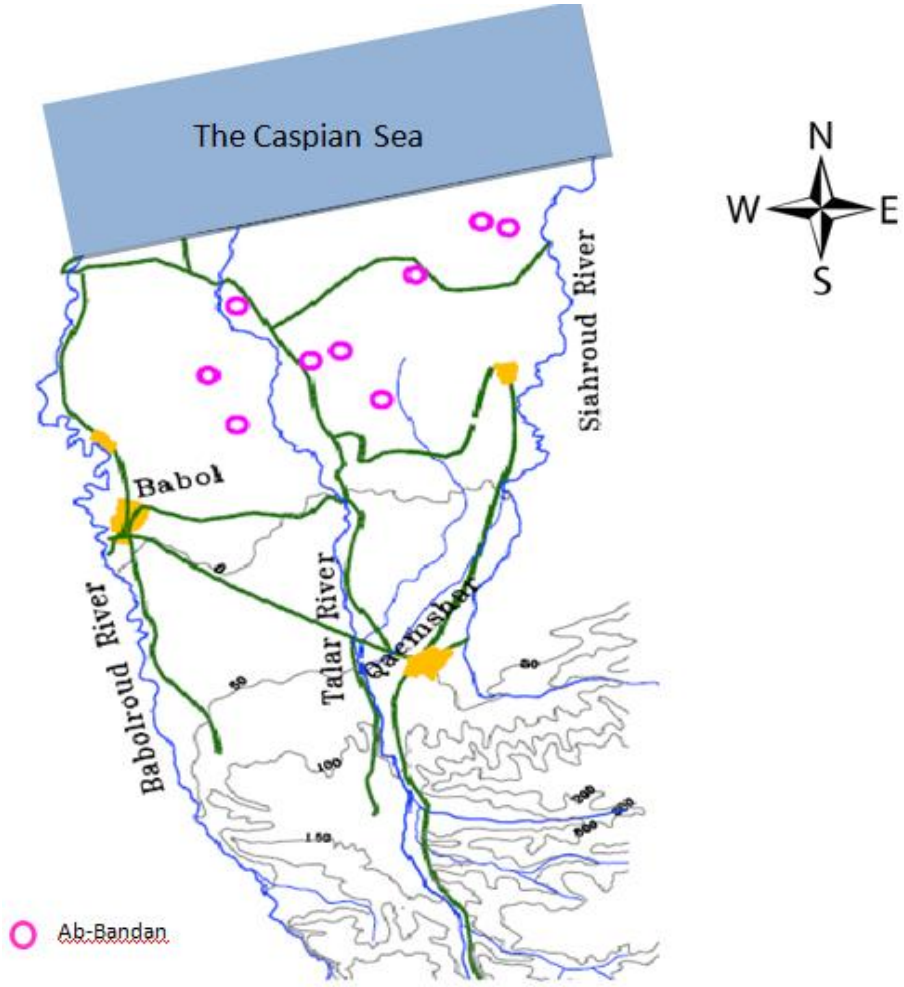


**Figure 1.** Map of studied area and sampling sites.

densities to increase the yield of production. Study area extends from Babolroud to Siaroud. Many abbandans created in this area such as Kharajisha, Ramenet, Esmaeelkola, Kordkola, Shrag-e-Larim, Anarmarz, Roshandan, Galeshkola and Langoor were selected to measure diazinon toxin (Figure 1). All mentioned abbandans feed mainly by Babolroud, Talar and Siaroud rivers and somewhat through rainfall, drainage and underground water.

Diazinon is an organophosphorus (OP) compound and a broad-spectrum insecticide that is used as a pesticide in agriculture and non-agriculture activities. This pollutant has been detected in freshwater, seawater, point-source discharges, and storm-water runoff in urban and agricultural areas (Kawai et al., 1984; Talebi, 1998; Bailey et al., 2000; Konstantinoun et al., 2005; USEPA, 2005; Luo et al., 2008). Diazinon is the main insecticide used to control stem boring caterpillar of rice (*Chilo supressalis*), lice, blowflies, ked, ticks in sheep, cattle, goats, dogs and so on. It controls aphids, caterpillars, moths, butterflies, various worms, locusts, grasshoppers and scale in pastures, orchards, vegetables and field crops. This finding indicates that pesticides, especially diazinon, are widespread in the Northern provinces and can possibly have adverse effects on aquatic ecosystem health.

It is moderately persistent but also mobile in the environment. After December 31, 2004, it became unlawful to sell outdoor, non-agricultural diazinon products in the United States (USEPA, 2005).

In the present study, the general objectives are to evaluate seasonal distribution of diazinon in different aquatic ecosystems, identify higher local concentrations to improve suitable agricultural activities toward sustainable development.

## MATERIALS AND METHODS

Nine Abbandans including Kharajisha, Ramenet, Esmaeelkola, Kordkola, Shrag-e-Larim, Anarmarz, Roshandan, Galeshkola and Langoor were selected among many Abbandans for measuring diazinon concentration as one of the organophosphorus toxins in Mazandaran. All Abbandans were chosen near rivers in order to establish an equal condition in sampling. All samples were collected

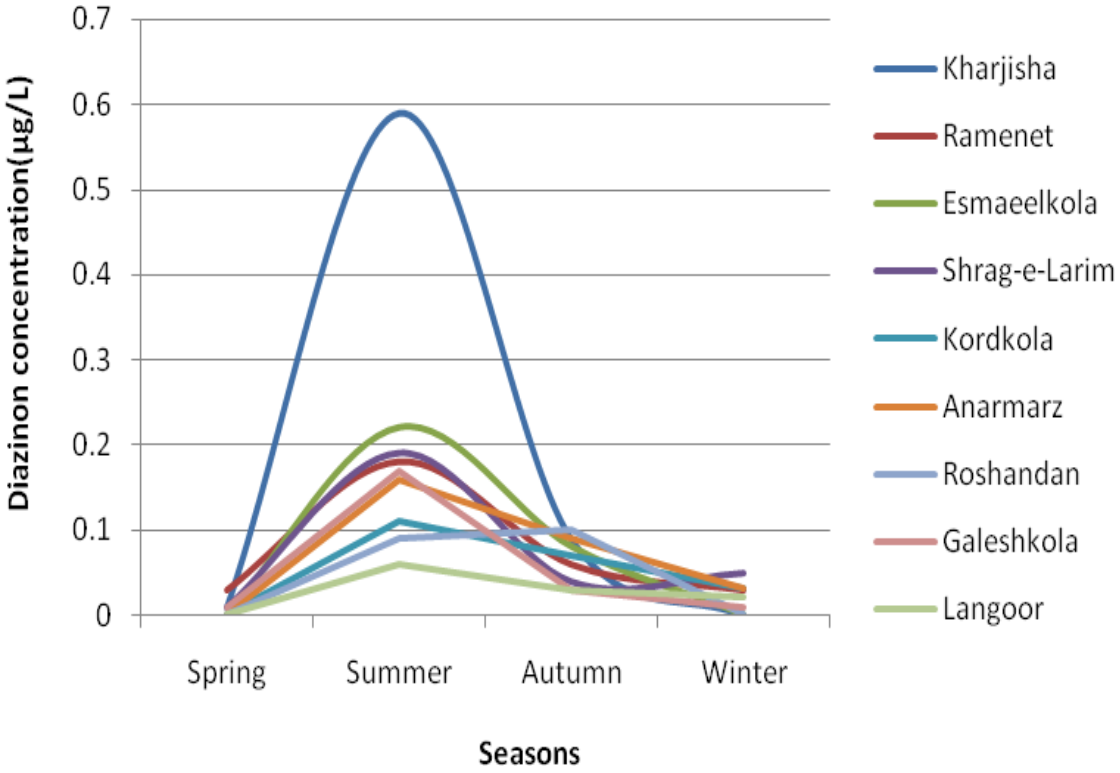


**Figure 2.** Diazinon seasonal fluctuation in Ab-bandans.

from designated stations every mid-month and collected from middle, across and depth of the Abbandans. Details on the sample sites selection and protocols have been employed as stated by Bartram and Balance (1996) and the general locality of nine selected stations is displayed in Figure 1. Sampling, containers, preservation and the transferring of samples were performed according to the methods described by APHA (1999), USEPA (2007) and Zhang (2007). At each station, duplicate water samples were collected in 1,000-mL glassy bottles. Samples were extracted without any filtration according to the method described by Zweig and Devine (1969) and Zweig (1972). Determination of diazinon in water using solid-phase micro-extraction (SPME) with gas chromatography-mass spectrometry detection (GC-MS) was investigated and detected by a flame photometric detector (FPD).

Extraction of water samples was carried out by direct immersion of the PDMS/DVB fiber in the 4mL sample contained in a 5-mL clear glass vial under magnetic stirring for 45 min at 60°C. Sample agitation was done at 1150 rpm by a magnetic stirrer. Then the fiber was removed from the sample solution and immediately inserted into the GC injector for GC-MS analysis. SPME fibers were desorbed in the splitless mode for 5 min at 250°C. GC-MS was performed with a Shimadzu (Shimadzu, Kyoto, Japan) equipped with a split-splitless injector and connected to a quadrupole mass spectrometer. Data handling and system operations were controlled by the GC–MS Solution software. Separation was carried out using a DB-5 MS capillary column (30 m × 0.25 mm, 0.25 µm, containing 5% phenylmethylpolysiloxane).

For the chromatographic determination, helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. Injector temperature was kept at 250°C in splitless mode (5min), and oven temperature was programmed as follows: initial temperature 100°C (hold 2 min), 20°C/min to 180°C, and 10°C/min to 250°C (hold 2 min). The total SPME-GC–MS analysis time is 60 min.

## RESULTS

Due to the extensive application of insecticides in the Caspian coasts of Iran (rice paddies in particular), this investigation was carried out to obtain the necessary data and information on the concentration of diazinon. Agricultural chemicals can contaminate surface water resources by runoff into streams and lakes or by the lateral movement of chemicals through unsaturated or saturated soil media to bodies of surface water (Shirmohammadi and Knisel, 1994). Accordingly, one of the biggest challenges for Abbandans and rivers are chemical pollution because it is one of the most critical threats to the aquatic ecosystem (Wei et al., 2006). The levels of diazinon concentration in Abbandans and rivers are shown in Figures 2 and 3, respectively. The highest concentration was 0.59 µg/L in Kharajsha and 0.23 µg/L in Siaroud during summer when diazinon is usually used as a pesticide in the rice fields (Talebi, 1998; Ghassempour et al., 2002). As a matter of fact, the highest distribution pattern of diazinon residue occurs in July, when diazinon is used in the fields to provide protection from pests before harvesting. Based on the field survey, harvesting occurs in late September, and thereafter, generally, diazinon and other pesticides do not show up in the study area (Tables 1 and 2).

Diazinon concentrations in this area were less than concentrations from some locations in the world (Ahmadi et al., 2011) (Table 3).

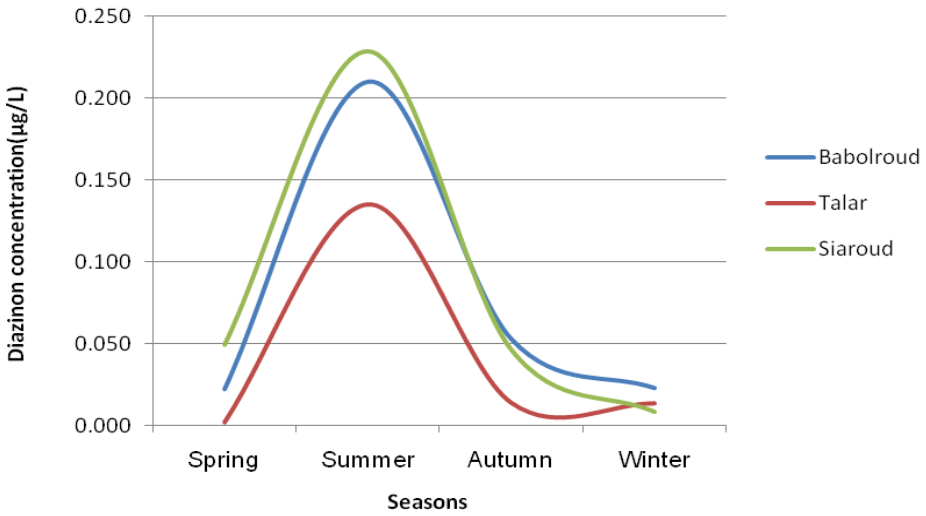


**Figure 3.** Diazinon seasonal fluctuation in rivers.

**Table 1.** Mean values of diazinon (µg/L) during four seasons in Abbandans

| | Spring | Summer | Autumn | Winter |
|---|---|---|---|---|
| Kharajisha | 0.01 | 0.59 | 0.09 | ND |
| Ramenet | 0.03 | 0.18 | 0.06 | 0.03 |
| Esmaeelkola | ND | 0.22 | 0.08 | ND |
| Shrag-e-Larim | 0.01 | 0.19 | 0.04 | 0.05 |
| Kordkola | ND | 0.11 | 0.07 | 0.03 |
| Anarmarz | ND | 0.16 | 0.09 | 0.03 |
| Roshandan | ND | 0.09 | 0.1 | ND |
| Galeshkola | 0.01 | 0.17 | 0.03 | 0.01 |
| Langoor | ND | 0.06 | 0.03 | 0.02 |

**Table 2.** Mean values of diazinon (µg/L) during four seasons in rivers.

| | Babolroud | Talar | Siaroud |
|---|---|---|---|
| Spring | 0.02 | ND | 0.05 |
| Summer | 0.21 | 0.14 | 0.23 |
| Autumn | 0.05 | 0.01 | 0.05 |
| Winter | 0.02 | 0.01 | 0.01 |

**Table 3.** Concentrations of diazinon (ng/L) in rivers from various sites in the world.

| Location | Concentration level (ng/L) | Reference |
|---|---|---|
| Selangor River, Malaysia | 116.1–510.0 | Leong et al. (2007) |
| Kalamas River, Greece | ND to 775 | Konstantinou (2006) |
| Kurose River, Japan | < 2–89 | Derbalah et al. (2003) |
| Susquehanna River, USA | 28 (max) | Liu et al. (2002) |
| Sacramento and San Joaquin River, USA | 10–1,690 (max range) | Giddings et al. (2000) |
| Hendo Khale River, Iran | 62–270 | Talebi (1998) |

more in summer than in other seasons of all nine Abbandans. They have similar trend of Diazinon concentration; it increases in spring gradually and reaches peak in aquatic ecosystems during summer. Similar studies (Khoobdel et al., 2008) have shown significant difference of diazinon and azinphos-methyl concentration during summer and other seasons in Qarahso and Gorganroud Rivers ($P<0.05$). The results showed that the value of diazinon in water samples was ND to 0.59µg/1. Kharajisha, however, shows more increasing trend as much as 3-10 times compared to others during summer. On the other hand, rivers that feed these Abbandans have also more concentration in summer (Figure 3); in fact, there is direct correlation between diazinon concentration in rivers and Abbandans.

According to British Colombia guideline, diazinon must be lower than 0.1 µg/L to protect fresh water aquatic life from short-term and lethal effects (www.env.gov.bc.ca). To protect fresh water aquatic life from long-term and sub-lethal effects, Diazinon concentrations should be lower than 0.003 µg/L and our results exceeded this value. In the Caspian Sea, there are six commercially valuable sturgeon species, four of which produce 90% of the world's caviar (Hosseini et al., 2008). The Babolroud River is one of the most important breeding habitats for sturgeon species and it provides spawning grounds for them. The high concentration of diazinon pesticide is a threat to the Babolroud River ecosystem and may contaminate the fish products consumed by humans.

Diazinon application decreases when cultivation season ends and its concentration decreases concurrently in aquatic ecosystems. As a matter of fact, rivers are not the only source of Abbandan's Diazinon, so agriculture drainage of adjacent lands also enters into them. For example, Siaroud has more concentration in summer; so it is expected to have the highest amount of diazinon in adjacent Abbandans. Kharajisha in Babolroud adjacency reveals more than others. When summer ends, diazinon application reduces gradually and its concentration falls in autumn similarly. Winter and spring have lower use in terms of crop pattern so its value is always lower in rivers and Abbandans as well.

Results show surface water concentration of diazinon is highest in summer months for rivers and abbandans. Diazinon is extremely low in abbandans from the initial sampling in April because its application had not yet started in the study area. After starting to use pesticide, the highest level of diazinon was detected in July compared to all other sampling times.

## Conclusion

In most rice paddies in Mazandaran Province, diazinon is applied to control stem boring caterpillar of rice (*Chilo suppressalis*). It comprises about 23% of all used pesticides in Mazandaran and Golestan provinces (Abbasian et al., 2012). The existence of agricultural activities has a main role on surface and underground water resources pollution in Mazandaran province. The main problems are the high groundwater table in the region, and consumption of this water by local people as well as the anthropogenic side effects of water pollutants. Physical and chemical properties of the studied diazinon such as fumigation characteristic as well as the ecolo-gical conditions and soil type influence the reduction and eventual removal of the insecticides during the cultivation and harvest periods (Arjmandi et al., 2010). Retention time varies strongly in water, soil and biota. For example, a 2008 study by Ezemonye et al. showed decreasing order of occurrence of the diazinon as follows: fish > sediment > water. The fate of Diazinon in river depends on water outflow and degradation. In Tajan River, the si-mulated results showed Diazinon insecticide of about four months (Ahmadi, 2001). Two weeks after spraying, Diazinon reveals more than standard amount in common carp (*Cyprinus carpio*) and Chub (Leuciscus cephalus) in Qezel Ozan of Zanjan (Hamidi et al., 2012). Busher's study (2005) revealed that diazinon residue is more than admissible amount in rivers of Mond, Shahpour and Dalaky (Shayeghi et al., 2007).

Limited data indicated that yellowtail (*Seriola quinqueradiata*), a marine teleost, was 84X more sensitive to Diazinon than were 4 species of freshwater fishes, as judged by LC-50(48 h) values, and by its inability to biotransform Diazinon to nontoxic metabolites within one hour (Fujii and Asaka, 1982). Diazinon has not been detected in marine waters, but the potential exists for contamination of estuarine areas from agricultural and urban runoff (Eisler, 1986). Simulation of retention time of diazinon in river showed that highest concentration of diazinon affected fauna. The bioaccumulation of diazinon was studied in bluegill sunfish (*Lepomis macrochirus*) according to US EPA data requirements (Fackler, 1988). The steady state bioaccumulation factors were determined to be 470, 540 and 500X for the edible, non-edible and whole fish tissues respectively. Elimination of diazinon from these tissues was rapid, with half-life of between 1 and 3 days, indicative of rapid depuration.

It is very important to note that the rates of pesticide application in Mazandaran Province threaten both surface and underground ecosystems and this study can be a warning for potential decreases in ecosystem biodiversity. Water sampling from 10 shallow wells located in seven villages was carried out during summer and autumn of 2006. The results of the study showed the residue of Diazinon in groundwater of Mahmoud Abad area was 0.002 to 0.572 µg/l. In some samples the concen-tration of diazinon residues in water samples was higher than WHO maximum residue limits (0.µg/l) (Khazaei et al., 2010).

Obtained findings in this research reveal that farmers are applying huge amounts of pesticides and it shows the weakness of management and lack of an exact control and supervision on rate of pesticide consumption. Also it is impossible to forbid organ phosphorous pesticides

usage in present condition, but this research emphasizes the importance of combinative (natural chemical) pest control to decrease the application of these kinds of pesticides. This controlling style can be a peaceful method between natural resources and agro ecosystems associated with sustainable development aspects.

