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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Spatio-temporal variation of the zooplankton community in a tropical wetland (Bhoj Wetland), Bhopal, India

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Bhopal, the capital city of the state of Madhya Pradesh, India is famous for its numerous lakes. The Bhoj Wetland is a wetland of international importance (Ramsar Site). In the two years of study period, a total of 82 zooplankton species were recorded from February 2008-January 2010. Out of which 66 species were recorded in the 1st year (2008-2009), and 70 species documented during the 2nd year (2009-2010). The zooplankton population belongs to five major groups namely: Rotifera (46%), followed by Cladocera (34%), Protozoa (10%) and Copepoda and Ostracoda contributing 6 and 4%, respectively. With regards to Rotifera, in the major peak of June 2009 (38 species), was dominated by the various species of *Brachionus* and *Keratella.* The population density data revealed that cumulative site mean density ranged from 84 to 1579 Ind. l^{-1}, with an overall mean of 399 Ind. l^{-1}. The 24 months of mean site density indicated a major peak of 1579 Ind. l^{-1}, in June 2009, with 47 and 43% contribution from Copepoda and Rotifera. However, among Copepoda, *Cyclops* and Nauplius larvae were major contributors to this peak and among Rotifera, *Brachionus caudatus* and *Keratella tropica* were dominant contributors. The comparative cumulative mean data on zooplankton in Bhoj Wetland indicate that during the 1st year, the diversity was 31 species and 30 species in the 2nd year. The mean density, during the 1st year was 276 Ind.l^{-1} that increased to 468 Ind.l^{-1} in the 2nd year. This variation in density during two years may be attributed low water volume caused by drought conditions in the second year. Bhoj wetland is under eutrophic state as a result of human stress in the catchment area. Further, increase of plant nutrients in the wetland waters is deteriorating its ecological condition.Hence, it is imperative to focus on preservation of these endangered habitats to achieve ecological sustainability.

Key words: Zooplankton, diversity, density, indicator species, BhojWetland.

INTRODUCTION

Water is one of the basic needs of mankind and is vital to all forms of life, it exist in lentic and lotic habitats. Tropical wetlands have played an important role for humankind (Junk, 2002; Bhat et al., 2014). These are characterized by a large number of ecological niches and harbour a significant percentage of world's biological diversity. Wetlands

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are among the most productive ecosystems in the world (Thomas and Deviprasad, 2007). Wetlands are highly productive systems which support diverse plant and animal communities. They are shallow and can warm up quickly, and aquatic vegetation can thrive, because light frequently penetrates to the bottom of the water column. The aquatic vegetation creates ideal habitat for invertebrates, fish and waterfowl. Wetlands are highly variable systems, differing in water flow, depth, extent and type of vegetation and available nutrients (Lougheed and Chow-Fraser, 1998). Zooplankters are the microscopic, free-swimming animalcule components of an aquatic eco-system, which are primary consumers on phytoplankton. Zooplankton community is cosmopolitan in nature and they inhabit all freshwater habitats of the world. Zoo-plankton diversity and density refers to variety within the community (Jalilzadeh et al., 2008). Verma and Munshi (1987) have suggested that zooplankton provide the main food item of fishes and can be used as indicators of the trophic status of a water body.

Rotifers, cladocerans, copepods, protozoa and ostracods constitute the major groups of zooplankton. Zooplankton plays an important role in energy transfer and occupies a central position in the trophic link between primary producers and higher trophic levels (Tunde, 2009). The influence of environmental factors, chemical conditions of hydro-geology of aquatic ecosystem cause changes in the composition of zooplankton and influence their densities, and so, they are also termed as bioindicators of the physical and chemical conditions of aquatic environments. The members of zooplankton are important for their role in the aquatic food chain (Cadjoet al., 2007). The factors on the basis of bioindicators are evaluated through the qualitative and quantitative condition, relative success, community structure (composition) trophic structure or environmental heterogeneity and species interactions (Holyoaket al., 2005). According to Ferrar (1972), the primary productivity fluctuates with changes in environmental factors and grazing by zooplankton. Trivediet al. (2003) disclosed that places of low zooplankton population usually have rapidly multiplied phytoplankton population. Zooplankton distribution is non homogenous. Some are mainly found in the littoral waters while others are in selected limnetic waters. Hakanson et al., (2003) attributed this to food availability and avoidance of predators. The review of limnological literature indicates limited information available on the eco-logy, diversity and role in aquatic productivity of inland aquatic environs of India (Sharma and Sharma, 2008). The dominant species and their seasonality are highly variable in different ecosystem according to their nutrient status, age, morphometry and other location factors (Rajashekhar et al., 2009).

The objectives of this study are i) to study the fluctuations of zooplankton abundance of the Bhoj Wetland, ii) to understand the impact of pollution on zooplankton community in the Bhoj Wetland. In this investigation, the data of zooplankton density and diversity in a tropical wetland system (Bhoj Wetland) was studied for two years.

Study area

Bhopal, the capital city of the state of Madhya Pradesh, India is famous for its numerous lakes. Of these, the most important are the Upper and Lower Lakes, which have commonly been designated as Bhoj Wetland (Figure 1). The Bhoj Wetland is a wetland of international importance. The Upper Lake basin comprises of a submergence area of about 31.0 sq. km and a catchment area of 361 sq. km., whereas the Lower Lake basin comprises of a submergence area of 0.9 sq. km and catchment area of 9.6 sq. km. While Lower Lake is surrounded on all sides by dense urban settlements, only about 40% of the fringe area of Upper Lake has dense human settlement and the rest is sparsely populated having cropping as the major land use. The Upper Lake spread over longitude 77°18'00" to 77°24'00" E and latitude 23°13'00" to 23°16'00" N, whereas the considerably smaller Lower Lake is spread over 77°24'00" to 77°26'00" E and latitude 23°14'30" to 23°15'30" N. The Upper Lake was created in the 11th century by constructing an earthen dam across Kolans River, the main feeding channel of the lake with the objective of supplying potable water for the city dwellers. The wetland also supports a wide variety of flora and fauna. Several species of phyto and zooplankton, macrophytes, aquatic insects, amphibians, fishes and birds (resident as well as migratory) are found in theBhoj Wetlands. Considering its ecological importance, Ramsar site was declared by the Government of India in 2002. Increasing anthropogenic activities in the catchment during the second half of the last century has resulted in environmental degradation of the wetland.

Investigations on the ecology of Bhoj wetland of Madhya Pradesh indicate that this man-made wetland is under severe degradation pressure. Siltation, solid waste disposal and weed infestation, dumping of agricultural waste, hospital waste disposal and idol immersion in the wetland during the festival season pollutes the wetland ecosystem beyond the tolerable limits of any aquatic system.

MATERIALS AND METHODS

Water samples were collected on monthly basis for a period of two year. For the present study, nine sampling points in the Bhoj Wetland were selected and each point, taking into account the human activities such as washing, bathing, fishing and boating, etc. the outlets, inlets, morphometric features and growth of aquatic vegetation etc., and other important factors considered during the selection of the sampling sites (Table 1).

The water samples were collected in one liter polyethylene canes of the surface waters by the boat between 8 am to 12 pm from the selected sites during first week of every month of the Bhoj Wetland. For the quantitative analysis of zooplankton, water was collected from the surface with minimal disturbance and filtered through a No. 25 bolting silk cloth, net of mesh size 63 µm. Ten liters of water were filtered and concentrated to 100 ml and were preserved by adding 2ml of 4% formalin simultaneously. The quantitative analysis of zooplankton was done by using Sedgwick-Rafter cell with dimensions of 50mm x 20mm x 1mm, following the method given in

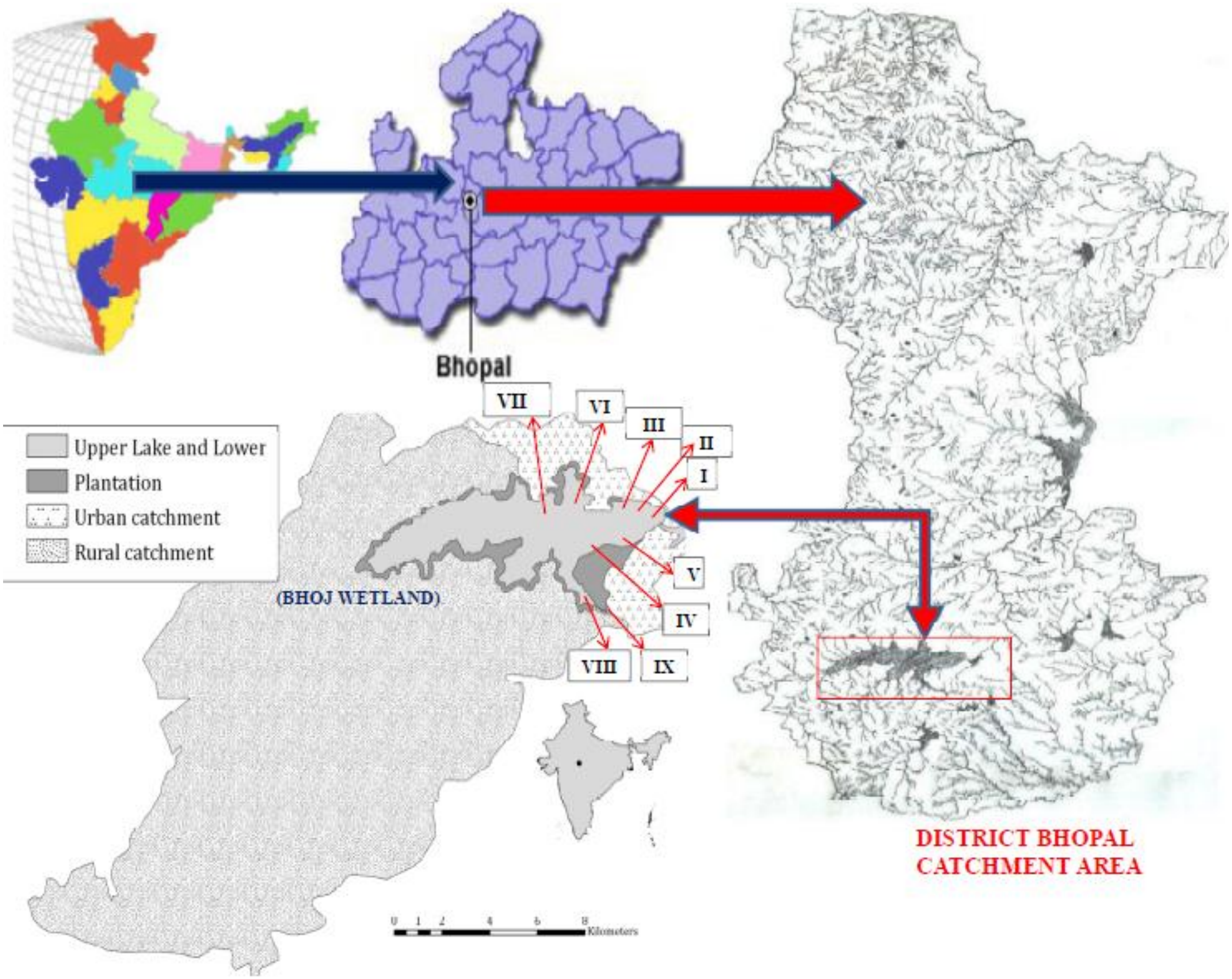


Figure 1. Map of India indicating location of Madhya Pradesh State and also indicating location of study area (Bhoj wetland), Bhopal.

Table 1. Some of the features of the sampling sites.