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# Statistical analysis of hydrological properties and genetic toxicity of Maheshara Lake

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**The purpose of this study was to assess the water qualities and genotoxicity of Maheshara Lake situated near Gorakhpur, India. The lake receives many types of pollutants from two industries, agricultural run-off and domestic sewages. A total of five sampling stations were established for measurement of water pollution and the measurement of cytogenetic alterations *in vivo* is considered an initial step in the risk assessment procedures for genotoxic agent. A study was conducted to assess the cytogenetic changes in an airbreathing fish, *Channa punctatus* inhabiting the polluted water of Maheshara Lake. In the present study, significantly ($p<0.05$) higher micronuclei formation was observed in these groups of fishes which receive lake water thus indicating induced mutation in fishes living in Maheshara Lake water. It might be the presence of genotoxic chemicals in water, which is responsible for the DNA damages in fishes.**

**Key words:** Maheshara Lake, genotoxic, micronuclei, *Channa punctatus*.

## INTRODUCTION

In nature, water is one of the most abundantly available resources, which has been exploited by the man. Water is considered as a good disposable site of city sewage and also of industrial wastage. Quality of water provides current information about the concentration of various solutes at a given place and time. In this fast growing age, declining availability of usable fresh water is the major concern in terms of water quantity and quality (Boyd and Tucker, 1998)

In toxicity test, the genotoxicity test in fishes, amphibians and other aquatic organisms serves as an effective tool in monitoring environmental quality. In aquatic ecosystem, genotoxic pollutions refer to the introduction of contaminants having mutagenic, teratogenic and carcinogenic potentials into its principle media (Fagr et al., 2008). The compounds like heavy metals (Matsumoto et al., 2005), microbial toxins (Smith, 1996) and pesticides as fungicides (Srivastava and Singh, 2013), insecticides and so on are considered as genotoxicants that come from indiscriminate disposal of sewage, industrial wastes and human activities. These genotoxicants have been reported to cause mutations because of their strong affinity to bond with DNA and resulting in the formation of DNA adducts and micronuclei. These genomic losses in organisms induce various disease. Therefore, now a day detection of genotoxic effects in aquatic organisms has gained importance (Hayashi et al., 1998; Kushwaha et al., 2012).

The micronucleus (MN) test, due to its simplicity, is one of the most applicable techniques to identify genomic alterations in environmental animals (Hayashi and Bolognesi, 2011). Micronuclei express in dividing cell that either contains chromosome breaks lacking centromere or whole chromosomes, which unable traveling to spindle poles during mitosis. At telophase, a nuclear envelope forms around the lagging chromosome and fragments which uncoil and gradually assume morphology of an interphase nucleus. With an exception, they are smaller than the main nuclei in the cell, hence termed as micronuclei.

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Fishes response to toxic agents similar to higher vertebrates can allow the assessment of substances that are potentially hazardous to humans (Grisolia and Carderio, 2002; Kushwaha et al., 2012). Due to chemical pollutants, the low amount of DNA per cell, the large numbers of small chromosomes, and the low mitotic activity in many fish species impaired the metaphase analysis of chromosomal damage and sister chromatid exchanges are demonstrated (Pavlica et al., 2000). The MN test, due to its potentiality to be applied in any proliferating cell population regardless of the karyotype of the species use, are successfully applied in fish for evaluating the genotoxic activity of xenobiotic agents and of complex environmental mixtures in laboratory as well as in field studies (Hayashi and Bolognesi, 2011).

The Gorakhpur City lies between Lat. 26° 13´N and 27° 29´N and Long. 83° 05´ E nad 83° 56´E near the bank of River Rapti. The Maheshara is a tributary of Rapti, is an important water body of Gorakhpur, India region. In this region, people use synthetic pesticides for pest control in agricultural fields especially in paddy fields, which received the effluents of the two industries (metal workshop and fertilizer enterprises), domestic sewage and agricultural run-off. Lake water is also used for bathing, cleaning of utensils, washing of cloths and disposable of industrial wastage. These activities have been subjected to a strong biotic pressure all along the periphery and have turned into an eutrophic stage. The water of the lake is declining in quality posing threat to these people. It has also noticed that the highly polluted water of Maheshara Lake affect the population of aquatic fauna especially fishes. Therefore, the study on the fish genotoxicity in Maheshara Lake was lacking, hence the present study had aimed at examining the water quality and fish genotoxicity of lake river system in Gorakhpur, India. The findings from the study will benefit the planning and management of sustainable fisheries and conservation of natural resources.

## MATERIALS AND METHODS

### Site selection

Five study sites were selected as shown in Table 1. Site 1 is agricultural runoff point. This site was chosen because there was paddy fields surrounding the lake and local farmers had used several pesticides in the fields and by surface run off these pesticides enter into body tissues of fishes and alter the biochemical pathways and also induce genetic disorders, Site 2 was metal workshop point selected because the effluents of this industry continuously reach in the lake, Site 3 was domestic water point, lake water has also been used for keeping house hold activities by the surrounding people who are inhabitant, Site 4 was sewage point, most of the city sewage discharge into the lake and site 5 was industrial effluent point. There was another industry, which effluents also reach in the lake.

### Collection of water sample

In Gorakhpur city, seasonal variations were found. May-June have extremely hot day while November-December are extremely cold day and July-August is a rainy month. Hence, water samples were collected in these months. Water samples were collected early in morning (6:00to 8:00am) from May-June (2013), July-August (2013) and November-December (2013). From each site, 10 samples of water were collected and averages of each site are presented in Table 1. Temperature and pH of water samples were measured in the field immediately with the help of mercury glass thermometer and portable pH meter, respectively. Other physico-chemical parameters were analyzed in the laboratory within 5 h of collection. All the parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids and total dissolved solids were examined by using standard methods APHA (2005).

### Collection of fishes

For genotoxic experiment, fishes were collected with the help of expertise of local fisher folk. Sampling was carried out at all the five sites. Different types of gear including cast net, gill net and drag net and other local conveners were used. *Channa punctatus* is edible fish and mostly found in lake at all sites.

### Experimental designs for micronucleus test assay (MNT)

*C. punctatus* were collected from different sites of Maheshara Lake. For experiment, 9 fishes were collected from all sites and divided into three groups. An average number of micronuclei is shown in Table 2.

### Slide preparation

Blood smear slides were prepare by the method of Das and Nanda (1986) with some modifications. Peripheral blood samples were obtained from caudal vein of fish. For each groups, fishes used and blood smear were prepared. At each assessment, 2500 cell/fish were analyzed, 7500 erythrocytes for each group. Slides were dried in air, fixed in absolute methanol for 10-15 min, and stained in Giemsa (pH 7.0) for 1-2 h; washed with DDW and air-dried. Permanent preparation was made by mounting in DPX. They were screen in oil immersion objective (100x) and micronuclei was observed under microscope (OLYMPUS CX21i) with camera (Magnus MIPS USB 5MP).

### Chemicals and Instruments

Chemicals such as Di-sodium hydrogen orthophosphate (17549) from Merck chemicals, Potassium Di-hydrogen orthophosphate (39619K05) from Merck chemicals, Giemsa stain (44034G25 G09Y/1009/0907/71) from SD Fine chemicals India, Glycerol, Xylene and Methanol from SD Fine chemicals India were used. The instruments used in the study are Microscope (OLYMPUS CX21i) purchased from Japan, Camera used in the experiment (Magnus MIPS USB 5MP) was purchased from Japan, Hot Air Oven (LIMCO equipment Limited).

### Statistical analysis

The frequencies of abnormalities were determined for each group. MN frequencies (MN‰) are calculated as follows:

$$MN‰ = \frac{\text{Number of cells containing micronucleus} \times 1000}{\text{Total number of cells counted}}$$

**Table 1.** Physico-chemical parameters of water sample at different sites of Maheshara Lake.

| Parameter | May-June | | | | | July-August | | | | | November-December | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
| pH | 8.02±0.11 | 6.25±0.05 | 10.14±0.12 | 10.04±0.10 | 10.0±0.09 | 7.92±0.10 | 5.15±0.01 | 7.80±0.07 | 7.50±0.07 | 7.85±0.06 | 7.10±0.06 | 5.57±0.02 | 7.0±0.05 | 6.91±0.04 | 7.15±0.06 |
| Temp | 32.2±0.10 | 35.3±0.12 | 37.0±0.20 | 35.41±0.18 | 35.0±0.16 | 30.0±0.09 | 32.4±0.11 | 34.1±0.12 | 32.0±0.10 | 31.5±0.13 | 26.12±0.10 | 28.5±0.14 | 28.0±0.13 | 28.71±0.12 | 28.0±0.10 |
| DO | 2.85±0.13 | 1.80±0.09 | 1.95±0.10 | 2.0±0.12 | 1.82±0.09 | 5.20±0.12 | 4.61±0.09 | 4.90±0.10 | 5.10±0.26 | 4.0±0.06 | 6.0±0.17 | 5.60±0.14 | 5.85±0.15 | 6.03±0.18 | 5.35±0.12 |
| BOD | 20.33±0.14 | 40.0±0.20 | 30.40±0.18 | 41.66±0.24 | 25.0±0.16 | 28.35±0.17 | 33.3±0.20 | 31.03±0.19 | 35.56±0.22 | 30.54±0.18 | 31.78±0.19 | 36.29±0.23 | 33.04±0.20 | 39.83±0.28 | 32.29±0.21 |
| COD | 82.10±1.02 | 120.23±1.14 | 100.43±1.01 | 125.56±1.12 | 90.25±1.14 | 113.34±1.16 | 133.20±1.09 | 124.12±1.14 | 142.27±1.05 | 122.19±1.12 | 127.14±1.10 | 145.17±1.06 | 132.19±1.04 | 159.32 ±1.07 | 129.16±1.13 |
| TSS | 317±1.11 | 303±1.13 | 447±1.24 | 353±1.15 | 385±1.20 | 213±1.08 | 235±1.13 | 278±1.10 | 245±1.12 | 274±1.14 | 184±1.06 | 198±1.09 | 210±1.13 | 201±1.10 | 206±1.11 |
| TDS | 154.25±1.17 | 150.0±1.12 | 186.75±1.20 | 163.25±1.15 | 171.25±1.17 | 193.12±1.19 | 187.32±1.20 | 232.32±1.34 | 200.23±1.28 | 210.56±1.22 | 220.15±1.25 | 212.32±1.21 | 265.34±1.42 | 228.45±1.30 | 236.12±1.32 |

DO = Dissolve oxygen (mg/l), BOD = biological oxygen demand (mg/l), COD = chemical oxygen demand (mg/l), TSS = total suspended solids (mg/l), TDS = total dissolve solids (mg/l), Temp=Temperature (°C).

Then the mean ± standard error for each group was calculated. Student's t-test was employed for compareson of control and experimental animals.

Correlation coefficients between different pairs of parameters were calculated by using the following formula and t-test was applied for checking significance.

$$r = \frac{n(\Sigma xy)-(\Sigma x)-(\Sigma y)}{\sqrt{[n\Sigma x^2-(\Sigma x)^2][n\Sigma y^2-(\Sigma y^2)]}}$$

Where n = sample size.

## RESULTS

### Physico-chemical analysis

The physico-chemical characteristics of Maheshara Lake during the period of study at different sites are presented in Table 1.

#### *pH*

In the present study during May-June, the highest pH (10.14 ± 0.12) was recorded at Site-3 (domestic wastewater point) while lowest pH (6.25 ± 0.05) was recorded at Site-2 (metal workshop point). During July-Aug the highest pH (7.92 ± 0.10) was recorded at Site-1 (agriculture run off point) while lowest pH (5.15 ± 0.01) was recorded at Site-2 (metal workshop point). During November-December the highest pH (7.10 ± 0.06) was recorded at Site-1 (agriculture run off point) while lowest pH (5.57 ± 0.02) was recorded at Site-2 (metal workshop point).

#### *Water temperature*

Water temperature was a controlling factor for aquatic life. During May-June the highest temperature (37.0 ± 0.20°C) was recorded at Site-3 (domestic wastewater point) while lowest temperature (32.2 ± 0.10°C) was recorded at Site-1 (agriculture run off point). During July-Aug the highest temperature (34.1 ± 0.12°C) was recorded at Site-3 (domestic wastewater point) while lowest temperature (30.0 ± 0.09°C) was recorded at Site-1 (agriculture run off point). During November-December, the highest temperature (28.71 ± 0.12°C) was recorded at Site-4 (sewage waste point) while lowest temperature (26.12 ± 0.10°C) was recorded at Site-1 (agriculture run off point).

#### *Dissolve oxygen (DO)*

Dissolve oxygen was one of the most important parameters. Concentrations of dissolved oxygen support a great diversity of aquatic organisms. During May-June the highest concentration of DO (2.85 ± 0.13mg/l) was recorded at Site-1 (agriculture run off point) while lowest concentration of DO (1.80 ± 0.09 mg/l) was recorded at Site-2 (metal workshop point). During July-August, the highest concentration of DO (5.20 ± 0.12 mg/l) was recorded at Site-1 (agriculture run off point) while lowest concentration of DO (4.0 ± 0.06 mg/l) was recorded at Site-5 (industrial provision point). During November-December, the highest concentration of DO (6.03 ± 0.18 mg/l) was recorded at Site-4 (sewage waste point) while lowest concentration of DO (5.35 ± 0.12 mg/l) was recorded at Site-5 (industrial provision point).

#### *Biological oxygen demand (BOD)*

Biological oxygen demand test show how much oxygen is being consumed. During May-June the highest value of BOD (41.66 ± 0.24 mg/l) was recorded at Site-4 (sewage waste point) while lowest value of BOD (20.33 ± 0.14 mg/l) was recorded at Site-1 (agriculture run off point). During July-August the highest value of BOD (35.56 ± 0.22 mg/l) was recorded at Site-4

**Table 2.** Correlation between different pairs of parameters.

| Pair of parameter | r-value | t-value |
|---|---|---|
| pH and temp. | 0.4020 | 7.60 |
| pH and DO | -0.0112 | 1.95 |
| pH and BOD | 0.0884 | 1.53 |
| pH and COD | -0.1516 | 2.65 |
| pH and TSS | 0.8320 | 25.9 |
| pH and TDS | 0.8434 | 26.9 |
| DO and BOD | -0.3459 | 6.38 |
| DO and COD | -0.5854 | 12.5 |
| DO and TSS | -0.3428 | 6.32 |
| DO and TDS | -0.3063 | 5.57 |
| BOD and COD | 0.9984 | 31.27 |
| BOD and TSS | -0.1557 | 2.73 |
| BOD and TDS | -0.1673 | 2.93 |
| COD and TSS | -0.1815 | 3.19 |
| COD and TDS | -0.1905 | 3.36 |
| TSS and TDS | 0.9998 | 86.58 |

(sewage waste point) while lowest value of BOD (28.35 ± 0.17 mg/l) was recorded at Site-1 (agriculture run off point). During November-December, the highest value of BOD (39.83 ± 0.28 mg/l) was recorded at Site-4 (sewage waste point) while lowest value of BOD (31.78 ± 0.19 mg/l) was recorded at Site-1 (agriculture run off point).

### *Chemical oxygen demand (COD)*

On other hand, Chemical oxygen demand (COD) range during May-June was highest (125.56 ± 1.12 mg/l) at Site-4 (sewage waste point) lowest (82.10 ± 1.02 mg/l) Site-1 (agriculture run off point). During July-August, the highest value reached up to (142.27 ± 1.05 mg/l) at Site-4 (sewage waste point) lowest (113.34 ± 1.16 mg/l) at Site-1 (agriculture run off point). During November-December, the highest reached up to 159.32 ± 1.07 mg/l at Site-4 (sewage waste point) while lowest value reached up to 127.14 ± 1.10 mg/l at Site-1 (agriculture run off point).

### Total suspended solids

During May-June, Total suspended solids recorded from (303 ± 1.13 mg/l) to (447 ± 1.24 mg/l). During July-August, it recorded as from 213±1.08 mg/l to 278 ± 1.10 mg/l. During November-December, it recorded from 184 ± 1.06 to 210 ± 1.13 mg/l. Total dissolve solids was from 150.0±1.12 to 186.75 ± 1.20 mg/l during May-June. During July-August, it was from 187.32 ± 1.20 to 232.32 ± 1.34 mg/l. During November-December, it was from 212.32 ± 1.21 to 265.34 ± 1.42 mg/l.

### Micronuclei assay

The result of micronucleus in peripheral erythrocytes of *C. punctatus* at different sites of Maheshara Lake are shown in Table 3 and Figure 1. At Site 1, which was agricultural runoff point, the micronuclei frequencies was recorded as 9.60 ± 0.04. At Site 2, The metal workshop point, induction of micronuclei in fish *C. punctatus* was observed as 13.33 ± 0.05. Site 3, domestic water point, the induction of frequencies in peripheral blood samples were 19.86 ± 0.08. Site 4 was sewage point and the induction of frequencies in peripheral blood samples were also 22.13 ± 0.09, and At Site 5, the Industrial effluent point observed frequency was 13.86 ± 0.06. In all the groups receiving polluted water, frequencies of micronuclei were observed to be significantly higher ($p<0.05$) when compared with the control. The maximum induction in micronuclei frequency was observed at Site 4 and minimum was observed at Site 1. The hierarchy of micronuclei frequencies at different sites were as follows: Site 4 > Site 3 > Site 5 > Site 2 > Site 1 as compared to the control.

## DISCUSSION

Different water quality parameters with significant correlation coefficients are given in Table 2.

### pH

pH is an important limiting chemical factor for aquatic life. The $H^+$ or $OH^-$ ion activity may disrupt aquatic organisms biochemical reactions by either harming or killing the lake organisms. Correlation analysis showed that pH had a significant positive relationship with temperature (r = 0.4020, t = 7.60), BOD (r = 0.0884, t = 1.53), TSS (r = 0.8320, t = 25.9) and TDS (r = 0.8434, t = 26.9) and negative correlation between D.O. (r = 0.0112, t = 1.95) and

**Table 3.** Frequencies of micronuclei (MN) observed in fish *C. punctatus* at different sites of Maheshara Lake.

| Site | Total number of cells studies | Total number of MN | | | Average percentage (%) aberration* ±SE |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 3 | |
| Control (freshwater fish) | | 02 | 01 | 01 | 0.8±0.05 |
| Site 1 | | 21 | 28 | 23 | 9.60±0.04 |
| Site 2 | 2500cell/fish | 34 | 30 | 36 | 13.33±0.05 |
| Site 3 | | 50 | 46 | 53 | 19.86±0.08 |
| Site 4 | | 56 | 53 | 57 | 22.13±0.09 |
| Site 5 | | 38 | 32 | 34 | 13.86±0.06 |

*, Significant (P<0.05) when Student's 't' test was applied between treated and control groups.