Station no./name	Description of the stations
Station 1 (Kamla Park)	This station is situated on eastern end of the wetland. It is subjected to maximum anthropogenic pressure. The Idol immersion activity at this site has been reduced after developing PrempuraGhat particularly for immersion activity.
Station 2 (G. Medical College)	It is situated close to the inlet of Shaheed Nagar Nallah adjacent to Gandhi Medical College.
Station 3 (Koh e Fiza)	There is an intake point for water supply in this area. This station is also the site of Tazia immersion.
Station 4 (Van Vihar)	This station represents the area that comes under protected forest (Van Vihar). The station is comparatively free from human intervention and other anthropogenic activities.
Station 5 (Yatch Club)	This is the boating station, where maximum human interaction takes place. Tourists start their motor and paddle boats from this station, and a crowd of tourists can be observed from morning till evening at this station.

Table 1. Contd

Station 6 (Bairagarh)	This station of Bhoj Wetland is situated near Bairagarh where substantial inflow of domestic sewage can be seen. The area has become shallow due to high density of free floating, emergent, and submerged macrophytes.
Station 7 (Sehore side)	A lot of agricultural land surrounds this station in Bhoj Wetland. Most of the catchment area consists of agricultural land. Because of this all the fertilizers, pesticides and agricultural residues used in the fields find their way as run off into the wetland waters.
Station 8 (PrempuraGhat)	This is the Idol immersion station. During the Hindu religious festivals, lots of idolsare immersed in water.
Station 9 (Nehru Nagar)	This station is highly influenced by anthropogenic and cattle activities. The run-off from the catchment area adds nutrients to the wetland. The region is covered with high density of emergent/submerged macrophytes. The run-off from the catchment area also adds considerable quantities of nutrients to the wetland.

APHA (2000). 1 ml of concentrated sample was taken in a Sedgwick-Rafter counting cell and the entire contents were counted. The identification of aquatic biota (zooplankton) was done following the standard works and methods of Edmondson (1959), Needham and Needham (1962), Pennak (1978), Victor and Fernando (1979), Michael and Sharma (1988), Battish (1992) and Sharma (1999).The results are expressed as individuals/l (Wanganeo and Wanganeo, 2006).

$$\text{Number of zooplankton “n”} = \frac{\text{C x 1000 mm}^2}{\text{A x D x E}}$$

C = Number of organisms recorded; A= area of field of microscope; D= depth of field (SRC depth) in mm;E = number of fields counted.

$$\text{Number of zooplankton/l} = \frac{\text{n x Vol. of concentrate (ml)}}{\text{Vol. (litres) of water filtered}}$$

Shannon diversity index

This index is an index applied to biological systems derived from a mathematical formula used in communication area by Shannon-Weaver, 1947.

$H' = -\Sigma\ [(n_i / N) \times (\ln n_i / N)]$

H': Shannon Diversity Index; n_i: number of individuals belonging to i species; N: total number of individuals.

Simpson diversity index

Itisa diversity indices derived by Simpson in 1949 (Mandaville, 2002). Simpson index values (Δ) are between 0-1. But while calculating, final result is subtracted from 1 to correct the inverse proportion.

$1 - \Delta = [\ \Sigma\ n_i (n_i - 1)\ ] / N (N-1)$

Δ : Simpson diversity index; n_i: number of individuals belonging to i species; N: total number of individuals.

Margalef diversity index

It has no limit value and it shows a variation depending upon the number of species. Thus, it is used for comparison of the sites (Kocataş, 1992).

$d = (S-1) / \ln N$

d: Margalefdiversity index; S: total number of species; N: total number of individuals.

Pielouevenness index

It was derived from Shannon index by Pielou in 1966. The ratio of the observed value of Shannon index to the maximum value gives the Pielou Evenness index result. The values are between 0–1. When the value is getting closer to 1, it means that the individuals are distributed equally (Pielou, 1966).

$J' = H' / H'_{max}$

J':Pielou evenness index; H': the observed value of Shannon index; H'_{max}:lnS; S: total number of species.

RESULTS AND DISCUSSION

Zooplankton dynamics

In the two years of study, a total of 82 zooplankton species were recorded from February 2008-January 2010. Out of which 66 species were recorded in the 1styear (2008-09), and 70 species documented during the 2ndyear (2009-2010) (Table 2). The above zooplankton population (82 species) belongs to five major groupsviz:Rotifera (46%), followed by Cladocera (34%), Protozoa (10%), and Copepoda and Ostracoda contributing 6 and 4%, respect-tively (Table 3 and Figure 2).

Annual mean trends

Diversity: Studies on Zooplankton dynamics carried out for 24 months (from February 2008 to January 2010), revealed a total of 82 species in Upper basin of Bhoj Wetland. The present study indicated significantly higher zooplankton diversity as compared to earlier reports, Verma et al. (2009) recorded 36 species, Pani et al. (2000) reported 29 species. However, Agarker et al. (1994) reported higher diversity of 78 species from the same waterbody. The

Table 2. List of Zooplankton species of Bhoj Wetland (2008-2010).

Zooplankton species	2008-2009	2009-2010
Cladocera	**19**	**26**
Alona sp.	+	+
Alonelladentifera	+	+
*Alonella*sp.	+	+
Bosminalongirostris	+	+
*Bosmina*sp.	+	+
Bosminopsisdeitersi		+
*Ceriodaphnia*sp.	+	+
Chydorussphaericus		+
Chydorusventricosue	+	
*Chydorus*sp.	+	+
*Conochiloides*sp.	+	
Daphnia sp.	+	+
Diaphanosomabrachyurum		+
Diaphanosomaexcisum		+
Diaphanosomasarsi		+
*Diaphanosoma*sp.	+	+
*Leydgia*sp.	+	+
*Macrothrix*sp.	+	+
Moinamacrocopa	+	+
Moinamicrura		+
*Moina*sp.	+	+
*Moinadaphnia*sp.	+	+
Pleuroxusaduncus		+
*Scapholebris*sp.		+
Sidacrystallina		+
*Sida*sp.	+	+
*Simocephalus*sp.	+	+
*Streblocerus*sp.	+	+
Rotifera	**35**	**33**
*Ascomorpha*sp.	+	+
*Asplanchna*sp.	+	+
Asplanchnopsis	+	+
Brachionusangularis	+	+
Brachionusangulosum		+
Brachionuscalyciflorus	+	+
Brachionuscaudatus	+	+
Brachionusfalcatus	+	+
Brachionusforficula	+	+
Brachionusquadridentata	+	+
Brachionusurceus	+	
*Cephalodella*sp.	+	+
*Colurella*sp.	+	+
*Conochilus*sp.	+	+
*Filinia*sp.	+	+
*Gastropus*sp.	+	+
*Harringia*sp.	+	+
*Hexarthra*sp.	+	+
Keratellacochlearis	+	+
Keratellatropica	+	+

Table 2. Contd

Zooplankton species	2008-2009	2009-2010
*Keratella*sp.	+	
*Lecane*sp.	+	+
*Lepodella*sp.	+	+
*Monostyla*sp.	+	+
*Mytilina*sp.	+	+
*Philodina*sp.	+	+
*Platyias*sp.	+	+
*Ploesoma*sp.	+	
*Polyarthra*sp.	+	+
*Rotaria*sp.	+	+
*Scaridium*sp.	+	+
*Synchaeta*sp.	+	
Tetramastixapoliensis		+
Trichocercalongiseta	+	+
*Trichocerca*sp.	+	+
Trichotria sp.	+	+
Triploceroslimnias	+	
*Trochosphaera*sp.		+
Protozoa	**4**	**6**
*Actinophyrus*sp.	+	
*Arcella*sp.		+
*Centropyxis*sp.	+	+
*Climacostomum*sp.		+
*Colpidium*sp.	+	
*Oxytricha*sp.	+	+
*Coleps*sp.		+
*Verticella*sp.		+
Copepoda	**5**	**4**
Cyclops sp.	+	+
*Diaptomus*sp.	+	+
*Mesocyclops*sp.	+	+
Nauplius larvae	+	+
Unidentifiedcopepod	+	
Ostracoda	**3**	**1**
*Cyprinotus*sp.	+	+
*Cypris*sp.	+	
*Stenocypris*sp.	+	
Overall Total	**66**	**70**

Table 3. Percentage of different groups of Zooplankton.

S/No.	Zooplankton group	Percentage (%)
1	Rotifera	46
2	Cladocera	34
3	Protozoa	10
4	Copepoda	6
5	Ostracoda	4
Total percent		100%

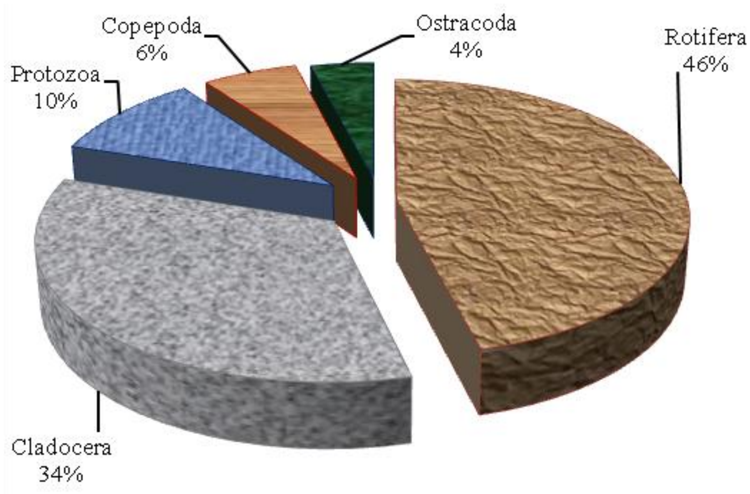


Figure 2. Percent contribution of different groups of zooplankton.