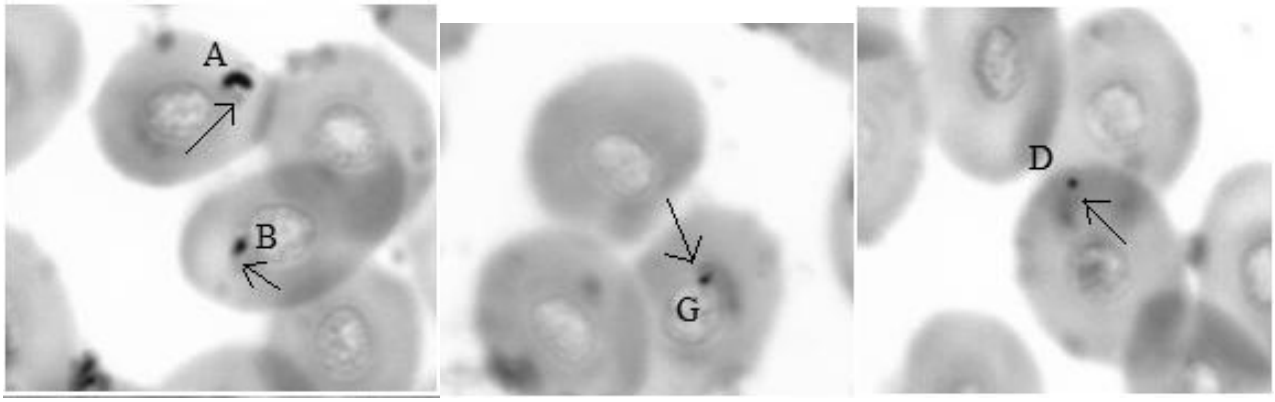


**Figure 1.** the Micronuclei (MNi) in blood of *C. punctatus*. A = Notched nuclei; B, G = lobed nuclei; D = micronuclei.

COD (r = 0.1516, t = 2.65). Change in pH can change the aspects of water chemistry, e.g. as pH increases (at Site 3), smaller amounts of ammonia is needed to reach a level that is toxic to fish. As pH decreases (at Site 2), the concentration of metals in water may increase because higher acidity increases their ability to be dissolved from sediments into the water and fishes were affected. Sim (2004) and Sujaul et al. (2012) have reported similar findings. The acidity of natural lake ecosystems due to high rich organic matter and metals were reported by Sujaul et al. (2012), Wetzel (1983) and Chapman (1988).

## Temperature and dissolve oxygen (DO)

There are many factors that can influence the lake temperature. Water temperature can fluctuate seasonally, daily, and even hourly, especially in smaller sized streams or lake. Temperature affects the concentration of dissolved oxygen in water body. The dissolve oxygen in water is often attributed to the fact that the oxygen is dissolve more during the period of high catabolic activities by photosynthesis. DO decreases as turbidity, TDS and TSS increases (Joshi et al., 2009). The concentration of dissolve oxygen in lake is also affected by velocity of water, aquatic plants, TDS, TSS organic waste and urban human activities. In the present study, D.O. has strong significant negative correlation with BOD (r = 0.3459, t = 6.38), COD (r = 0.5854, t = 12.5), TSS (r = 0.3428, T = 6.32) and TDS (r = 0.3063, t = 5.57). Summer is usually the most crucial time for dissolve oxygen levels because stream flows tend to lessen and water temperature tend to increase. In the present study, the minimum DO concentration was observed in month of May-June at different sites when compared with months of July-August and November-December. In general, DO levels less than 3 mg/l are stressful to most aquatic organisms. Most fishes die at 1-2 mg/l. However, fish can move away from low DO areas. Water with low DO from 2-0.5 mg/l are considered hypoxic, waters with less than 0.5mg/l are anoxic.

## Biological oxygen demand (BOD) and chemical oxygen demand (COD)

A quantitative relationship exists between the amount of oxygen required to convert a definite amount of any organic matter to $CO_2$, water and $NH_3$ (Joshi et al., 2009). BOD is an indication of the organic load and it is a pollution index especially for water bodies receiving organic effluents (Ndimele, 2012). There are significant negative correlation between BOD and TSS (r = 0.1557, t = 2.73)

and BOD and TDS (r = 0.1673, t = 2.93). Whereas significant positive correlation was observed in BOD and COD (r = 0.9984, t = 31.27). The value of BOD and COD were increased with increase in the pollution loads. Correlation analysis showed that COD had a significant negative relationship with pH (r = 0.1516, t = 2.65), DO (r = 0.5854, t = 12.5), TSS (r = 0.1815, t = 3.19) and TDS (r = 0.1905, t = 3.36).

**Total suspended solids (TSS) and total dissolve solids (TDS)**

The highest total suspended solids were recorded in month of May-June which vary from 303 ± 1.13 to 447 ± 1.24 mg/l. During July-August, it recorded from 213 ± 1.08 to 278 ± 1.10 mg/l and in November-December, it recorded from 184 ± 1.06 to 210 ± 1.13 mg/l. Total dissolve solids was recorded as 150.0 ± 1.12 to 186.75±1.20 mg/l during May-June. During July-August, it recorded from 187.32 ± 1.20 to 232.32 ± 1.34 mg/l. During November-December, was from 212.32 ± 1.21 to 265.34 ± 1.42 mg/l. Correlation analysis showed that TSS and TDS had a significant positive relationship (r = 0.9998, t = 86.58). With the above observation we concluded that due to the deposition of metals, pesticides residues and other organic wastages in the lake, pH of lake water become disturbed and it affects the vegetation in lake. Due to heavy deposition of organic waste and increase in concentration of ammonia, lake has eutrophication condition and this BOD level increase and DO level decrease, affect fish diversity and its health too. Similar results in biological aspect and physico-chemicals parameters were documented by several authors (Joshi et al., 2009; Sinha and Biswas, 2011; Shinde et al., 2011; Sujaul et al., 2012).

**Micronucleus**

Micronucleus (MN) test in fish erythrocytes of polluted water bodies were demonstrated to be a sensitive biomarker to detect genotoxic damage induced by complex mixture of contaminants in water. These complex mixtures of pollutants are responsible for multiple effects in aquatic organisms as it affect the functions of many organs, reproductive status, species survival, population size and ultimately biodiversity too (Bickham et al., 2000; Dixon et al., 2002). These carcinogenic and mutagenic compounds present in polluted water because of the discharge of industrial, agricultural and urban wastages may exert damage beyond that of individual and may be active through following generations. For ecotoxicological studies fishes are reported to be suitable organisms because they play different roles in the heterotrophic web, undergo bioaccumulation, and respond to mutagens at low concentration such as environmental pollutions (Cavas and Ergene-Gozukara, 2005; Kushwaha et al., 2012).

In field study, MN test is one of the most applicable techniques to identify genomic alterations in aquatic animals because this procedure is technically easier and more rapid than the microscopic analysis of chromosomal aberrations in metaphase (Fagr et al., 2008). Biomonitoring studies were carried out in native lake fish species *C. punctatus* at different sites. Consequently, maximum scoring in MN-frequency was observed in fish at Site 4 (fish received water from sewage waste) then Site 3 (fish received water from domestic water), Site 5 (fish collected from industrial effluent), Site 2 (fish received water from Metal workshop) and Site 1 (fish received water from agriculture run off). However, a number of studies applying the MN test in erythrocytes of fish in polluted water failed to reveal the impact of associated with well known genotoxic mixtures in polluted water such as heavy metals, pesticides or hydrocarbons. Many factors may be responsible for scoring of MN such as age, sex size, species, metabolic capacity, DNA repair efficiency and defense mechanisms, development of adaptive mechanisms of tolerances to chemical stress and rate of dead or damaged cells to maintain normal physiological conditions or inhibition of nuclear division which is required for MN expression (Rodriguez et al., 2003; Cavas and Ergene-Gozukara, 2005). Formation of MN occurred during anaphase from lagging acentric chromosome or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks or at anaphase malsegregation of whole chromosomes which may also lead to MN formation. Malsegregation of whole chromosomes has resulted to the hypomethylation of repeat sequences in centromeric and pericentromeric DNA, defects in kinetochore proteins or assembly, dysfunctional spindle and defective anaphase checkpoint genes (Mateuca et al., 2006; Fenech, 2007). Malsegregation of chromosomes and formation of MN probably incorporated with inappropriate kinetochore protein assemble at centromere (Bull et al., 2008). Kinetochore proteins such as CENPA and CENPB are greatly affected by methylation of cytosine and histone also. Defects in kinetochore proteins leads to defects in microtubules interactions of chromatids during spindle formation and this mutation caused MN formation during Anaphase (Ieniet al., 2008). Other mechanisms that could lead to MN formation from acentric fragments Include simultaneous excision repair of damage or inappropriate base incorporated in DNA (e.g. uracil) that are in proximity and on opposite complementary DNA strands. Such simultaneous excition repair events, particularly if the gap-filling step is not completed, leads to DNA-double strands breaks and MN formation. This process can greatly enhance the blood MN assay DNA break or MN formation treated with cytosine arabinoside during $G_1$ phase of the cell cycle which inhibit the gap filling step of excision repair (Fenech and Crott 2002).

Several workers (Smith, 1990; Vigana et al., 2002; Rodriguez et al., 2003; Lemos et al., 2005) observed similar results in genotoxic patterns of polluted water in

other fish species.

## Conclusion

The result of the present investigation on genotoxic potential of the polluted water of Maheshara Lake suggested a serious concern about its potential to damage aquatic organisms especially fish. However for safeguarding aquatic environments and organisms, several studies are needed to explore the biological consequences of DNA damage in fish due to polluted water of Maheshara Lake.

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*Full Length Research Paper*

# Spatial-temporal distribution and limnology of crustaceans in a tropical freshwater lake, Nigeria

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**Information on biological communities is important to assess the qualities of aquatic systems receiving wastewater. Crustacean communities have a cosmopolitan distribution, short life cycle and are useful for the general monitoring of certain aspects of the environment, such as eutrophication, pollution, warming trends and long-term changes which are sign of environmental disturbance. In this study, spatial-temporal and limnology of crustacean in a tropical freshwater lake was carried out between 2010 and 2011 at three stations. Identification of all the sampled organisms was done using taxonomic keys. The sampled crustaceans were made up of 7 species (*N* = 820): *Daphnia* (30.44%), *Napilius* (18.33%), *Camptocerus* (14.30%), *Eurycerus* (8.54%), *Bosmina* (9.27%), *Canthocamptus* (8.57%) and *Cyclops* (10.51%). Phytoplankton sampled were composed of six families: *Bacillariophycea*, *Chlorophyceae*, *Cryptophyceae*, *Cyanophyceae, Dinophyceae* and *Xanthophyceae* while 12 species of macrophytes were sampled (*Kyllingn squamalata, Nymphaea lotus, Acroceras zizanioid, Cyperacea difformislinn, Alchonea*. The physico-chemical, plankton abundance, macro flora and fauna in Opi Lake fell within the productive values for aquatic ecosystem and indicated that the lake is eutrophic, crustaceans and biotic/abiotc factors are example of natural freshwater habitat.**

**Key words**: Opi Lake, crustaceans, phytoplankton, macro invertebrate, calcium.

## INTRODUCTION

Crustaceans constitute an invaluable source of calcium, iodine, amino acids, lipids, fatty acids, minerals and enzymes and are therefore inexpensive ingredients to replace fishmeal for cultured fish. Crustaceans are also considered healthy for the circulatory system because of lack of significant levels of saturated fat in shrimp (Ferdinard, 1994). The cholesterol content in shrimp actually improves the ration of LDL to HDL cholesterol and lowers triglycerides (Elizabeth et al., 1996). The study of crustacean communities seems to be particularly useful for the evaluation of ecosystem status. Distibution of crustacean is useful for the general monitoring of certain aspects of the environment, such as eutrophication, pollution, warming trends and long-term changes which are sign of environmental disturbance (Rutherford et al., 1999; Soberman et al., 2000). Crustaceans as bioindicators are very convenient in providing ecological indices. Due to short lifecycles, crustaceans can quickly respond to environmental modifications, being excellent key group (Boltovoskoy, 1999). Evurunobi (1984) looked at the phytoplankton and physiochemical while Hare and Carter (1984, 1986) dwelt on the diet and seasonal fluctuations of

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Benthos of Opi Lake. Inyang (1995) overviewed the fish fauna with particular reference to the Biology of *Tilapia zilii* and Nweze (2003) worked on the phytoplankton production, while Echi et al. (2009) investigated the coparasitism and morphometric of three clinostomatids of the Lake. There has been little study on spatial-temporal distribution and limnology of crustaceans in lowland tropical lentic ecosystems. Identification of fauna and flora taxa to generic level is often impossible and information on spatial and temporal variations in these populations is usually lacking. This limited information can be contrasted with the increasing interest in tropical aquatic systems both for practical purposes, as sources of power, protein and parasites, and for theoretical reasons. Parallel to this is an increasing concern over the accelerating pace of man's alteration of tropical ecosystems through such activities as deforestation and the use of biocides. The impacts of such activities have proved difficult to predict, given the present lack of knowledge about the natural state of these systems. Hence the present study was undertaken to describe spatial-temporal changes in limnology and crustaceans, macro invertebrates and phytoplankton populations.

## MATERIALS AND METHODS

### Study area

Opi Lake is a typical freshwater lake located between 6° 45' 0''-45' 28''N and 7° 29' 35'' E (GPS N 06.75275*, E 007.49104) in the valley of river Uhere, Northeast of Nsukka, Enugu State, Nigeria. The lake is about 300 m from Uhere River. The vegetation and climate of the lake has been described by Hare and Carter (1984). The lake has no permanent inlet but during the flood period the lake overflows through a small channel at the southern end. The western side of the lake has a wide beach overgrown with saprophytes dominated by *Crytosperma senegalenses* (Scholt); *Jussiaea repens Var diffusa* (Fordk) and *Rynchospora* species. Its surface area and maximum depth fluctuates seasonally and ranges between 1.3 and 2.0 and 2.0 ha and 3.9 m, respectively (Inyang 1995). The mid lake deposit is mixed with coarse organic matter from the marginal vegetation on the other part of the shoreline.

Three sampling stations were selected based on the nature of the lake (Figure 1). Station 1 situated in the southern or overflow end which have more vegetation, shade and receive in runoff during heavy rainfall and outlet. Station 2 situated in the middle of the lake and has lesser vegetation and lesser shade while station 3 was at the northern end with least vegetation and no shade. Sampling was done within 8 am and 12 noon once in a month.

### Physico-chemical

Physicochemical parameters of the Opi Lake were studied between 2010 and 2011. In each station at least five sample collections were taken at different locations. All *in situ* determinations and collections of water samples were made during mid-morning (10-11 am) local time. The water depth was measured using a graduated sounding line. A "HACH" conductivity meter (model 16300) was used for the conductivity measurement. The hydrogen ion concentration was measured using a compensated pH meter Kent Model 6025.

Transparency was measured using a secchi disc. Total ions, hardness, total alkalinity, calcium, colour, turbidity, were analyzed using 'HACH' portable laboratory test kits. Free $CO_2$ was determined in the field titrimetrically using 0.0027 N NaOH and phenolphthalein indicator. The chemical oxygen demand (COD) was estimated by running blank 70 ml (double distilled water to 50 ml of water). This was heated to boiling point and then 5 ml of $KMnO_4$ plus 5 ml KI + 10 ml 2 M $H_2SO_4$ + 1 ml of starch solution were added. These combinations were titrated with 0.1 M $Na_2S_2O$ until blue colour disappeared which was the end point. Temperature was measured immediately in the field using mercury in glass bulb thermometer and read to the nearest 0.1°C. Dissolved oxygen (DO) was also determined in the field using a dissolved oxygen meter. Samples for the analysis of other variables were then taken to the laboratory in clean 2.51 polyethylene bottles, and preserved in a deep freezer until analysis. The concentrations of calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), iron ($Fe^{2+}$) in the samples were determined using atomic absorption spectrophotometer (Buck 200A mode) at their characteristic wavelengths of 589, 766, 422, 285, 214 and 283 p.m, respectively. The concentrations of $NO_3^-$ and $PO_4^{3-}$ were determined using the colorimetric method (M201 CcamSpec Visible Spectrophotometer) as describe by APHA (1998). Total dissolved solids (TDS) was measured by the filtration, evaporation (at 105°C) and weighing (APHA 1998).

### Macro invertebrate sampling

Macro invertebrate were sampled with the use of equipment such as scoop net which have a fine mesh aperture of 60 µM. The stations, samplings were done within the entire range of habitats available (open waters, shallow water over hard and soft bare benthos or over submerged aquatic macrophytes, along shores of habitat and also masses of aquatic debris and vegetation which were collected drained slightly and spread out in order to see and collect some organisms that crawled out from these material). A collection in five-eight minutes from a variety of location within each station was done. After collecting the aquatic organisms, they were transported to Hydrobiology Unit Department of Zoology and Environmental Biology, University of Nigeria, Nsukka for preservation and identification.

### Plankton sampling

In each of the sampling station, sample was concentrated into 20 ml by draining water out through another 55 µ mesh net size to prevent loss of plankton. Then the plankton samples collected were filtered through another net of about 64 µ mesh net size (Avoaja, 2005). To separate the zooplankton from the phytoplankton, the zooplankton samples were collected in the specimen bottles, labelled and preserved in 4% formalin. The phytoplankton was equally preserved in the bottles with 4% formalin. These samples were allowed to stand for at least 24 h before analysis. During the analysis of the zooplankton, the supernatant was carefully pipette off and the zooplankton sample was concentrated to 10 ml volume. 1 ml was carefully viewed under an Olympus binocular microscope; model CH 0337331. Identification of the phytoplankton and zooplankton was done by keys (Jeje and Fernando, 1986; Nweze, 2005; Robert, 2003; Smile, 2008).

### Identification of crustaceans

Identification of specimens were sorted and identified with suitable keys (Jeje and Fernando, 1986; Robert, 2003; Smile, 2008).

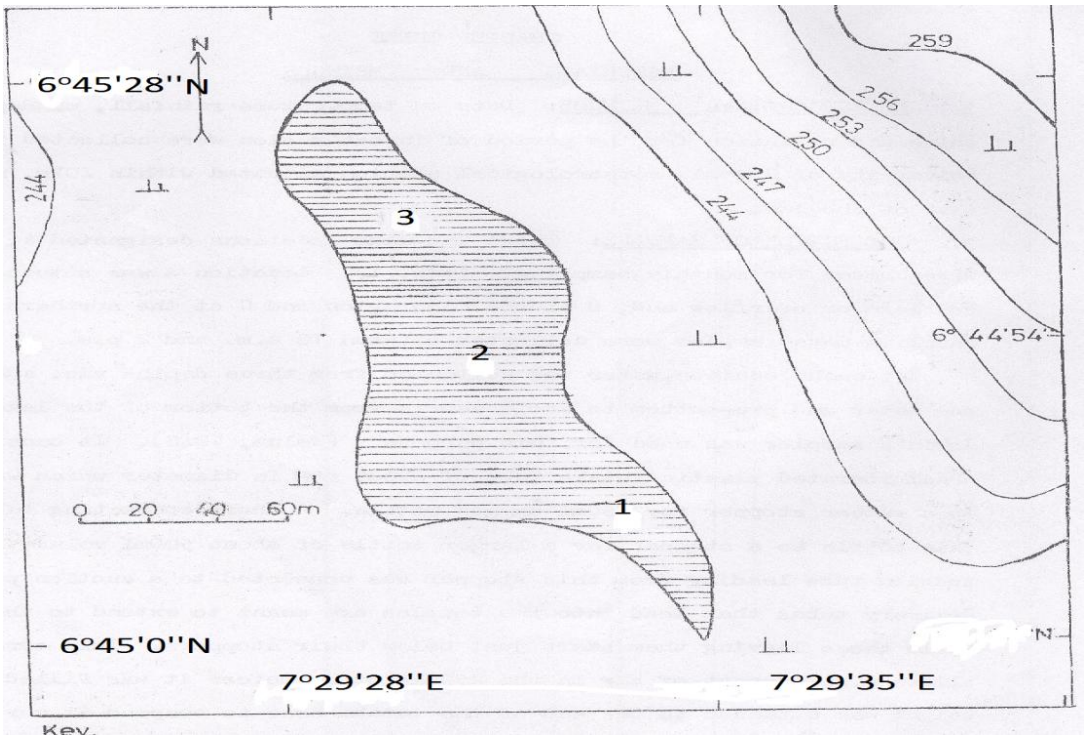


**Figure 1.** A sketch map of Opi Lake with its geographical features and sampling locations 1, 2 and 3.

**Statistical analyses**

This was done with SPSS version 17, T-test for seasonal variation of crustaceans and physico-chemical, relationship within crustaceans and other aquatic fauna, flora and physico-chemical were determined using correlation analysis.