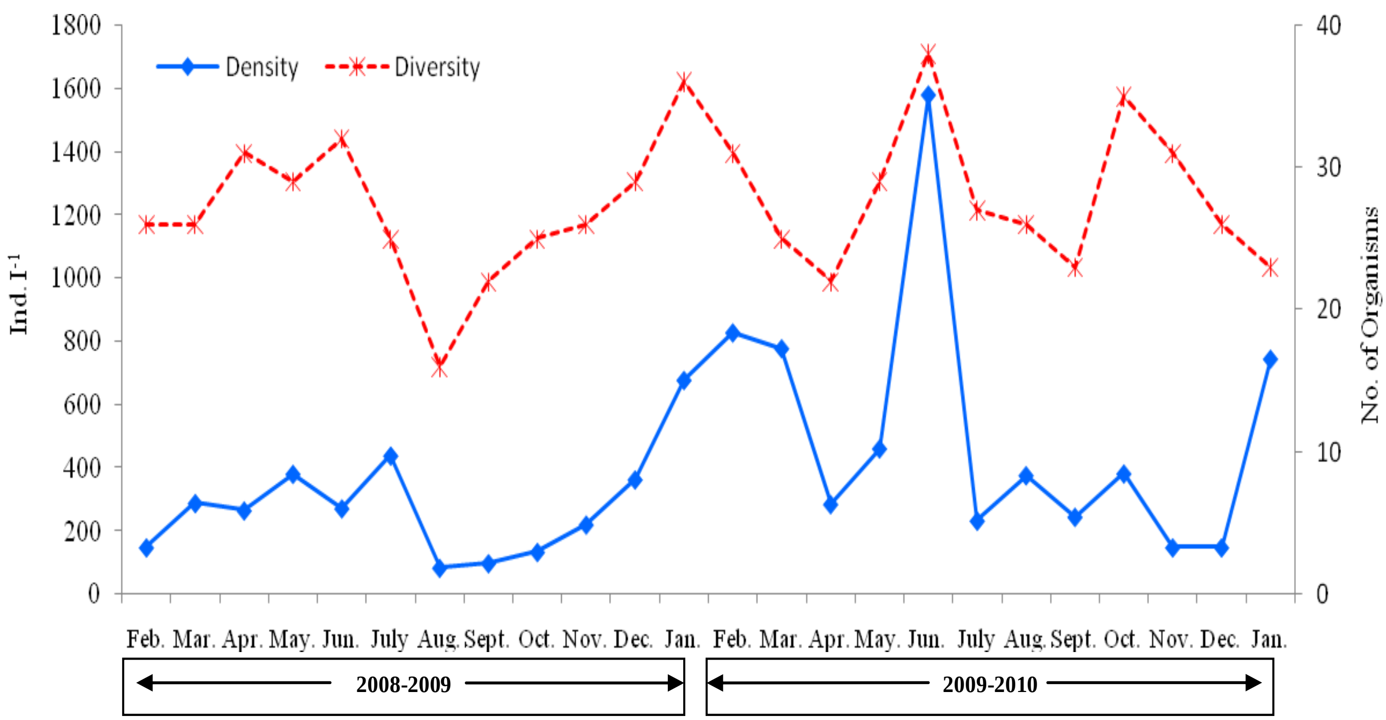


Figure 3. Comparison between diversity (no. of species) and density (Ind. l^{-1}) of Zooplankton.

current study varying exhaustive, was able to record much higher zooplankton diversity during the period of 2008-2010. The cumulative site mean diversity ranged from 16 to 38 species with a mean value of 28. A major peak of 38 species was recorded in the June 2009 with two minor peaks of 36 species (January 2009) and 35 species (October 2009). Rotiferacontributed to this diversity to the tune of 47, 58 and 51% in three peaks recorded in June, January and October, respectively. The data further indicated that among Rotifera, the major peak of June 2009 (38 species) was dominated by the various species of *Brachionus*and *Keratella,* these species also dominated minor peaks recorded during January and October 2009 (Figure 3).

The monthly variability of Zooplankton groups in terms of species number showed significant changes. The Rotifera recorded ≥60 species in March 2009 and September 2009 and in remaining months, it was mostly within 40-60 species, while minimum of < 40 species was

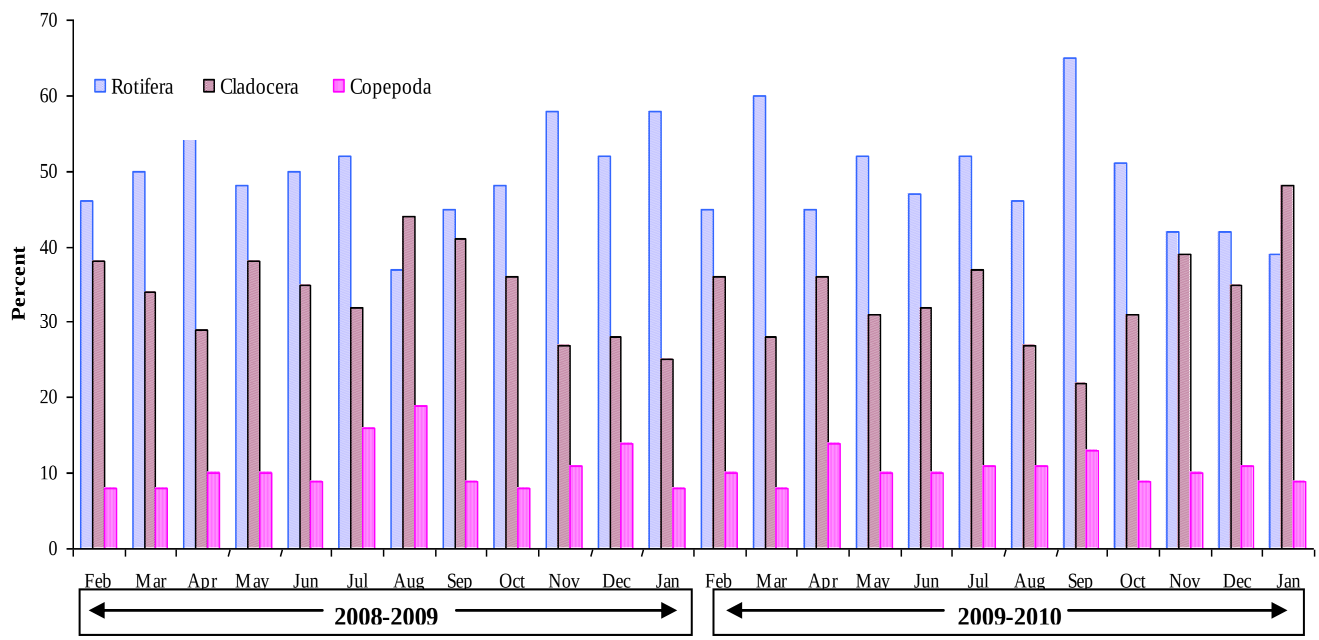


Figure 4. Group wise percent contribution of Zooplankton diversity.

recorded in August 2008 and January 2010. The Cladocera, encountered >40 species on three occasions viz, August, September 2008 and January 2010, while in remaining months the species number ranged between 30-40, and <30 was noted in April, November, December and January 2008 and March, August and September 2009. The Copepoda was significantly different and the overall species were restricted to <20 in the study period (Figure 4).

Zooplankton group Dynamics: On the basis of mean percentage contribution of different zooplankton groups, during the present study, among the total of 82 species, Rotifera formed the highest number of species (46%) followed by Cladocera (34%), Protozoa (10%), Copepoda (6%) and Ostracoda (4%) (Figure 2).

In general, Rotifera registered highest number of species in terms of percentage during the period of study except in the month of August 2008 and January 2010. Cladocerans recorded second highest and followed by copepods. The maximum diversity and population density of rotifers recorded in the present study was mainly due to organic load and eutrophic condition. High rotifer population in the lake waters indicates enrichment due to direct inflow of untreated domestic sewage from adjacent area into the lake (Arora, 1966). Chandrashekhar (1998) recorded diversity of rotifers to be influenced by the different water quality and other chemical factors.