## RESULTS

### Physico-chemical parameters

Table 1 showed the mean values of physico-chemical parameters. The lake has relatively stable thermal regimes with a surface water temperature differential of 3.61°C between the extreme values. Transparency exhibited 1.5 fold mean variation in the total dissolved solids. The low transparency and high concentration of suspended solids recorded was an indication of periods of very low light penetration. The average water pH was almost neutral; the minimum and maximum were slightly acidic. The mean fluctuation was minimal and moderate for fish farming with a difference of 1.71 between the extreme. The lake was well oxygenated with mean of 5.83 mgl. Total alkalinity was mainly of the bicarbonate type with high values recorded during the onset of the dry season. Magnesium observation depicted 4.29 threshold variations while calcium had 2.08 fluctuation fold during the study. The concentration level of $CO_2$ was 21.5 fold mean variation. Total hardness depicted amplitude of 2.27 fold mean variation. The ranges in the levels of $NO_3$-$N_2$ and $PO_4$-P revealed change of 3.88 to 6.0, respectively. The $NO_3$-N exceeded those of $PO_4$-P during the month of study. TDs was constant during the study periods. The water level represented 14.65 fold fluctuations.

### Crustacean fauna

Sampled fauna of Opi Lake include zooplankton (crustacean, rotifer a fish egg and fish larvae), macro invertebrate (insect, Arachnid a and Hirudina) Crustacean were made up of seven genera – *Daphnia* (30.44%), *Naplius* (18.34%), *Captocerus* (14.30%), *Eurycerus* (8.56%), *Bosmina* (9.29%), *Canthocamptus* (8.56%) and *Cyclops* (10.51%).

### Correlations of physico-chemical parameters

Correlations between crustaceans and physico-chemicals are shown in Table 2. *Daphnia* and Magnesium showed negative relationship ($p < 0.01$), *Daphnia* and nitrate and *Daphnia* and iron observed a negative correlation ($p < 0.05$). Positive correlation was observed between *Eurycerus*, and *Cyclops* and calcium ($p < 0.05$).

### Phytoplankton density

The sampled flora were phytoplankton and macrophyte. Phytoplankton sampled were composed six families:

**Table 1.** Monthly mean values of physico-chemical.

| | Temp. ($^0$C) | Tran. (m) | Depth (m) | pH | DO (mg/L) | Alk. (mg/L) | Mag. (mg/L) | Cal. (mg/L) | T.H (mg/L) | Phosphate (mg/L) | Nitrate | Iron (mg/L) | TDS | Free $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oct. 2010 | 28.46 ± 0.81$^{cde}$ | 0.56 ± 0.06$^{bc}$ | 2.93 ± 0.30$^{g}$ | 6.89 ± 0.23$^{e}$ | 7.27 ± 0.25$^{f}$ | 29.42 ± 4.28$^{e}$ | 6.81 ± 0.46$^{a}$ | 11.70 ±1.95$^{ef}$ | 18.52 ± 2.18$^{a}$ | 0.28 ± 0.11$^{ab}$ | 0.14 ± 0.03$^{b}$ | 0.08 ± 0.01$^{a}$ | 10.00 ± 0.00 | 0.86 ± 0.35$^{f}$ |
| Nov 2010 | 28.07 ± 0.73$^{c}$ | 0.62 ± 0.13$^{cd}$ | 2.25 ± 0.38$^{fg}$ | 6.98 ± 0.15$^{e}$ | 7.24 ± 0.33$^{f}$ | 32.68 ± 2.72$^{g}$ | 7.22 ± 0.37$^{a}$ | 11.96 ± 1.38$^{f}$ | 19.18 ± 1.32$^{a}$ | 0.24 ± 0.04$^{ab}$ | 0.24 ± 0.00$^{cd}$ | 0.07 ± 0.09$^{a}$ | 10.00 ± 0.00 | 0.81 ± 0.35$^{ef}$ |
| Dec 2010 | 25.09 ± 1.06$^{a}$ | 0.67 ± 0.08$^{e}$ | 2.05 ± 0.16$^{df}$ | 6.33 ± 0.19$^{c}$ | 7.07 ± 0.11$^{f}$ | 20.81 ± 1.43$^{c}$ | 10.31 ± 1.25$^{b}$ | 10.11± 1.18$^{cd}$ | 20.40 ± 1.45$^{a}$ | 0.72 ± 0.16$^{d}$ | 0.27 ± 0.04$^{cde}$ | 0.29 ± 0.11$^{a}$ | 10.00 ± 0.00 | 0.77 ± 0.31$^{def}$ |
| Jan 2011 | 25.67 ± 0.71$^{a}$ | 0.62 ± 0.03$^{cd}$ | 1.84 ± 0.32$^{cd}$ | 6.58 ± 0.15$^{d}$ | 6.22 ± 0.89$^{d}$ | 26.41 ± 1.87$^{d}$ | 25.28 ± 2.56$^{d}$ | 7.27 ± 1.84$^{a}$ | 32.53± 4.28$^{c}$ | 0.28 ± 0.09$^{ab}$ | 0.22 ± 0.09$^{c}$ | 0.78 ± 0.15$^{c}$ | 10.00 ± 0.00 | 1.13 ± 1.07$^{f}$ |
| Feb 2011 | 28.94 ± 0.30 $^{def}$ | 0.62 ± 0.02$^{cd}$ | 1.22 ± 0.24$^{b}$ | 6.16 ± 0.10$^{b}$ | 2.34 ± 0.22$^{b}$ | 27.44 ± 1.98$^{d}$ | 25.93 ± 1.42$^{d}$ | 6.09 ± 0.76$^{a}$ | 32.02± 2.06$^{c}$ | 0.72 ± 0.16$^{d}$ | 0.22 ± 0.03$^{cd}$ | 0.36 ± 0.11$^{abc}$ | 10.00 ± 0.00 | 0.22 ± 0.25$^{abc}$ |
| Mar 2011 | 29.12 ± 0.45$^{efg}$ | 0.62 ± 0.04$^{cd}$ | 1.03 ± 0.24$^{ab}$ | 6.04 ± 0.17$^{b}$ | 2.29 ± 0.25$^{b}$ | 27.26 ± 1.80$^{d}$ | 25.27 ± 2.23$^{d}$ | 6.66 ± 0.89$^{a}$ | 31.93 ± 2.65$^{c}$ | 0.65 ± 0.13$^{cd}$ | 0.23 ± 0.02$^{cd}$ | 0.72 ± 0.20$^{bc}$ | 10.00 ± 0.00 | 0.21 ± 0.25$^{abcd}$ |
| Apr 2011 | 29.78 ± 0.62$^{g}$ | 0.63 ± 0.02$^{c}$ | 1.04 ± 0.25$^{ab}$ | 6.57 ± 0.13$^{d}$ | 1.44 ± 0.17$^{a}$ | 11.56 ± 0.96$^{a}$ | 22.69 ± 1.68$^{c}$ | 6.50 ± 0.33$^{a}$ | 29.13 ± 1.81$^{b}$ | 1.03 ± 0.42$^{e}$ | 0.08 ± 0.05$^{a}$ | 0.38 ± 0.08$^{ab}$ | 10.00 ± 0.00 | 0.06 ± 0.04$^{ab}$ |
| May 2011 | 29.20 ± 0.75$^{g}$ | 0.42 ± 0.04$^{a}$ | 0.90 ± 0.26$^{a}$ | 6.56 ± 0.18$^{d}$ | 3.22 ± 0.53$^{c}$ | 18.32 ± 1.49$^{b}$ | 21.76 ± 2.18$^{c}$ | 6.60 ± 0.29$^{a}$ | 28.36 ± 2.37$^{b}$ | 0.45 ± 0.19$^{bc}$ | 0.30 ± 0.09$^{e}$ | 0.33 ± 0.06$^{a}$ | 10.00 ± 0.00 | 0.04 ± 0.01$^{a}$ |
| Jun 2011 | 28.33 ± 0.29$^{cd}$ | 0.52 ± 0.15$^{b}$ | 1.63 ± 0.26$^{c}$ | 5.28 ± 0.16$^{a}$ | 7.31 ± 0.14$^{f}$ | 25.98 ± 0.86$^{d}$ | 28.51 ± 1.61$^{e}$ | 13.53 ± 1.02$^{g}$ | 42.04 ± 1.83$^{g}$ | 0.12 ± 0.02$^{a}$ | 0.28 ± 0.05$^{de}$ | 0.44 ± 0.10$^{ab}$ | | |
| Jul 2011 | 28.28 ± 0.96$^{cd}$ | 0.58 ± 0.05$^{bc}$ | 1.72 ± 0.21$^{c}$ | 5.27 ± 0.19$^{a}$ | 6.13 ± 0.24$^{d}$ | 18.82 ± 1.25$^{b}$ | 28.79 ± 1.53$^{e}$ | 10.58 ± 1.67$^{de}$ | 39.34 ± 1.98$^{e}$ | 0.24 ± 0.10$^{ab}$ | 0.23 ± 0.08$^{cd}$ | 0.33 ± 0.02$^{a}$ | | |
| Aug 2011 | 27.11 ± 0.26$^{b}$ | 0.60 ± 0.10$^{bcd}$ | 1.76 ± 0.28$^{c}$ | 5.37 ± 0.28$^{a}$ | 6.59 ± 0.31$^{e}$ | 18.38 ± 1.25$^{b}$ | 28.21 ± 0.76$^{e}$ | 8.55 ± 1.04$^{b}$ | 36.75 ± 1.68$^{d}$ | 0.57± 0.32$^{cd}$ | 0.31 ± 0.01$^{e}$ | 0.32 ± 0.04$^{a}$ | | |
| Sep 2011 | 27.86 ± 0.47$^{c}$ | 0.64 ± 0.10$^{de}$ | 1.89 ± 0.29$^{cd}$ | 5.43 ± 0.13$^{a}$ | 7.02 ± 0.09$^{e}$ | 17.42 ± 0.88$^{b}$ | 29.14 ± 0.79$^{e}$ | 9.01 ± 0.63$^{bc}$ | 38.16 ± 1.14$^{de}$ | 0.62 ± 0.39$^{cd}$ | 0.28 ± 0.03$^{cde}$ | 0.38 ± 0.02$^{ab}$ | | |

The mean values with the same superscript on the same column are not significantly different ($p$ > 0.05).

*Bacillariophycea*, *Chlorophyceae*, *Cryptophyceae*, *Cyanophyceae, Dinophyceae* and *Xanthophyceae* while 12 species of macrophytes were sampled (*Kyllingn squamalata, Nymphaea lotus, Acroceras zizanioid, Cyperacea differmislinn, Alchonea cordifolra, Echinochloa stagnica, Panicum laxum*, *Hydrolytica, Sagrttaria* species and *Braseria shreberia*). Among the phytoplankton families, *Chlorophyceae* had the highest percentage composition in all the 3 stations while *Xanthophyceae* had the least recorded percent. *C. differmislinn*, *A. cordifolra and E. stagnica* were not present in station 3 while *P. laxum* was not present in station 2 among the macrophyte (Table 3). There was no significant difference in the mean density of Phytoplankton across the station (p > 0.05) as shown in Table 3.

Among crustaceans and phytoplankton, *Daphnia* and *Dinophyceae*, showed positive correlation as well as Cyclops and *Cryptophyceae,* while *Eurycerus* and *Xanthophyceae* had a negative correlation (< 0.05), as represented in Table 4.

**Mean density of sampled macro invertebrate**

Among the 23 species of macro invertebrate sampled, 19 species were identified to species level while 4 (insects) were unidentified. The mean densities of 14 species were not significantly different ($p$ > 0.05) within the 3 sampling stations. The mean density of *Ranatra fusca* in station 3 was significantly different from Stations 1 and 2, while *Nepa* spp. mean density in 1 and 2 were significantly different ($p$ < 0.05), as shown in Table 5.

**Table 2.** Correlation matrix of relationship between crustaceans and physico-chemical parameters.

| | Temp | Trans. | Depth | pH | DO | Alka. | Mag. | Cal | TD | Phos. | Nit. | Iorn | TD | Free $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D.* | 0.586** | -0.419 | 0.133 | 0.478** | 0.489** | 0.383* | -0.412** | 0.423* | -0.328 | -0.308 | -0.385* | -0.396* | A | 0.102 |
| *N.* | 0.178 | -0.235 | 0.135 | 0.178 | 0.330* | 0.126 | -0.286 | 0.263 | 0.241 | -0.125 | -0.274 | -0.346* | A | 0.050 |
| *C.* | 0.017 | -0.174 | -0.045 | -0.091 | 0.024 | 0.299 | -0.240 | 0.284 | -0.170 | 0.187 | -0.251 | -0.146 | A | -0.109 |
| *E.* | 0.323 | -0.037 | 0.242 | 0.389* | 0.293 | 0.191 | -0.443** | 0.369* | -0.401* | -0.215 | -0.327 | 0.487** | A | -0.141 |
| *B.* | 0.241 | -0.111 | 0.105 | 0.006 | 0.192 | 0.009 | -0.265 | 0.390* | -0.158 | 0.062 | -0.224 | -0.240 | A | -0.126 |
| *Can.* | 0.250 | -0.212 | 0.072 | 0.230 | 0.243 | 0.280 | -0.061 | 0.129 | -0.019 | -0.175 | -0.224 | -0.177 | A | -0.136 |
| *Cy.* | 0.224 | -0.048 | 0.100 | 0.297 | 0.305 | 0.309 | -0.199 | 0.422* | -0.066 | -0.229 | -0.077 | -0.328 | A | -0.293 |

**- Significant at $P < 0.05$,*- significant at $P < 0.05$ and a- variance is constant. *D = Daphnia, N = Naplius, C = Camptocerus, E = Eurycerus, B = Bosmina, Can = Canthocamptus* and *Cy =Cyclops.*

Positive correlation was observed between F, Z1 and *Naplius* and *Naplius* (Table 6).

## DISCUSSION

The ranges and fluctuations of values for physico-chemical parameters recorded in Opi Lake during the investigation were within the range of physico-chemical parameters values, of natural and man-made freshwaters for optimal growth and survival for aquatic life in tropical Africa (Adeniji, 1973; Adebisi, 1981; Boyd, 1981; Eyo and Ekwuonye, 1995; Nweze, 2003; Odo, 2004; Avoaja, 2005). The low recorded values of water temperature in the months of December and January might be attributed to the cooling effects of harmattan wind during the period when the environment including waters were cold as suggested by Biswas (1973), Evurunobi (1984) and Avoaja (2005). The rise in water temperature observed in February, March and April in the lake agrees with Biswas (1973) that in early dry season, there is usually an increase in water temperature. The observed high transparency in the dry season could be attributed to reduced rainfall and low wind speed leading to calm weather conditions (Livingston and Melack, 1979; Howard-Williams and Ganf, 1981; Evurunobi, 1984; Odo, 2004). The recorded low transparency during the rain in the lake was due to inflow and runoff (which are major sources of water into the lake as noted by Hare and Cater (1984) and Evurunobi (1984) which brought in humus materials, suspended matter and probably colloidal iron that lowered light penetration and this was not different from the findings of Adeniji (1982) in Asa Lake, Evurunobi (1984) in Ogelube Lake and Oluasanya (1988) in Opa Reservoir. The pH of most natural waters falls in the range of 4.0 to 9.0 and much more often in the range of 6.0 to 8.0 (Lind, 1979). The range of pH (5.27 to 6.98) obtained in this research work was adequate for some aquatic life. The seasonal pattern of the DO content is similar to the previous findings of Nweze (2003), Ayoade et al. (2006) and Echi et al. (2009). There was a negative correlation of DO and temperature which agreed with Adebisi (1981) and Eyo and Ekwonye (1995) that increase in water temperature reduces the DO content as a result of increased DO demand of aquatic fauna which is caused by high metabolic activities. The range of magnesium 6.81–29.14 mg/L (as a common constituent nutrient of natural freshwaters is essentially important for plant growth and development) recorded was within the tolerable range for natural freshwater in the tropics (Avoaja, 2005). The insignificant difference in the seasonal content of calcium found in Opi Lake during the research work could be associated with minor amount of calcium content of flood water which is usually a major source of calcium to the water body as suggested by Evurunobi (1984). Magnesium and calcium ions in a lake form the total hardness of the water. The lake could be classified as soft lake since its calcium and magnesium content did not exceed 120 mg/L as stated by Lind (1979) and Mustapha and Omotosho (2005). The high $PO_4$ level in the dry and rainy seasons indicated pollution since it was above the United State Environment Protection Agency (USEPA) standard limit of 0.025 mg/L in natural aquatic bodies (Davies et al., 2009) and this could be as a result of flood water from farmland surrounding the lake which brought in soil component associated with in fertilizer and animal (mainly cattle) faeces which are washed into the lake after grazing at the nearby farmland. The rainy season increase of nitrate could be as a result of enrichment of the water by nitrate ions during floodwater which was equally noted by Davies et al. (2009) but on the other hand was not in line with the findings of Odo (2004) where there was a dry season increase due to nitrate enrichment from previous rainy season. The sharp decrease of free $CO_2$ content in the months of April would possibly be as a result

**Table 3.** Mean density of phytoplankton.

| Specie | Station 1 | Station 2 | Station 3 | Total |
|---|---|---|---|---|
| *Bacillariophyceae* | 5.00 ± 1.00[a] | 4.75 ± 1.12[a] | 5.00 ± 1.02[a] | 4.92 ± 0.59 |
| *Chlorophyceae* | 12.92 ± 1.35[a] | 11.33 ± 1.42[a] | 11.50 ± 1.24[a] | 11.92 ± 0.76 |
| *Cryptophyceae* | 5.25 ± 1.33[a] | 4.08 ± 0.98[a] | 6.83 ± 1.25[a] | 5.34 ± 0.70 |
| *Cyanophyceae* | 11.42 ± 0.96[a] | 10.25 ± 1.07[a] | 10.17 ± 0.76[a] | 10.61 ± 0.53 |
| *Dinophyceae* | 5.50 ± 1.08[a] | 3.92 ± 0.70[a] | 3.83 ± 1.28[a] | 4.42 ± 0.60 |
| *Xanthophyceae* | 1.25 ± 0.37[a] | 1.66 ± 0.49[a] | 0.75± 0.41[a] | 1.06 ± 0.24 |

The mean density values with the same superscript on the same row are not significantly different ($p > 0.05$).

**Table 4.** Correlations between crustaceans and phytoplankton.

| | ***Bac*** | ***Chl*** | ***Cry*** | ***Cya*** | ***Din*** | ***Xan0.*** |
|---|---|---|---|---|---|---|
| *D* | -0.200 | -0.210 | -0.063 | -0.144 | 0.394* | -0.160 |
| *N* | -0.115 | -0.087 | -0.226 | -0.053 | 0.121 | -0.087 |
| *C* | -0.205 | 0.-0.024 | -0.258 | 0.134 | 0.051 | 0.099 |
| *E* | 0.246 | 0.212 | -0.323 | 0.117 | 0.307 | 0.407* |
| *B* | 0.220 | 0.218 | 0.301 | 0.075 | 0.310 | -0.063 |
| *Can* | 340* | 0.380* | 0.333 | 0.250 | 0.386* | -0.175 |
| *C* | 0.211 | 0.298 | 0.093* | 0.148 | 0.048 | 0.275 |

**- Significant at $P < 0.05$,*- significant at $P < 0.05$; *D = Daphnia, N = Naplius, C = Camptocerus, E = Eurycerus, B = Bosmina, Can = Canthocamptus* and *Cy =Cyclops; Bac = Bacillariophyceae, Chl = Chlorophyceae, Cry = Crptophyceae, Cya = Cyanophyceae, Din = Dinophyceae* and *Xan = Xanthophyceae.*

**Table 5.** Macro invertebrate mean (±SE) density per station.

| Specie | Station 1 | Station 2 | Station 3 | Total |
|---|---|---|---|---|
| *Arctocoriax interrupta* | 9.08 ± 2.73[a] | 10.58 ± 3.25[a] | 5.42 ± 1.90[a] | 8.36 ± 1.55 |
| Damselfly | 19. 33 ± 5.39[b] | 9.83 ± 2.52[ab] | 3.25 ± 1.12[a] | 10.81 ± 2.26 |
| *Ranatr fusca* | 4.92 ± 1.6[a] | 6.17 ± 2.11[a] | 4.67 ± 2.30[b] | 5.25 ± 1.14 |
| *Aeshna brevistyla* | 18.68 ± 5.8[a] | 14.42 ± 5.72[a] | 10.67 ± 3.17[a] | 14.58 ±2.88 |
| *Nepa species* | 1.50 ± 0.57[a b] | 0.33 ± 0.19[a] | 0.66 ± 0.26[ab] | 0.83 ± 0.23 |
| F | 10.25 ± 4.20[b] | 3.17 ± 1.88[ab] | 1.00 ± 0.62[a] | 4.81 ± 1.64 |
| *Helobata larvalis* | 0.92 ± 0.45[b] | 0.00 ± 0.00[ab] | 0.08 ± 0.08[a] | 0.33 ± 0.16 |
| *Coccinell species* | 0.17 ± 0.17[a] | 0.00 ± 0.00[a] | 0.08 ± 0.08[a] | 0.08 ± 0.06 |
| Water penny | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00 |
| *Argyronta aquatic* | 7.50 ± 2.92[a] | 5.08 ± 2.52[a] | 4.25 ± 2.10[a] | 5.61± 1.44 |
| Water strider | 2.00 ± 0.67 | 1.85 ± 0.53[a] | 0.79 ± 0.23[a] | 1.84 ± 031 |
| *Lethocerus americannus* | 2.17 ± 0.64[a] | 0.92 ± 0.50[a] | 1.33 ± 0.62[a] | 1.47 ± 0.34 |
| M | 0.08 ± 0.83[a] | 0.67 ± 0.31[a] | 0.00 ± 0.00[a] | 0.25 ± 0.69 |
| Leech | 3.00 ± 2.47[a] | 0.17 ± 0.11[a] | 0.58 ± 3.36[a] | 1.25 ± 0.84 |
| Water mite | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00 |
| *Antipodochlora braueri* | 2.92 ± 0.98[a] | 1.17 ± 0.56[a] | 1.17 ± 0.46[a] | 1.75 ± 0.42 |
| *Oniscigaster wakefieldi* | 0.58 ± 0.36[a] | 0.08 ± 0.08[a] | 0.25 ± 0.18[a] | 0.31 ± 0.14 |
| *Orectochilus orbisonorum* | 3.50 ± 2.30[a] | 1.58 ± 0.92[a] | 3.25 ± 1.28[a] | 2.78 ± 0.91 |
| W | 0.92 ± 0.50[a] | 0.17 ± 0.11[a] | 0.17 ± 0.11[a] | 0.42 ± 0.18 |
| Z1 | 0.42 ± 0.28[a] | 1.92 ± 1.14[a] | 0.50 ± 0.42[a] | 0.94 ± 0.42 |
| *Acroneuria cycorias* | 0.42 ± 0.28[a] | 1.92 ± 1.14[a] | 0.50 ± 0.42[a] | 0.94 ± 0.42 |

F, M, W, and Z1-unidentified species, Mean with same superscript on the same row are not significantly diff. ($p < 0.05$).

**Table 6.** Correlations between crustaceans and macro invertebrate.

| Macro invertabrate | Crustaceans | | | | | | |
|---|---|---|---|---|---|---|---|
| | Dap. | Nau. | Cam. | Eury. | Bos. | Canth. | Cyclops |
| *Arctocoriax interrupta* | -0.398* | 0.111 | 0.218 | 0.028 | -0.117 | -0.095 | -0.101 |
| Damselfly | 0.490** | 0.355* | 0.148 | -0.050 | -0.097 | -0.079 | 0.016 |
| *Ranatr fusca* | -0.471** | -0.137 | -0.098 | 0.009 | 0.075 | -0.230 | -0.065 |
| *Aeshna brevistyla* | -0.145 | -0.019 | 0.095 | 0.351* | 0.128 | 0.243 | 0.094 |
| *Nepa species* | -0.025 | -0.059 | 0.183 | 0.402* | 0.281 | 0.018 | 0.024 |
| F | 0.112 | 0.340* | -0.017 | 0.263 | 0.209 | 0.347* | -0.028 |
| *Helobata larvalis* | 0.130 | 0.014 | 0.307 | 0.434* | 0.155 | 0.222 | 0.192 |
| *Coccinell species* | 0.077 | 0.128 | 0.134 | 0.391* | 0.032 | 0.198 | 0.301 |
| Water penny | a | A | a | A | a | A | A |
| *Argyronta aquatic* | 0.246 | 0.005 | 0.095 | 0.079 | -0.225 | -0.048 | 0.056 |
| Water strider | 0.235 | 0.188 | 0.248 | 0.175 | -0.151 | 0.085 | 0.117 |
| *Lethocerus americannus* | 0.035 | 0.232 | -0.031 | 0.005 | 0.018 | 0.077 | 0.037 |
| M | -0.156 | 0.205 | 0.027 | -0.181 | 0.077 | 0.070 | -0.072 |
| Leech | 0.171 | -0.154 | 0.315 | 0.090 | 0.590** | -0.099 | 0.044 |
| Water mite | a | A | a | A | a | A | A |
| *Antipodochlora braueri* | 0.247 | 0.261 | -0.270 | 0.183 | 0.141 | 0.237 | -0.003 |
| *Oniscigaster wakefieldi* | -0.065 | 0.036 | 0.115 | 0.092 | -0.091 | -0.089 | 0.081 |
| *Orectochilus orbisonorum* | -0.056 | -0.046 | 0.127 | -0.069 | 0.248 | 0.064 | 0.002 |
| W | 0.275 | 0.094 | 0.214 | 0.237 | 0.595** | 0.247 | 0.015 |
| Z1 | -0.165 | 0.370* | 0.045 | -0.227 | -0.082 | -0.101 | -0.236 |
| *Acroneuria cycorias* | -0.165 | 0.370* | 0.045 | -0.227 | -0.082 | -0.101 | -0.236 |

**- Significant at $P < 0.05$,*- significant at $P < 0.05$ and a- variance is constant.

of lower content of alkalinity observed during that month, as it was suggested by Attama (2003) that free $CO_2$ goes a long way to enhance water alkalinity. The total number of 820 crustaceans comprising of 7 species (*Daphnia*, *Nauplius*, *Camptocerus*, *Eurycerus*, *Bosmina*, *Canthocamptus* and *Cyclops*) encountered in the study area appeared to be normal inhabitants of natural lakes, ponds, streams and artificial impoundment in Nigeria and tropical countries (Jeje, 1986, 1988; Mustahpa, 2009; Arimoro, 2010; Kolo et al., 2010). *Daphnia* with the highest percentage composition among the crustacean species could be as a result of its ability to survive and graze effectively on most phytoplankton and fellow zooplankton, and this is in consonance with Jeje (1986), Akin-Oriola (2003) and Mustapha (2009). Percentage composition of *Bosmina* was not in agreement with the finding of Imoobe and Adeyinka (2010) where *Bosmina* species was recorded to be more abundant than every other zooplankton crustacean and *Daphnia* being absent. Floral composition in the lake corresponds with Nigerian freshwater plants (Evurunobi, 1984; Hare and Cater, 1984; Nweze, 2003; Davies et al., 2009; Echi et al., 2009; Hassan et al., 2010; Achionyde-Nzeh and Isimaikaiya, 2010; Okayi et al., 2011). The correlations of crustaceans with phosphate and nitrate may not necessarily be a direct relationship of the species utilizing the nutrients, but could be attributed to the dependency of the phytoplankton (which serves as food for the crustaceans) on these nutrients. The insignificant negative correlation of all the crustaceans with transparency could result from low transparency of the water which hinders zooplankton growth and abundance (Mustapha, 2009). Alkalinity and pH were also found to favor crustacean growth and abundance in the lake as seen from the positive correlation of alkalinity and pH in all the species except *Camptocerus*. Negative (insignificant) correlation of most of the crustaceans with $CO_2$ could be the reason why dry season $CO_2$ content of the lake was higher and this did not agree with Mustapha (2009) study. Similar trend in the relationship between crustaceans and physico-chemical, phytoplankton and other zooplankton has been reported by many scientists such as Carpenter et al. (1985), Jeje (1986) Akin-oriola (2003) and Mustapha (2009). Feeding effects of *Daphnia*, *Eurycerus* and *Canthocamptus* were found to have significant positive effect on the some phytoplankton. As a result of no significant negative feeding effect observed between crustaceans and phytoplankton, the entire crustacean species were unable to reduce phytoplankton population density in their community, which could suggests the abundance of phytoplankton (food) throughout the period (Hare and Cater, 1984; Carpenter et al., 1985; Mustapha, 2009; Achionyde-Nzeh and Isimaikaiya, 2010). The study of crustacean ecology in Opi Lake, Nigeria has revealed that there are abundant

crustaceans (zooplankton) in the system; the organisms are highly diverse and seasonal in abundance and distribution. The crustacean fauna has been found similar to the species of crustaceans found in other Nigerian lakes and other freshwater bodies. The physico-chemical parameters, plankton abundance, macro flora and fauna in Opi Lake fell within the productive values for aquatic ecosystem and indicated that the lake is eutrophic. The study also revealed the interaction between crustaceans and biotic/abiotc factors is an example of a natural tropical freshwater habitat.

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# Effect of rainfall season on the chemical properties of the soil of a Southern Guinea Savanna ecosystem in Nigeria

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**Investigations were carried out on the effect of rainfall pattern on some soil chemical properties during 2011 in the Southern Guinea savanna ecosystem in Nigeria. The study was carried out in Oro Forest Reserve in Kwara State of Nigeria. Twenty plots were randomly selected for soil sampling at for different seasons namely: January (dry season), May (beginning of rains), September (peak of rains) and November (end of rains). Different soil depths were sampled: 0 to 5, 6 to 10, 11 to 15 and 16 to 20 cm at five randomly selected locations. The chemical properties that were mostly influenced by rainfall pattern are soil organic matter, total nitrogen, soil pH, available phosphorus, exchangeable cations (Ca, Mg and K), and cation exchange capacity (CEC). The two major seasons that show profound influence on soil properties are dry season (January) and peak of rainy season (September). Soil pH and available phosphorus were higher in dry season (January) and at the beginning of rainy season (May) and remain low at the peak of the rainy season (September). In contrast, soil organic matter and total nitrogen were low in dry season (January) due to burning of the vegetation. However, nitrogen content increased at the peak of the rainy season (September), due to nitrogen fixation. The increase in the total exchangeable bases (TEB) could be attributed to their importance in the tissue synthesis. There was decline in most soil nutrients during active growth of the woodland savanna trees. Therefore, the limitation of N, P, Ca, Mg, Na and K is most likely to occur in September (peak of rainy season).**

**Key words:** Soil properties, seasonal changes, Southern Guinea savanna, sampling depths.

## INTRODUCTION

At different spatial and time scales, vegetation cover helps in protecting the soil from harsh climatic conditions, mostly soil erosion. The presence of dense vegetation affords the soil adequate cover, thereby reducing the loss in macro and micro nutrients that are essential for plants growth and energy fluxes (Iwara et al., 2011). The influence of soil factors on the composition and distribution of savanna vegetation in Nigeria has been reported (Child, 1974; Muogholu and Isichei, 1991). Others have discussed relationship between soil characteristics and distribution of plant communities in the Guinea Savanna (Menaut et al., 1985; Cole, 1986; Abdul Ameed et al., 2004). The evidence of possible literature shows that vegetation associations within the Guinea Savanna and tree species reflect differences in soil texture, structure and mineral content (Abdul Ameed, 2005). Change in soil chemical properties in the form of P mineralization–immobilization of organic P, are strongly

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influenced by seasonal variations in temperature, moisture, plant growth and root activity, and by organic matter accumulation from litter fall (Perrot et al., 1990; Mc Gvath et al., 2000). Land cover changes affect also soil properties and biogeochemical processes (Ross et al., 1999; Zeng et al., 2009). Each of the rainy and dry seasons of the seasonal climate of the tropical ecosystem, is characterized by a number of ecological phenomena which set up series of processes which influence the biotic and edaphic components of the ecosystem. Soil moisture is highly affected by soil texture and its stability. Various soil texture types may cause preferential flow or water immobilization (KodeSova et al., 2006, 2007). Bodner et al. (2008) discussed the impact of the rainfall intensity, soil drying and frost on the seasonal changes of soil hydraulic properties in the texture related range. Suwardji and Eberbach (1998) studied both aggregate stability and hydraulic conductivities. They documented the lowest aggregate stability during the winter and increased in spring.

The estimation of soil available nutrient contents - in a complex heterogeneous system, is of a great pedological as well as ecological importance. Understanding spatial changes in soil nutrients is important, as they may differ markedly among identical locations subjected to natural and man-made disturbances. Vertical, horizontal and temporal distribution of nutrients in soils are controlled by a combination of factors viz, parent material, topography, soil management practices and rainfall and area seasons. Similar to this, land use patterns and vegetation play important role in soil nutrient transformations and fertility. Anthropogenic changes such as fire (Sharma, 1988) alter several processes in soil; physical (porosity, soil structure and aggregate stability and water repel-lency), chemical (soil organic matter, nutrient availa-bility and cycling, pH and C: N) and biological (microbial composition, soil faunal diversity and density, biomass productivity and carbon sequestration). The use of C: N as an indicator of ecosystem stability has necessitated precise estimations on the soil C at N pools worldwide.

During literature review, we did not come across any study on seasonal variability on soil chemical properties in the Southern Guinea savanna in Nigeria, except for the limited information on the effects of savanna burning of the dry season on soil litter and the chemical composition of soil (Egunjobi, 1970). Thus, the present investigation is an attempt to document the seasonal dynamics of the chemical properties of the tropical savanna wood land ecosystem in Nigeria.

## MATERIALS AND METHODS

### Site description

The present study was undertaken in 2011 at Oro Forest reserve, a woodland savanna of the Southern Guinea vegetation of Nigeria. It covers about 5414 ha which are located in the North-Eastern portion (Lafiagi Section) of the reserve, at a place approximately $08°53^{1}E$ and $05°22^{1}N$.

### Topography and drainage

Most of the land of Oro Forest Reserve fall within an altitude of 120 + 155 m and the few isolated high lands in the north-eastern part, range in height from 1100 to 1150 m. The main drainage system within the reserve is the Oro River – a tributary of River Niger, which is about 20 km to the north of the reserve.

### Geology

The main geological formation of the reserve is the pre-Cambrian Basement complex, composed of metamorphic and igneous rocks. This basement complex includes the oldest rocks known in Nigeria, the dominant clay mineral of the area was kaolinithe (a low activity clay) (Kowal and Knabe, 1972).

### Climate

Oro Forest Reserve has a typical seasonal climate characterized by a two peaked pattern of rainfall- a feature common to all savanna types occurring south of latitude 9°N (Kowal and Knabe, 1972). However, the duration of the well defined dry and wet seasons varies from year to year.

### Rainfall and temperature

The annual rainfall of 913.5 mm occurred between March and October. The rainfall within a year shows two maxima, the first one being 202.3 mm occurring in June, while the second one with 254.7 mm is in September 2010 (Figure 1). In 2011, the first maximum was 201.2 mm while the second maximum was 295.2 mm (Figure 2). The temperature was fairly constant for the year 2010 and ranged between 20.7 and 27.3°C for the minimums, while the mean monthly maximum temperature ranged between 22.5 and 27.6°C(Figure 1). In 2011, however, the temperature values ranged between 21.2and 25.3°C minimums, while the mean monthly maximum ranged between 23.5 and 28°C (Figure 2).

### Plot description and samples collection

The study site was located in the North-Eastern of the reserve. In the location of the forest, one hectare (100 x 100 m) was separated and divided into 100 plots of 10 × 10 m.

Twenty plots indicated plots were randomly selected for soil sampling at four different sampling periods viz: January (dry season), May (beginning of rains), September (peak of rains) and November (end of rains). Soil samples were collected at four different top soil depths; 0 - 5, 6 - 10, 11 - 15 and 16 - 20 cm at five randomly selected locations of each plot for one mean sample. A Dutch auger was used to collect soil sample which were carefully kept in well labeled plastic bags and sent immediately to laboratory for analysis.