The most abundant zooplankton group during the study was rotifera, the group, across all stations was dominated by different species of *Brachionus* and *Keratella,* and these are found extensively in eutrophic waters as reported (Arora, 1966; Berzins and Pejler, 1989). The diversity patterns greatly depend on the water temperature and availability of food in the waterbody. Phytoplankton populations constituting the essential component of the rotifera dietary spectrum, increase with higher water temperature in summer that impacts species diversity in the wetland. Maximum Rotifera members in the wetland may be due to optimal nutrient and temperature conditions and favourable DO content, as reported by Subla et al. (1992) and Padmanabha and Belagali (2006) in their investigations. The *Brachionus* has cosmopolitan distribution in India and during the present study, it was represented by (8) species namely: *Brachionu sangularis, Brachionus angulosum, Brachionus calyciflorus, Brachionus caudatus, Brachionus falcatus, Brachionus forficula, Brachionus quadridentata* and *Brachionus urceus.* Pejler (1977) and Fernando (1980) have also stressed that *Brachionus* was an important genera among tropical zooplankton community. Among the 8 species, *B. caudatus* was the most dominant, which is supported by the observations of Patil (1978),Isairasu and Mohandoss (1998) and Malathi (1999). Hutchinson (1967) observed that *Brachionus* species are very common in temperate and tropical waters, having alkaline pH. The dominance of *Brachionus*has been related to eutrophic nature and the presence of high organic matter in the water (Dadhich and Saxena, 1999; Sampaio et al., 2002). In other investigations Mageed (2008) and Uzma (2009) stated that presence of more than (5) species of *Brachionus*reflects eutrophication of water bodies. The Bhoj Wetland being a tropical water body is highly eutrophicand receives sewage

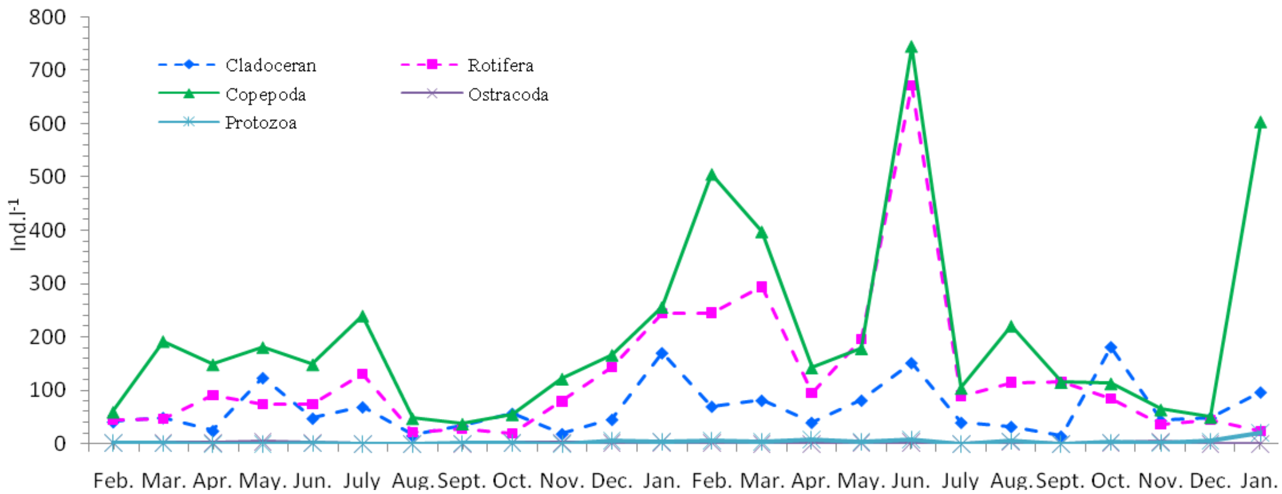


Figure 5. Variation in total density (Ind.l^{-1}) of Zooplankton during the period of study.

and other agricultural effluents from the catchment records dominance of *Brachionus*.

Density: The population density data revealed that cumulative site mean density ranged from 84 to 1579 Ind. l^{-1}, with an overall mean of 399 Ind. l^{-1}. The 24 months of mean site density indicated a major peak of 1579 Ind. l^{-1}, in June 2009, with 47 and 43% contribution from Copepoda and Rotifera (Figure 5). However, among Copepoda, *Cyclops* and Nauplius larvae were major contributors to this peak and among Rotifera, *B. caudatus* and *Keratella tropica* were dominant contributors. The two minor peaks of 828 and 744 Ind. l^{-1} were recorded in February 2009 and January 2010, respectively. Among Copepoda, *Cyclops* contributed significantly to the February 2009 and January 2010 peaks, to the tune of 61 and 81%**.**

It is reported that Copepoda are best adapted to eutrophic lakes (Gannon and Stremberger, 1978). In the present study, the high density of rotifers (43%) and of Copepoda (47%) provides a positive evidence of the progression of eutrophication. The significant density of copepod nauplii larvae in Bhoj Wetland was recorded during the warm period, indicating the role of high temperature in promoting the egg production and development. This is in agreement with Makino and Ban (2000) who reported that higher water temperature causes more rapid development and higher egg production while increased food density results in larger body size and higher egg production. Singh et al. (2002) reported that higher rotifer population occurs during summer and winter which might be due to higher trophic level of the lake, high summer temperature and low level of water in winter. In our investigation, *Keratella* and *Brachionus* are common rotifers with a wide range of tolerance to different physico-chemical conditions. *Cyclops* is regarded as a strictly pollution sensitive copepod (Bhatti and Rana, 1987). However, in our study, they were common genera at all stations. The occurrence of nauplii throughout the study period in wetland indicated extended reproductive phase of the cyclopoid,which is in agreement with the reports of Sharma(2011) and Sharma and Sharma (2011). The Bhoj is typically tropical wetland, with rotifers recording highest density and dominated mainly by *Brachionus* and *Keratella*. The members of rotifera are reported as good indicators of eutrophication and pollution (Saksena, 1987) and eutrophic lakes have been noted to harbor high density of *Brachionus*and *Keratella*(Arora, 1966).

The comparative cumulative mean data on zooplankton in Bhoj Wetland indicate that during the 1styear, the diversity was 31 species and 30 species in the 2ndyear. The mean density during the 1styear was 276 Ind.l^{-1} that increased to 468 Ind.l^{-1} in the 2ndyear. This variation in density during two years may be attributed to low water volume caused by drought conditions in the second year (Plate 1, 2, 3, 4). The maximum population density recorded in the 2ndyear also reflected a positive relationship with temperature, nitrate and phosphate concentrations. Similar observations were recorded by Paliwal (2005). The maximum population density of zooplankton in the 2nd year may also be attributed to greater availability of food namely: phytoplankton and suspended detritus. The factors like temperature, D.O play an important role in controlling the diversity and density of zooplankton (Edmondson, 1965; Baker, 1979). According to Kurbatova (2005) and Tanner et al.(2005), the pH more than 8 means highly productive nature of a water body, in the present study, the average pH recorded was 8.3, indicating water highly productive for zooplankton population.

Comparison between diversity and density: Comparing diversity and density of zooplankton in the present study,the two seem to be positively related, the data revealed highest diversity of 38 species and density of

1579 Ind. l^{-1} in June 2009, In August 2008, a minimum diversity of 16 species correspondedwith a low density of 84 Ind.l^{-1}. Drawing a comparison between two years on the cumulative mean trends, the data revealed that in the 1styear, the mean diversity was 27 and 28 in the second year; similarly the mean density in the 1styear was 281 Ind. l^{-1}, which increased to 518 units l^{-1} in the second year (Figure 3). The diversity/density pattern was similar in both years, but their relative abundances varied. The variability in density during two years may be attributed to hydrological change in the second year, mainly caused by acute drought conditions (Plate 1, 2, 3, 4). In summer season, low flow of water brings stability to the ecosystem and more availability of food due to production and decomposition of organic matter. The high diversity and density of zooplankton recorded in June 2009 may be related to high phytoplankton density during this period. It is documented that nutrient availability influence the abundance of rotifer and Copepoda (Kumar et al.,2004). Overall, the diversity and density of zooplankton depends upon the nutrient status of water body and enabling water quality. Sarkar and Chowdhury (1999) reported that variability in temperature, total alkalinity, phosphate, nitrate and pH does influence the growth of zooplankton. Low zooplankton diversity and density during the rainy season has been attributed by Okogwu et al. (2010) to turbulence generated by the excess water flows.

Site variability

Diversity: In the first year (2008-2009), the species number from different stations, ranged from 20 to 40, a maximum of (40 species) encountered at station VIII (Prempura Ghat), and minimum of 20 species at station VII (Sehore Side). In the second year (2009-2010),a mini-mum of 16 species were recorded at station VII and maximum of 52 species at station VIII (Figure 6).The peak diversity recorded at station VIII in both years, was due to dominance of rotifers and cladocerans species. The higher nutrient status at station VIII resulting from decom-position of macrophytes enabling higher diversity as well as density of zooplankton. Maximum zooplankton peak obtained at station VIII (Prempura Ghat) in the wetland may be due to high nutrients like (nitrate $\bar{X}$=0.62mg/l and phosphate $\bar{X}$=0.27mg/l) and favourable temperature and DO conditions, similar trend has been reported by Padmanabha and Belagali (2006). The progressive decrease in the zooplankton diversity at station VII during the 1styear may be attributed to drought from January 2009 to June 2009 (Plate 3).

Density: The zooplankton density at nine stations recorded a minimum of 67 Ind.1^{-1} and a maximum of 520 Ind.1^{-1} with overall mean density of 276 Ind.1^{-1} in the 1st year, whilein the 2ndyear, a minimum of 56 Ind.1^{-1} and a maximum of 1059 Ind.1^{-1} was recorded with mean density of 468 Ind.1^{-1}. The mean cumulative monthly density of different stations indicated major peaks of 520 and 1059 Ind. l^{-1} at station VIII in the 1stand 2nd year, respectively. Maximum contribution of 46 and 47% by Copepoda in the above peaks in both years was recorded. Among Copepoda, two major contributors were *Cyclops* (65 and 73%) and its Nauplius larvae (29 and 25%) in the 1stand 2ndyear, respectively (Figure 6). Joshi (1987) reported dominant population of Copepoda (*Cyclops*) throughout the year from Sagar Lake. Gupta (1989) reported Copepoda population throughout the year in Gulabsagar and Ganglooan water bodies of Jodhpur, this was attributed to enriched nature of waters. Syuhei-Ben (1994) stated that individual growth rate of Copepoda may also depend on temperature conditions. Gannon and Stemberger (1978) found that cladocerans and cyclopoid copepods are more abundant in nutrient enriched waters of the Great Lakes.

Two year comparison: Two years diversity/density data showeda positive relation, the station VIII recorded the maximum diversity/density (40 and 52 species and 520 and 1059 Ind. l^{-1}) in the 1stand 2ndyear (Figure 6). The *Cyclops* and its nauplius larvae, dominated the zooplankton density peak. In diversity peaks, *Brachionus* (7 and 6 species) recorded maximum during the 1stand 2ndyear and *Diaphanosoma* (3 species) only in the 2ndyear. Among rotifers, the numerical superiority was found to be high in the case of *Brachionus* (7 species), which is considered typical and most frequent in tropical environment (Nogueira, 2001; Mulani et al., 2009). In the present investigation, *Brachionus* species was frequently observed at all stations. This species is considered the indicator of eutrophication (Sampaio et al., 2002). Nogueira (2001) reported that *Brachionus* species as indicator of sewage and industrial pollution. Jana and Pal (1984) reported the abundance of *Diaphanosoma excisum* in water bodies having high organic content. Therefore, presence of *Diaphanosoma* at all the stations in the present study can also be considered as an indication of increased organic content in the water,from sewage and other agricultural effluents.