### Statistical analysis

A Dutch auger was used to collect soil sample which were carefully kept in well labeled plastic bags and sent immediately to laboratory for analysis.

a. Particle size analysis: This was done by hydrometer method

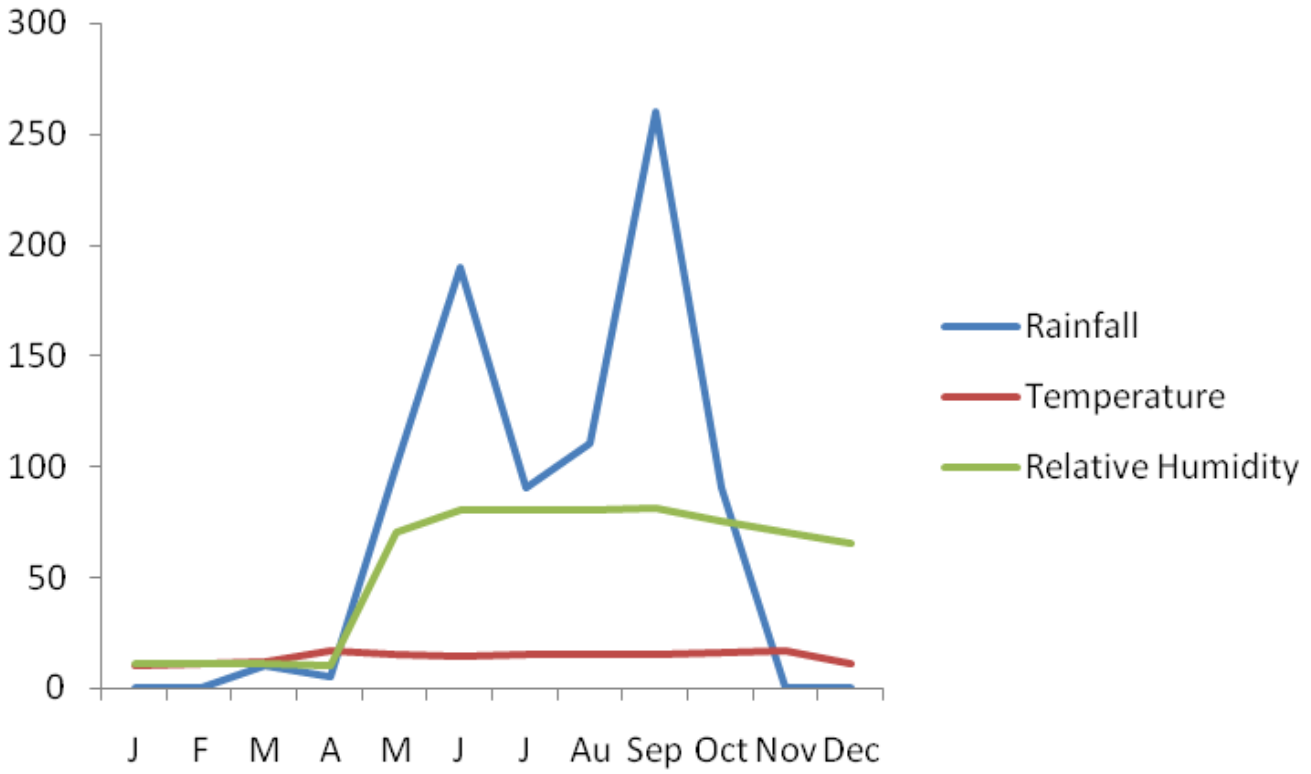


**Figure 1.** Rainfall (cm), temperature (°C) and relative humidity (%) distributions for 2010.

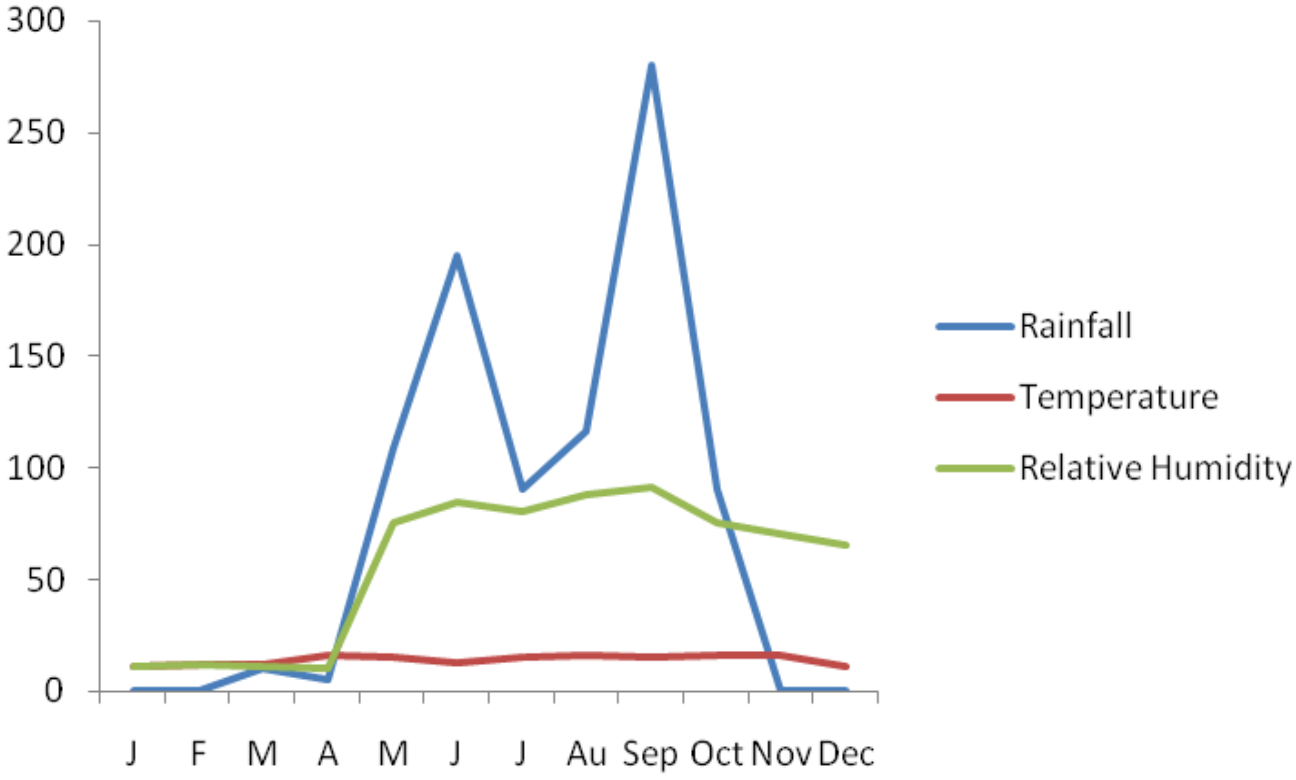


**Figure 2.** Rainfall (cm), temperature (°C) and relative humidity (%) distributions for 2011.

**Table 1.** The mean values (n=10) of some soil chemical properties across sampling time (0 - 20 cm).

| Time of sampling | Properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($H_20$) | OC (%) | OM (%) | N (%) | C/N | Ca | Mg | K | Na | TEB | Av. P | E.A | CEC |
| | Cmol/kg of Soil | | | | | | | | | | | | |
| A | $6.3^a$ | $0.46^d$ | $0.8^d$ | $0.05^c$ | $6.43^b$ | $3.0^d$ | $0.88^b$ | $0.31^b$ | $0.05^a$ | $5.33^a$ | $2.08^a$ | $0.15^b$ | $4.81^b$ |
| B | $6.3^a$ | $0.58^c$ | $1.00^c$ | $0.06^b$ | $6.65^a$ | $3.36^c$ | $0.84^c$ | $0.35^a$ | $0.04^b$ | $5.11^b$ | $2.08^a$ | $0.15^b$ | $4.81^b$ |
| C | $6.1^b$ | $0.66^b$ | $1.12^b$ | $0.06^b$ | $6.25^c$ | $3.48^b$ | $0.96^a$ | $0.29^c$ | $0.04^b$ | $4.51^d$ | $1.73^b$ | $0.16^a$ | $4.93^a$ |
| D | $6.1^b$ | $0.85^a$ | $1.47^a$ | $0.08^a$ | $6.42^b$ | $4.24^a$ | $0.96^a$ | $0.29^c$ | $0.04^b$ | $4.87^c$ | $1.73^b$ | $0.16^a$ | $4.93^a$ |

Means on the same column followed by the same letter are not significantly different at P ≤ 0.05; A = dry season (January); B = beginning of rains (May). C = peak of rains (September); D = end of rains (November); OC = organic carbon, OM = organic matter, N = total nitrogen, C/N = carbon : nitrogen ration, Ca = calcium, K = potassium, Na = sodium; TEB = total exchange bases, Av. P = average phosphorus, EA = exchange acidity and CEC = cations exchange capacity.

(Gee and Bauder, 1986) using sodium haxametaphosphate (calgon) as dispensing agent.

b. Chemical analysis: The soil samples were dried for few days sieved to pass through 2 mm mesh and chemically analysed. The pH (in water) was determined in a 1:2.5 solution (soil: distilled water) and was measured with a standard glass electrode. The organic carbon content of the soil was determined according to Walkey and Black (1965) dichromate oxidation method. The percentage organic matter content in the samples was calculated by multiplying the values of organic carbon by the conventional Van Bammeller factor of 1.724. Total soil nitrogen was determined by Macro kjeldahl methods (Bremner, 1965). Available phosphorus was extracted using Bray II method (Bray and Kutz, 1965) and determined by spectrophotometer.

Exchangeable Na, K, Ca and Mg were extracted with $BaCl_2$ 0.1 m (Hendershort, 1993) and analysed by atomic absorption. Exchangeable acidity was determined from 0.1 NaCl extracts and titrated with 1.0 N HCl.

Cation exchangeable capacity (CEC) was determined by summing up total exchangeable bases (TEBS) and total exchangeable acidity (TEA), which the:

Base saturation = TEB/CEC x 100

Where TEB = Total exchangeable bases; CEC = cations exchangeable capacity

#### Statistical analysis

The data were analyzed using two way analysis of variance (ANOVA) and means were separated by Duncan new multiple range test and student t- test was used to test the level of significance of some properties in the pre and post burn era at 5% level.

## RESULTS AND DISCUSSION

### Effect of sampling month/periods on some chemical properties

Across the months of sampling, significant differences were observed for both exchangeable acidity (EA) and CEC. It is however noted there was no significant differences between the dry season (A) and the beginning of raining seasons (B) as well as peak and end of raining seasons (Table 1). Significant differences were recorded in the soil pH, soil organic carbon, total nitrogen and available phosphorus across the sampling time. There were significant differences in the values of calcium, magnesium, potassium and total exchange bases across the seasons (Table 1). However, calcium values were higher in the peak and end of rainfall. Potassium values were more at the beginning of rains, while sodium values had the highest values during the dry season. Total exchange bases values were more during the dry season and at the beginning of rains.

### Effect of dry season on soil chemical properties

There were significant differences in soil pH, soil carbon, total nitrogen and available phosphorus among the sampling depths during dry season, (January A) p ≤ 0.05 (Table 2). The values of pH ranged from 6.2 to 6.3 and showed no appreciable trend down the depths, the organic carbon ranged from 0.31 to 0.63% at the 16-20 and 0-5 cm, respectively. It decreased down the depth. While the values of the available phosphorous ranged from 1.4 cmol/kg of soil to 2.8 cmol/kg of soil at 16-20 and 0-5 cm respectively, it decreased down the slope. In the same vein, there were significant differences among some of the parameters in the sampling depths at P ≤ 0.05 in this season, calcium (ca), magnesium (Mg), potassium (K) and EA decreased down the depths, the cation exchange capacity showed no appreciable trend down the depth. The sodium (Na) and total exchange bases (TEB) showed no significant difference during this season.

### Effect of beginning of rains on soil chemical properties

At the beginning of the rains (May B) (Table 3), soil pH, soil organic matter, total nitrogen, calcium, magnesium, potassium and total exchange bases, exchange acidity

**Table 2.** Soil chemical properties in dry season (January).

| Depth (cm) | Properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($H_20$) | OC (%) | OM (%) | N (%) | C/N | Ca | Mg | K | Na | TEB | Av. P | E.A | CEC |
| | Cmol/kg of Soil | | | | | | | | | | | | |
| 0-5 | 6.4$^{a}$ | 0.63$^{a}$ | 1.09$^{a}$ | 0.06$^{a}$ | 6.43$^{a}$ | 6.15$^{a}$ | 1.01$^{a}$ | 0.54$^{a}$ | 0.05$^{ab}$ | 7.75$^{ab}$ | 2.8$^{a}$ | 0.19$^{a}$ | 4.99$^{b}$ |
| 6-10 | 6. 3$^{b}$ | 0.56$^{b}$ | 0.97$^{b}$ | 0.05$^{b}$ | 6.92$^{b}$ | 4.13$^{b}$ | 0.87$^{b}$ | 0.32$^{b}$ | 0.05$^{ab}$ | 5.37$^{a}$ | 2.3$^{b}$ | 0.17$^{b}$ | 5.52$^{a}$ |
| 11-15 | 6.1$^{d}$ | 0.34$^{c}$ | 0.59$^{c}$ | 0.04$^{c}$ | 6.18$^{c}$ | 2.90$^{c}$ | 0.85$^{b}$ | 0.28$^{b}$ | 0.06$^{a}$ | 4.09$^{b}$ | 1.8$^{c}$ | 0.15$^{c}$ | 4.22$^{d}$ |
| 16-20 | 6.2$^{c}$ | 0.31$^{d}$ | 0.53$^{d}$ | 0.04$^{c}$ | 6.18$^{c}$ | 3.00$^{c}$ | 0.75$^{c}$ | 0.30$^{b}$ | 0.04$^{b}$ | 4.12$^{b}$ | 1.4$^{a}$ | 0.14$^{c}$ | 4.50$^{c}$ |

Means on the same column followed by the same letter are not significantly different at P < 0.05; OC = organic carbon, OM = organic matter, N = total nitrogen, C/N = carbon: nitrogen ration, Ca = calcium, K = potassium, Na = sodium; TEB = total exchange bases, Av. P = average phosphorus, EA = exchange acidity and CEC = cations exchange capacity. The description should be included into Methods chapter. It wouldn't be necessary use the same under each table.

**Table 3.** Soil chemical properties at the beginning of rain (May).

| Depth cm | Properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PH ($H_20$) | OC (%) | OM (%) | N (%) | C/N | Ca | Mg | K | Na | TEB | Av. P | E.A | CEC |
| | Cmol/kg of Soil | | | | | | | | | | | | |
| 0-5 | 6.4$^{a}$ | 0.73$^{a}$ | 1.26$^{a}$ | 0.07$^{a}$ | 6.14$^{a}$ | 2.41$^{a}$ | 0.86$^{a}$ | 0.53$^{a}$ | 0.04$^{a}$ | 6.84$^{a}$ | 2.8$^{a}$ | 0.19$^{a}$ | 4.99$^{b}$ |
| 6-10 | 6. 4$^{a}$ | 0.58$^{b}$ | 1.02$^{b}$ | 0.06$^{b}$ | 5.78$^{b}$ | 3.17$^{b}$ | 0.84$^{b}$ | 0.34$^{b}$ | 0.04$^{a}$ | 5.37$^{ab}$ | 2.3$^{b}$ | 0.17$^{b}$ | 5.52$^{a}$ |
| 11-15 | 6.1$^{b}$ | 0.57$^{b}$ | 0.98$^{b}$ | 0.05$^{c}$ | 6.43$^{c}$ | 2.66$^{c}$ | 0.84$^{b}$ | 0.27$^{c}$ | 0.04$^{a}$ | 4.09$^{b}$ | 1.8$^{c}$ | 0.15$^{c}$ | 4.22$^{d}$ |
| 16-20 | 6.1$^{b}$ | 0.42$^{c}$ | 0.72$^{c}$ | 0.04$^{d}$ | 8.24$^{d}$ | 2.20$^{c}$ | 0.84$^{b}$ | 0.25$^{d}$ | 0.04$^{a}$ | 4.12$^{b}$ | 1.4$^{d}$ | 0.14$^{c}$ | 4.50$^{c}$ |

Means on the same column followed by the same letter are not significantly different at P ≤ 0.05; OC = organic carbon, OM = organic matter, N = total nitrogen, C/N = carbon : nitrogen ration, Ca = calcium, K = potassium, Na = sodium; TEB = total exchange bases, Av. P = average phosphorus, E.A = exchange acidity and CEC = cations exchange capacity.

**Table 4.** Soil chemical properties at the peak of rain (September).

| Depth cm | Properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($H_20$) | OC (%) | OM (%) | N (%) | C/N | Ca | Mg | K | Na | TEB | Av. P | E.A | CEC |
| | Cmol/kg of Soil | | | | | | | | | | | | |
| 0-5 | 6.1$^{b}$ | 1.0$^{a}$ | 1.72$^{a}$ | 0.09$^{a}$ | 6.92$^{a}$ | 4.2$^{a}$ | 1.14$^{a}$ | 0.38$^{a}$ | 0.05$^{a}$ | 5.77$^{a}$ | 2.4$^{a}$ | 0.11$^{ab}$ | 7.04$^{a}$ |
| 6-10 | 6. 3$^{a}$ | 0.69$^{b}$ | 1.12$^{b}$ | 0.09$^{a}$ | 5.87$^{c}$ | 3.78$^{b}$ | 0.96$^{b}$ | 0.27$^{b}$ | 0.04$^{ab}$ | 5.05$^{b}$ | 1.8$^{b}$ | 0.07$^{b}$ | 5.12$^{b}$ |
| 11-15 | 6.1$^{b}$ | 0.53$^{c}$ | 0.91$^{c}$ | 0.06$^{b}$ | 5.57$^{d}$ | 2.76$^{c}$ | 0.92$^{c}$ | 0.27$^{b}$ | 0.04$^{ab}$ | 3.99$^{c}$ | 1.5$^{c}$ | 0.14$^{a}$ | 4.13$^{c}$ |
| 16-20 | 6.1$^{b}$ | 0.41$^{d}$ | 0.71$^{d}$ | 0.04$^{c}$ | 6.52$^{b}$ | 2.18$^{d}$ | 0.81$^{d}$ | 0.22$^{c}$ | 0.03$^{b}$ | 3.24$^{d}$ | 1.2$^{d}$ | 0.13$^{ab}$ | 3.42$^{d}$ |

Means on the same column followed by the same letter are not significantly different at P ≤ 0.05; OC = organic carbon, OM = organic matter, N = total nitrogen, C/N = carbon : nitrogen ration, Ca = calcium, K = potassium, Na = sodium; TEB = total exchange bases, Av. P = average phosphorus, E.A = exchange acidity and CEC = cations exchange capacity.

and cation exchange capacity showed significant differences at p ≤ 0.05 among the sampling depths. While sodium, total exchange bases showed no significant differences among the sampling depths. Organic matter, magnesium potassium available phosphorous and exchange acidity decreased down the depths. However, soil pH, calcium and cation exchange capacity (CEC) did show any appreciable variation down the depths.

### Effect of peak of rains on soil chemical properties

At the peak of rainfall (September C) (Table 4), organic matter, nitrogen, calcium, magnesium, potassium, available phosphorous, total exchange bases, exchange acidity and cation exchange capacity showed significant differences among the sampling depths at p ≤ 0.05. They also decreased down the depths. Soil pH and sodium did not show any appreciable variations down the depths.

### Effect of end of rains on soil chemical properties

At the end of rains, (November D) (Table 5), soil pH and exchange acidity showed significant differences and increased down the sampling depths at p ≤ 0.05. While the soil organic matter, nitrogen, calcium, magnesium

**Table 5.** Soil chemical properties at the end rain (November).

| Depth cm | Properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($H_20$) | OC (%) | OM (%) | N (%) | C/N | Ca | Mg | K | Na | TEB | Av. P | E.A | CEC |
| | Cmol/kg of Soil | | | | | | | | | | | | |
| 0-5 | 6.1[a] | 1.23[a] | 2.12[a] | 0.09[a] | 7.24[a] | 6.2[a] | 1.14[b] | 0.38[a] | 0.05[a] | 6.77[a] | 2.4[a] | 0.12[ab] | 7.04[a] |
| 6-10 | 6.1[a] | 0.73[c] | 1.25[c] | 0.07[c] | 6.22[b] | 3.92[b] | 0.96[a] | 0.27[b] | 0.04[b] | 5.05[b] | 1.8[c] | 0.07[b] | 5.12[b] |
| 11-15 | 6.1[a] | 0.61[d] | 0.55[d] | 0.06[d] | 6.19[c] | 3.26[d] | 0.92[c] | 0.27[b] | 0.04[b] | 3.99[c] | 1.5[c] | 0.14[a] | 4.13[c] |
| 16-20 | 6.1[a] | 0.81[b] | 0.40[b] | 0.05[b] | 6.03[d] | 3.57[c] | 0.81[d] | 0.22[c] | 0.03[c] | 3.24[d] | 1.2[d] | 0.14[a] | 3.42[d] |

Means on the same column followed by the same letter are not significantly different at P ≤ 0.05; OC = organic carbon, OM = organic matter, N = total nitrogen, C/N = carbon : nitrogen ration, Ca = calcium, K = potassium, Na = sodium; TEB = total exchange bases, Av. P = average phosphorus, E.A = exchange acidity and CEC = cations exchange capacity.

sodium total exchange bases, available phosphorous and cation exchange capacity showed significant differences and decreased down the soil depths at p ≤ 0.05.

## DISCUSSION

### Effect of sampling periods/months on some soil chemical properties

The changes in soil pH over the seasons which was higher in the first two seasons (dry and beginning of rains), might be attributed to the little rainfall and probably due to dry season burning in January which is annual occurrence in the savanna woodland, while the ash released from the accumulated litter following burning in January, caused a slight rise in the soil pH. In the same vein, the dissolution of the ash after early rains in May could be the major reason for slight increase in soil pH at this time (Ekinci, 2006). The distribution of organic matter which was higher during the peak might be due to higher rainfall at this period which favors litter decomposition and accumulation of soil organic matter. While the distribution of soil nitrogen which appeared to increase down the depths during the end of rains might be due to leaching of nitrates down the depths (Giacomo, 2005). The increase of total exchange bases during dry and beginning of rains might be attributed in part to little or no rainfall which reduced leaching of soluble cations and burning which in annual occurrence in the savanna woodland, as burning have been found to have increased soluble cations (Giacomo, 2005). The concentration of available soil phosphorous and exchange acidity at the upper depths during dry and beginning of rains could be due to absence of rainfall which led to little or no leaching of available phosphorous and exchange acidity.

### The effect of sampling depths and rainfall patterns on some soil chemical properties

#### *Soil pH*

The results of the study revealed the effect of sampling depths and rainfall patterns on soil pH, soil organic carbon, total nitrogen and available phosphorus in a tropical Guinea woodland savanna. The distribution of soil pH which decreased down the depths in dry season and the beginning of rains might be due to little or no rains which resulted in little or no movement of cations down the profile in part and might be due to the distribution of soil organic matter which serves as store house for the exchange bases. Also, it might be due to the distribution of exchange acidity, which decreased down the depths. However, at the peak of rainfall (September) and end of rains (November) soil pH tends to increase down the sampling depths, due to vertical movement or translocation of dissolved cations as well as low organic matter down the depths as a result, soil pH increased down the depth.

#### Organic matter

The low of soil organic matter in the dry season of the year (January) might be due to low rains and burning that usually occurs in the area. Also, soil organic matter tends to decrease down the depths, as a result of low decomposition down the profile, due to little or absence of soil microorganisms that are responsible for the decomposition.

Complete consumption of the organic material on the forest floor in the studied area indicates that a very high intensity fire occurred at the study area in January 2011, which accounted for the low organic matter in January. However, the establishment of post fire vegetation, through both re-sprouting and seedling establishment was rapid on the burnt area during the peak of rainfall (September) (Table 3). This enhanced litter fall, hence more organic matter (Litton Santakes, 2002).

#### Total nitrogen

Other studies have reported variable results in relation to rainfall and fire effects on the nitrogen content of soils (Wan et al., 2001). Nitrogen is easily lost from system during an intense fire, as it volatilizes at temperatures as

low as 200°C (White et al., 1973). This might be the reason why there was low nitrogen content in January (dry season) (Table 2). Actual losses of nitrogen due to volatilization have been estimated to vary from 75 kg/ha (Klemmendson, 1976) to 907 kg/ha (Grier, 1975). A likely explanation for decreased soil nitrogen in May (beginning of rains) (Table 2) might be due to leaching of nitrates during the beginning of rains as a result of absence of vegetation and consumption of litter layer led to increased infiltration rates in the burned soils.

The increased nitrogen contents at the peak of rains (September) (Table 4) and at the end of rains in November (Table 5), could be best explained by a possible increase in activity of nitrogen fixing microbes. Evidence exists to show that increased biological nitrogen fixation along with increased mineralization rates occur during rainy season, which resulted in increased nitrogen content at this time (Bergeron et al., 2002)

**Exchangeable cations**

The distribution of exchangeable bases (calcium, magnesium, potassium, sodium) showed decreased down the depths might be due in part to the higher organic matter at the upper depths during the dry and beginning of rains. The little or no rainfall at these periods might be due to the accumulation of the respective cation at the upper depth. At these periods, there was little or no leaching of these cations (Tables 2 and 3). At the peak rains, total exchange bases were low and decreased down the depths; this could be attributed to these elements being utilized by the regenerating plants, since these are reputed for their vigorous regeneration and growth following annual fires (Hopkins, 1974).

Considering the importance of these elements in tissues synthesis, there are enough indications to show that the disappearance of these elements could be due to the synthesis of plant tissues in newly flushing suckerings and sprouts from the plants of the herbaceous layer and possibly the flowers of ligneous savanna plants (Hopkins, 1974). At the end of rains, however, the vigorous tree growth might have been decreased which accounted for high exchange bases.

**Available phosphorus**

A large portion of the nutrient reserve in most forest ecosystems is contained in the organic material on the forest floor (Wagner and Wolf, 1998). The slight increase in phosphorus in dry season (January) (Table 2) and in May (beginning of rains) (Table 3), may be due to fire. De Ronde (1990) found that a high intensity wild fire resulted in an immediate increase in phosphorus level in the southern Cape Forestry Region of South Africa.

The distribution of available phosphorus between soil layers showed that it was fairly constant in dry season (January) and at the beginning of raining season (May) (Table 3) and decreased sharply during the peak of rainfall (September) (Table 4). This might be attributed to the growth of plants and accumulation of biomass during growing season (Styles and Coxon, 2007).

**Exchange acidity and cation exchange capacity**

Exchange acidity values were slightly low which might be the reason for slight acidity of the area and decreased down the depths due to the distribution of soil organic matter and little or no rainfall during dry and beginning of rains which might lead to little or no leaching. The increase in exchange acidity down the depths might be due to leaching of the elements down the depths due to high rainfall.

The low values of cation exchange capacity (CEC) in the dry and beginning of rains in the study area might be due to the distribution of soil organic matter, as soil organic matter was found to have influenced the distribution of cations exchange capacity. Also, organic matter had been identified as a store house for cations (Schlecht et al., 2006). In the same vein, cation exchange capacity decreased down the depths probably as a result of the decreased of soil organic matter down the depths.
The increased cations exchange capacity during the peak and end of rains might also be due to higher rainfall which favours rapid decomposition of dead plant materials that lead to accumulation of soil organic matter (Fatubarin, 1980).

**Conclusion**

This study shows that in Southern Guinea savanna in Nigeria, rainfall seasons have great effect on soil chemical properties. Van Reordivijii and Ong (1999), hypothesized that land use systems would most likely achieve long term sustainability, by mimicking patterns of resource use in the natural systems. The chemical properties that were mostly influenced by rainfall patterns were soil organic matter, total nitrogen, soil pH, available phosphorus, exchange cations (Ca, Mg and K), CEC. The two major rainfall seasons that showed profound influence on soil properties in the study area are dry season (January) and peak of rainy season (September). Some pH and available phosphorus values were higher in dry season (January) and at the beginning of rainy season (May). They were low during the peak of rains. In contrast, soil organic matter and total nitrogen were low in dry season (January) due to burning in the location. However, nitrogen content was later increased at the peak of rain due to nitrogen fixation activities. The decrease in the total exchangeable bases (TEB) during the peak of rainy season could be attributed to the importance in the tissue synthesis during this period. It was observed that there were decline in the soil nutrients except soil nitrogen during the peak of rainy season, which also coincided with the active growth period of

forest trees (no data). Therefore, the limitation of N, P, Ca, Mg, K, to tree growth is most likely to occur in September (peak of rainy season) (Chen et al., 2006). Protection of the litter layer is strongly recommended to ameliorate soil degradation and nutrient limitation in the study area, since litter layer was not only the main source of soil organic matter and available nutrients, but also the regulator of soil microbial activity (Chen et al., 2003).

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*Full Length Research Paper*

# Plankton diversity in Krishna River, Sangli, Maharashtra

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**Quantitative assessment of plankton was carried out in distinct sites of the river Krishna, district Sangli during January 2011 to December 2012. Phytoplankton diversity was observed in five groups, that is, Cyanophyceae, Bacillariophyceae, Chlorophyceae, Hydrocharitaceae and Desmidiceae including 53 species. Among them, Chlorophyceae was dominating with 22 species. Diversity of zooplanktons included, Cladocera, Rotifera, Protozoa, Nematoda, Aostraca, Schizopyrenida and Copepoda as major groups with 25 genera. Rotiferans were dominating with 9 diversified species. The relationship between plankton, diversity indices and water quality status of the river systems was discussed.**

**Key words:** Planktonic diversity, pollution, Krishna River.

## INTRODUCTION

Biological density of ecosystem was found to be the best indicator of healthy aquatic ecosystem. Aquatic contaminants as fertilizers and detergents were helpful for excessive growth of algae (Roy, 1996). Reid (1961) reported that, planktonic populations on which whole aquatic life depends is directly or indirectly governed by many biological conditions and tolerances of organisms to variations in one or more of these conditions. Phytoplankton in the aquatic community serves as a food for development and growth of zooplankton. Phytoplankton diversity appeared as a paradox (Hutchinson, 1967). Major diversity of zooplankton and phytoplankton with their composition varied with seasonal differentiation and production of meroplanktons as eggs, larvae and juveniles of the benthos, nekton, etc (Walsh, 1978).

Lotic systems are flow regime found to be one of the important factor associated with zooplankton diversity (Pace et al., 1992; Basu and Pick, 1996). Most zooplanktons are filter feeders that use their appendages to strain bacteria, algae and other fine particles of water (Thilak, 2009). Researchers described taxonomic and bivolume characteristics of riverine phytoplanktonic communities (Descy and Gosselain, 1994; Rojo and Alvarez, 1994; Reynolds and Descy, 1996).

Comparatively, lentic systems with its species composition and community structure of phytoplankton in lotic systems are not much focused on (Basu and Pick, 1996; Piirsoo et al., 2008). Species diversity in ecosystem was found to be directly related to abundance or equitability (Odum, 1983). The plankton in the ecosystem is useful as bioindicators for assessment of pollution status.

Taking account of literature and carried work, we have decided to assess planktonic diversity from river Krishna (Sangli) in terms of taxonomic richness and density which would help in understanding diversity indices of biological community. Obtained data was comparatively discussed in relation to pollution status of river Krishna. The present investigation was conducted to analyze plankton diversity mass from Krishna River, Sangli which shows level of contamination in the aquatic bodies under study.

## MATERIALS AND METHODS

### Study area and geographical location

Sangli District is present in the western part of Maharashtra. it is geographically located at 16.8670°N latitude and 74.5670°E longitude, surrounded by Satara and Solapur districts

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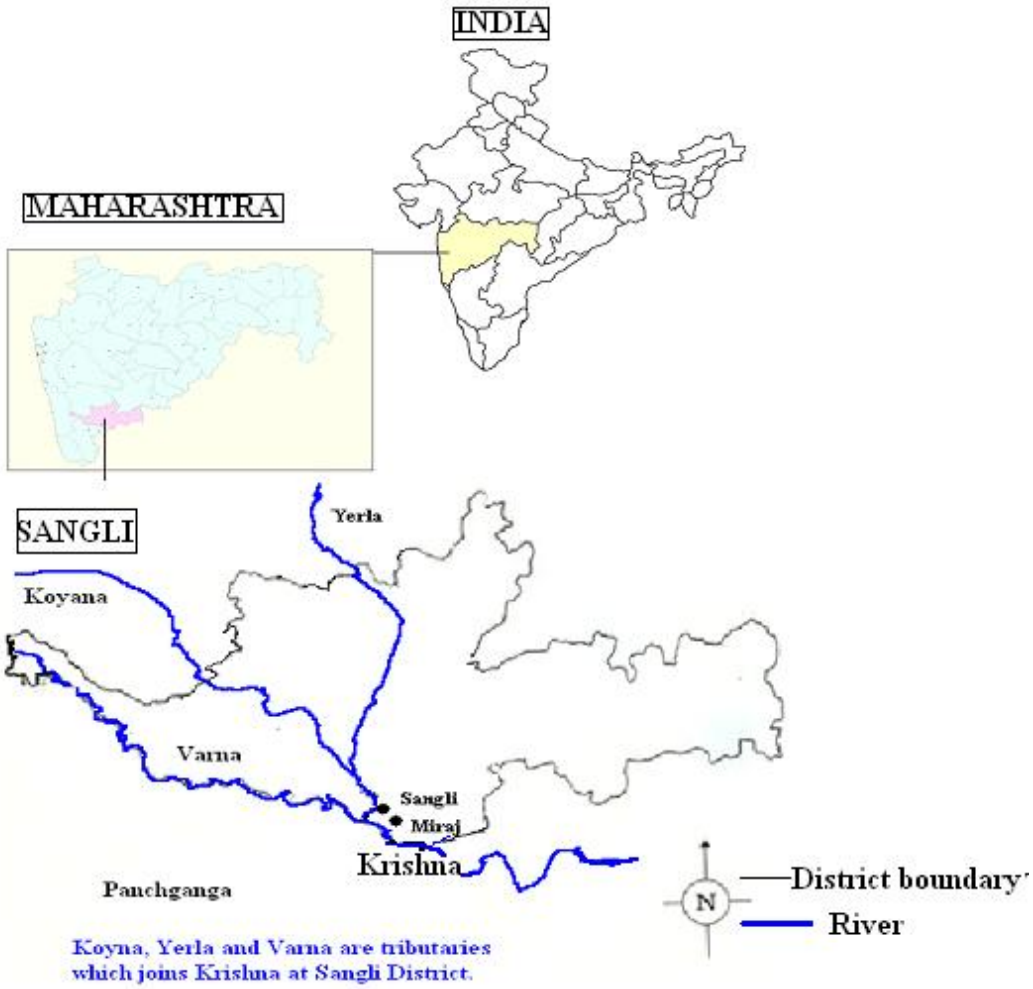


**Figure 1.** District Sangli with Krishna River.

to the north, Bijapur District, Karnataka to the east, Kolhapur and Belgaum, Karnataka districts to the south, and Ratnagiri District to the west. Sangli district is situated around river basins of the Warna and Krishna. Sangli City is the district headquarters and total area is 8,578 km² (3,312 sq mi). The district is 24.51% urban.

#### Collection site

Krishna River is the major lotic system of the district, it is considered as the longest rivers in India, measuring about 1300 km in length. Approximately 105 km of riverine flow covers the district. The river originates at Mahableshwar passes through Sangli and conjoins the sea in the Bay of Bengal at Hamasaladevi (Andhra Pradesh) (Figure 1). The mean annual discharge of water is 67305 million $m^3$ and its drainage area is 2, 68786 sq.km of which 26.8% is in Maharashtra, 43% is in Karnataka and 29.4% is in Andhra Pradesh (Rao, 1979; CWC).

#### Field sampling and analysis

Samples were assessed in each season monthly from the sampling stations. Collection of plankton was made by filtering 50 L of water sample through bolting silknet No.25 (64 µ). Water samples were collected in Amber – coloured bottle to prevent discoloration of algae. Samples were preserved in Lugol's iodine solution (1v/100v) and 70% alcohol which maintain the fragile structure of animals and also helpful for settling the sample. Sedge Wick – Rafter counting cell at 100x magnifications was used for quantitative analysis of phytoplankton and zooplankton (Sedgwick, 1988). Routine analyses of physicochemical parameters were carried out.

## RESULTS

### Phytoplankton analysis

Phytoplankton of freshwater habitat as green algae, blue green algae, diatoms, desmids etc. are important among aquatic flora. They are ecologically significant as they form the primary link in food chain for all aquatic animals (Misra et al., 2001). The present study showed five groups of phytoplanktonic population as Chlorophyceae, Bacillariophyceae, Cyanophyceae, Hydrocharitaceae and Desmidiceae (Figure 2). Comparatively, Chlorophyceae was dominating with 22 species in it. Next to Chloro-

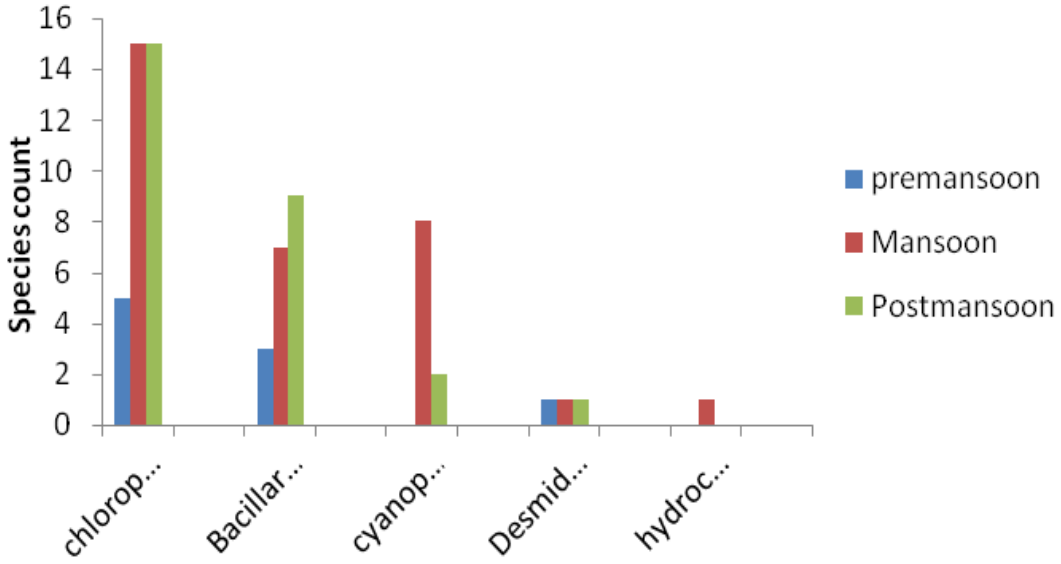


**Figure 2.** Season wise comparison of phytoplankton species.

phyceae, family Bacillariophyceae included eleven species whereas Cyanophyceae showed ten diversified species in the selected area. Hydrocharitaceae and Desmidiaceae showed only one species from each family. Family wise diversity in the selected aquatic body was as follows:

**i) Chlorophyceae**: Family Chlorophyceae showed 22 species which included, *Spirogyra, Chlorella, Ankistrodesmus, Pediastrum simplex, tetraspora, Scemedesmus, Nitella, Microspora, Zygnema, Ulothrix, Mougeotia, Coelastrum, Cosmarium, Tetrahedron, Treubaria, Micractinium, Pochycladon, Anthrodesmus, Volvex, Sphaerocystis, Gonatozygon , Gonatozygon* and *Netrium.*
**ii) Bacillariophyceae**: Bacillariophyceae showed 11 species and was found to be maximum in postmansoon season and minimum in mansoon period. They included *Synedra, Suriella, Tabellaria, Stauroneis, Navicula, Amphore, Diatoma, Fradilaria, Asterionella, Cyclotella, Cymbella.*
**iii) Cyanophyceae**: Cyanophyceae has 10 species: *Gomphosphaeria, Anacystis, Geotrichum, Botryococcus, Phormidi, Oscillate, Rivularia, Gloeotrichia, Synechocystis, Chroccoccus.*
**iv) Desmidiaceae**: Desmidiaceae showed least dominancy with one species: *Closterium* among phytoplanktonic families.
**v) Hydrocharitaceae**: Similar to Desmidiaceae, Hydrocharitaceae also showed only one species, that is, *Hydrilla*.

Phytoplanktonic population in the working area showed order of dominancy among the species with regards to number as follows: Chlorophyceae > Bacillariophyceae > Cyanophyceae > Desmidiaceae and Hydrocharitaceae.

**Zooplankton analysis**

The major group of zooplankton observed during study period was Cladocera, Rotifera, Protozoa, Nematoda, Aostraca, Schizopyrenida and Copepoda (Figure 3). Quantified data related to species diversity showed two species of Cladocera, eight species of Protozoa, nine species of Rotifera, three species of Copepoda and only one species of Nematode, Aostraca and Schizopyrenida in assessment area. Quantified data was as follows:

**i) Rotifera:** Rotifers, these are tiny wheel animals, considered as natural water purifiers because they perform clean up service in the slow moving aquatic bodies. In the study, Rotiferans were found dominant with 9 species, that is, *Cocconeis, Ascomorpha, Diacranophorus, Branchionus Caudatus, Branchionus falcatus, Keratella quadrata, Philodina, Pterodina, Sinantheria.*
**ii) Protozoa:** Protozoans are the smallest and first aquatic organisms in the form of zooplanktons. They are second dominant group with eight diversified species in the present investigation as *Chillodenella, Bursaria, Tetrahynema, Prorodon, Metapus, Verticella, Spirostomum, Slentor.*
**iii) Copepoda:** Copepods constitute planktonic group of both freshwater and marine habitats. In the present work, we found three free living groups viz. *Calanoids, Cyclopoids* and *Harpacticoides.* Copepods presented three species: *Cyclopod, Nauplius* and *Diatomus.*
**iv) Cladocera:** *Cladocerans* are commonly known as water fleas. These are minute crustaceans generally ranging in size from 0.2 - 5.0 mm. They belong to order *Cladocera* of subclass *Brachiopoda* under subphylum *Crustacea* including 11 families (Raghunathan and Suresh Kumar, 2002). Order *Cladocera* included two types of species during the present study, that is, *Sida*

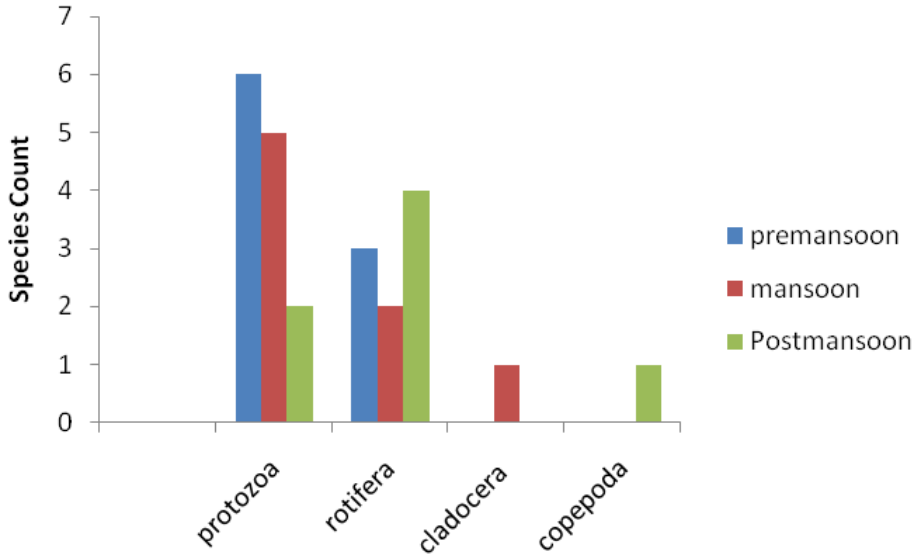


**Figure 3.** Season wise comparison of Zooplankton species.

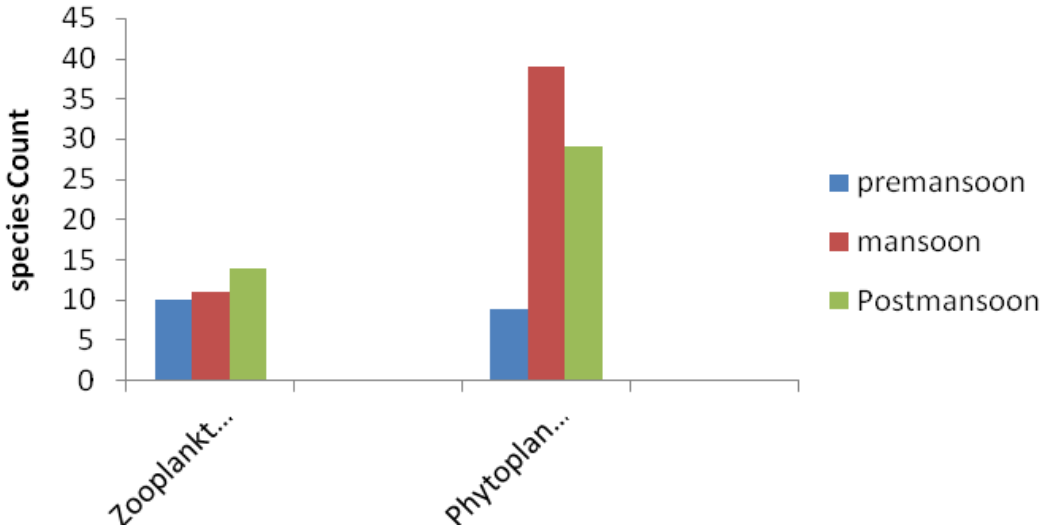


**Figure 4.** Season wise comparison chart of phytoplankton and zooplankton population.

and *Daphnia.*

**v) Nematoda**: showed one type of species, that is, *Heterodera*

**vi) Aostraca**: showed one type of species, that is, *Streptocephalus*

**vii) Schizopyrenida**: showed one type of species, that is, *Naegleria*

Among zooplankton in the study area, the order of dominance in diversified groups was as follows: Rotifera > Protozoa > Copepoda > Cladoecera > Nematoda > Aostraca > Schizopyrenida.

Plankton study showed seasonal variation in all sampling sites, as per their nutrient status, age, morphometry and other physicochemical factors. The statistical data in the present study showed that planktonic population was maximum in postmansoon and mansoon season as compared to premansoon season (Figure 4). Percent composition of phytoplankton throughout the study period showed Chlorophyceae 22%, Bacillariophyceae 11%, Cyanophyceae 10%, Hydrocharitaceae 1% and Desmidiaceae 1% (Figure 5). Relatively, zooplankton showed Protozoa 8%, Rotifera 9%, Copepoda 1%, Cladocera1%, Nematoda 1%, Schizopyrenida 1%, Anostraca 1%

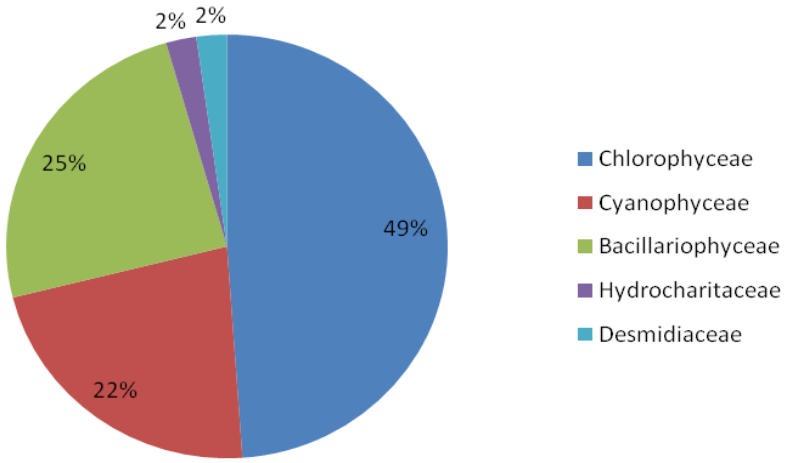


**Figure 5.** Percent composition of phytoplankton (Jan 2011 - Dec 2012).

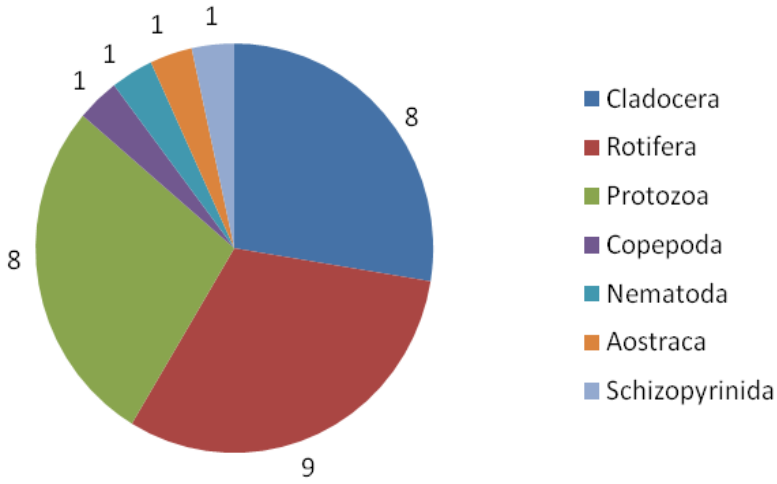


**Figure 6.** Percent composition of zooplankton (January - December, 2011).

(Figure 6). The study on plankton diversity showed some genera which act as bioindicators of organic pollution hence it can be said that the water body is slightly organically polluted. Tables 1 and 2 show results of phytoplankton and zooplankton analysis in the three sampling sites of river Krishna.

## DISCUSSION

Distribution patterns of phytoplankton were strongly correlated with environmental factors (Lepisto et al., 2004). The relationship between phytoplankton diversity and environmental factors has great importance in assessment of pollution status (Buric et al., 2007) and identification of the main factors controlling phytoplankton in a particular water body was essential for choosing an appropriate method for maintenance of desired ecosystem state (Peretyatko et al., 2007). In the present study, we found that, species from family Chlorophyceae was dominating to others with 22 species. Chlorophyceae had algal diversity and it is one of the important indicator of water quality (Jena et al., 2008). Bhivgade et al. (2010), observed Chlorophyceae as a dominant species than other zooplanktons in Nagzari tank, Beed. Similar results were reported by Prescott (1939) and Patil et al. (1983). Venkateswarlu (1969) observed maximum proportion of Chlorophyceae during winter in river Moosi, Hyderabad.

Bacillariophyceae showed 11 species and was found to be maximum in postmansoon season and minimum in mansoon period. Thiruganamoorthy and Selvaraju (2009) documented abundant count of Bacillariophyceae in mansoon season which was lowered in premansoon.

Mchugh (2003) reported Bacillariophyceae as dominant life form in phytoplanktons and largest group of biomass producer on earth which has much more estimated diversity than 100,000 species.

**Table 1.** Enumeration of phytoplankton occurring at the study site throughout the year under premonsoon, monsoon and postmonsoon seasons.

| Phytoplankton | Season | | |
|---|---|---|---|
| | Premonsoon | Monsoon | Postmonsoon |
| **1. Cyanophyceae** | | | |
| Gomphosphaeria | – | + | – |
| Anacystis | – | + | – |
| Geotrichum | – | – | + |
| Botryococcus | – | + | – |
| Phormidi | – | + | – |
| Oscillate | – | + | – |
| Rivularia | – | + | – |
| Gloeotrichia | – | + | – |
| Synechocystis | – | – | + |
| Chroccoccus | – | + | – |
| **2. Bacillariophyceae** | | | |
| Synedra | + | + | + |
| Suriella | – | + | + |
| Tabellaria | – | + | + |
| Stauroneis | – | + | – |
| Navicula | – | + | + |
| Amphore | – | + | – |
| Diatoma | + | – | + |
| Fradilaria | – | + | + |
| Asterionella | – | – | + |
| Cyclotella | + | – | + |
| Cymbella | – | – | + |
| **3. Chlorophyceae** | | | |
| Spirogyra | – | + | – |
| Chlorella | + | + | + |
| Ankistrodesmus | + | + | + |
| Pediastrum simplex | + | + | + |
| Tetraspora | – | + | – |
| Scenedesmus | + | + | + |
| Nitella | – | – | – |
| Microspora | – | + | – |
| Zygnema | – | + | + |
| Ulothrix | – | + | + |
| Mougeotia | – | + | – |
| Coelastrum | – | + | + |
| Cosmarium | + | – | + |
| Tetrahedron | – | + | + |
| Treubaria | – | + | – |
| Micractinium | – | + | + |
| Pochycladon | – | + | + |
| Anthrodesmus | – | – | + |
| Volvex | – | – | + |
| Sphaerocystis | – | – | + |
| Gonatozygon | – | – | + |
| Netrium | – | + | – |
| **4. Hydrocharitaceae** | | | |
| Hydrilla | – | + | – |
| **5.Desmidiaceae** | | | |
| Closterium | + | + | + |

In this work, Cyanophyceae showed diversity of 10 species. Barhate (1985) and Zafar (1967) considered that high percentage of dissolved oxygen is favourable for growth and development of Cyanophyceae and were recorded with seven species at Nagzari tank. Maximum growth and population was noted during the post mansoon season. Desmidiaceae showed only one species with least dominancy. Likewise Desmidiaceae, Hydrocharitaceae, also showed only one type of species in the study area.

Zooplanktons serve as important aquatic organisms, occurred abundantly in all types of aquatic habitats and has vital role in energy transfer of aquatic ecosystems (Altaff, 2004). Zooplankton constitutes important food item of many omnivorous and carnivorous fish (Shrifun, 2007).

Comparatively, Rotiferans were dominant group including nine diversified species in it, among Protozoa, Copepod, Nematode, Schizopyrenida, Anostraca and Cladocera. The density of zooplankton showed a distinct seasonal variation with each group and maximal and minimal peaks (Kiran et al., 2007). Rotifer has important role in energy flow and nutrient cycling, accounting for more than 50% of the zooplankton production in some freshwater systems (Saler and Sen, 2002).

In the present study, 9 species of Rotiferans were recorded, similarly, Jindal et al. (2010) observed 6 species of Rotifer in Hill stream Nogli at Rampur Bhusnar, District, Shimla. Rotifers were dominantly found in Hawkesbury–Nepean River (Kobayashi et al., 1998). Padmarabha et al. (2007) reported diversity indices of Rotifers for the assessment of pollution in Kukkarahalli and Karanji lakes in Mysore Karnataka State. Jeelani et al. (2005) documented relation of species diversity and seasonal distribution of Rotifers in Dal lake, Jammu and Kashmir. Certain species and genera of Rotifer were bioindicators of water quality, eutrophic status and productivity (Sladecek, 1983).

*Protozoa* showed eight species with next dominant to Rotifer; similarly, protozoan dominance was recorded in Gurha Brahmara, Jammu with different eleven species (Dutta et al. 2009), whereas Pathak and Mudgal (2004) observed two dominant species of Protozoa in Virla reservoirs, Madhya Pradesh. Zooplanktonic analysis in river Chenab has showed dominance of Protozoa and coincides with of findings of Zutshi (1992), Ali et al. (2003), Sharma (2009) and Rathore (2009).

Depth of water, transparency, pH and predators determine the distribution and abundance of Copepods (Confer et al., 1983; Patalas, 1971). Raghunathan (1983) reported that Copepods were found in ponds, lakes, rivers and reserviours. In our study, Copepods showed only one type of species, that is, *Cyclopoid*. Similarly, four species of Copepods were observed by Suresh et al. (2009) from Tungabhadra River. Patil and Goudar (1989) reported seven species of Copepods from aquatic bodies of Dharwad district. One to the three species was observed by Kamble et al. (2013) from Krishna River. Some genera of Copepod and Cladocerans were cosmopolitan in their distribution, while others were restricted to some continents

**Table 2.** Enumeration of Zooplankton occurring at the study site throughout the year under premonsoon, monsoon and postmonsoon seasons.

| Zooplankton | Season | | |
|---|---|---|---|
| | Premonsoon | Monsoon | Postmonsoon |
| **1. Cladocera** | | | |
| Sida | _ | + | _ |
| Daphnia | + | + | + |
| **2. Rotifera** | | | |
| Cocconeis | _ | _ | + |
| Ascomorpha | _ | + | _ |
| Diacranophorus | + | _ | + |
| Branchionus Caudatus | + | _ | _ |
| Branchionus falcatus | + | _ | + |
| Keratella quadrata | _ | _ | + |
| Philodina | _ | + | _ |
| Pterodina | | | |
| Sinantheria | | | |
| **3. Protozoa** | | | |
| Chillodenella | + | _ | _ |
| Bursaria | + | _ | _ |
| Tetrahynema | + | + | _ |
| Prorodon | + | _ | _ |
| Metapus | + | + | + |
| Verticella | + | + | + |
| Spirostomum | _ | + | _ |
| Slentor | _ | + | _ |
| **4. Copepoda** | | | |
| Cyclopoid | _ | _ | + |
| Nauplius | + | - | + |
| Diatomus | + | _ | + |
| **6. Nematoda** | | | |
| Heterodera | _ | + | _ |
| **7.Anostraca** | | | |
| Streptocephalus | _ | + | + |
| **8. Schizopyrenida** | | | |
| Naegleria | | | |

(Brooks, 1959; Williamson, 1991).

According to Uttangi (2001) Cladocerans preferred to live in clear waters. Cladocerans showed only one species in the present study. Our result matches with observations of Dutta and Verma (2010) who noted three genera of Cladocera from river Chenab. Seven species were reported from Tungbhadra River (Suresh et al., 2009) whereas Kamble et al. (2013) documented 4 species of Cladocera from Krishna River ghat, Miraj, Maharashtra. Green et al. (2005) in their study reported Cladocerans abundance with five diversified species. Korovchinsky et al. (2008) carried out work on the global diversity of Cladocerans and reported that, high diversity of Cladocerans was found in littoral zone of lotic as well as temporary water bodies.

Phytoplanktons are primary producers and very useful tools for the biomonitoring of water body with regard to its pollution status (Stoermer, 1977). Some of the genera such as Oscillate, Chrococcus, Cyclotella, Scenedesmus, Navicula etc were bioindicators of pollution.

According to Venkateswaralu (2006), presence of *Chlorella vulgaris* indicated contamination of aquatic bodies. In the diversity, *Chlorella* under Chlorophyceae was found to be dominant species in the assessment indicating that water quality of selected aquatic body is slightly polluted by mixing of organic material. Our result coincides with observations of Palmer (1969) who reported that Oscillatoria, Scendesmus, Navicula are found in organic rich aquatic bodies. Some of the species like *Euglena* and *Phacus* were found in highly polluted aquatic bodies.

Among Rotifers, Branchionus was the dominant genus. Genus Brancionus indicate eutrophic aquatic body (Sladecek, 1983) and hence its abundant presence is considered as biological indicator for eutrophication (Nogueira, 2001).

Certain species and genera of rotifers were used as indicators of water quality, eutrophic status and productivity of an aquatic ecosystem (Sladecek, 1983). Hence the present study gives the season wise distribution of diversity among the phytoplankton and zooplankton from the given study area which can be useful as a data to identify one of the biological aspect of the same.

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