Among the different groups, Rotifera varied from 25 to 62% with lowest at station VII (Sehore side) and highest at station IV (Van Vihar) in the first and second year, it varied from 38 to 58% with minimum at station VII (Sehore side), and maximum at station VI (Bairagarh) respectively, (Figure 7). Across the overall station trend in zooplankton diversity was Rotifera > Cladocera > Copepoda. The highest rotifera species diversity was observed at stations IV and VI, characterized by dense well developed macrophyte stands, which provides shelter, varied niches and comparatively good quality water as evinced by the physico-chemical properties of water (Robinson, 2004). The sufficient nutrient availability and other favourable conditions result in dominance of

Station I (Kamla Park) during full tank level

Station I (Kamla Park) receded water level during summer

Station II (Gandhi Medical College) during full tank level

Station II (Gandhi Medical College) during dry phase

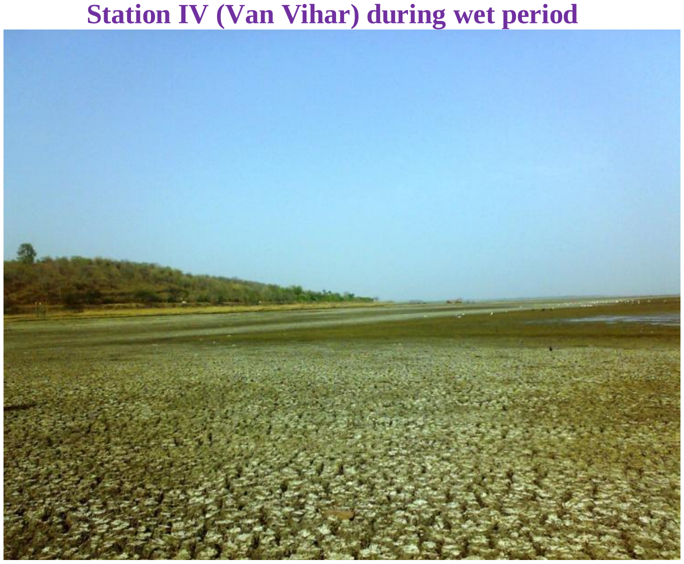
Station III (Kohe Fiza) during full tank

Station III (Kohe Fiza) during dry phase

Plate 1. Some evidence about drought conditions in the second year

Station IV (Van Vihar) during wet period

Station IV (Van Vihar) during dry phase



Station IV (Van Vihar) during dry phase

Station IV (Van Vihar) during dry phase

Station V (Boat Club) during full tank

Station V (Boat Club) during dry phase

Plate 2. Some evidence about drought conditions in the second year

Station VI (Bairagarh) during full tank level

Station VI (Bairagarh) during dry phase

Station VII (Sehore Side) during full tank level

Station VII (Sehore Side) during dry phase

Station VIII (Prempura Ghat) during full tank

Station VIII (Prempura Ghat) during dry phase

Plate 3. Some evidence about drought conditions in the second year

Station IX (Nehru Nagar) during full tank level **Station IX (Nehru Nagar) during dry phase**

Plate 4. Some evidence about drought conditions in the second year

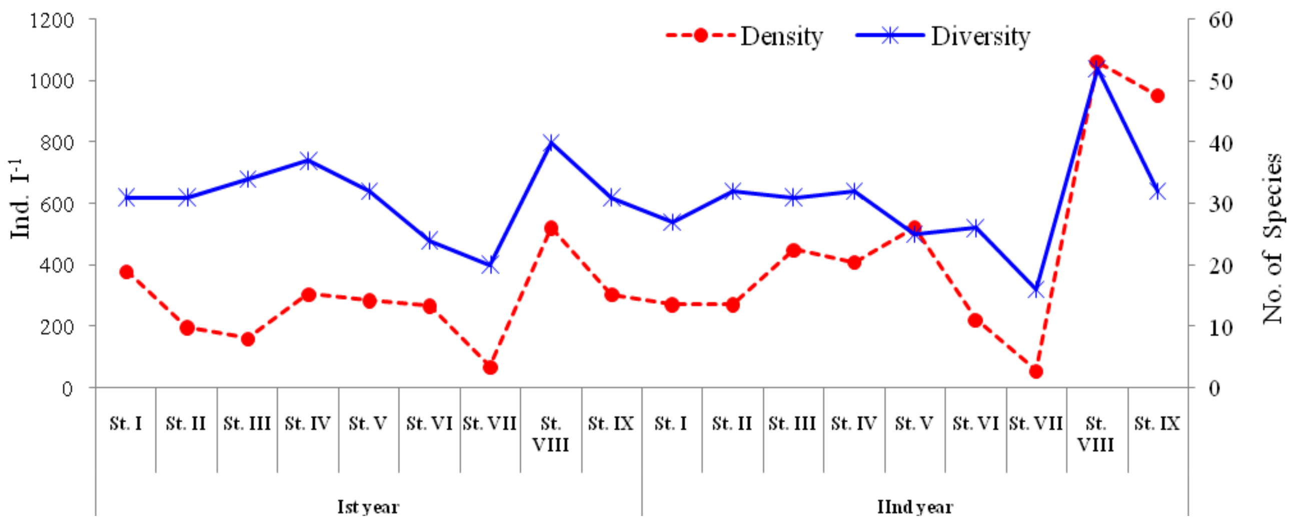


Figure 6. Comparison of diversity (no. of species) and density (Ind. l^{-1}) of Zooplankton at various stations.

rotifers while lower diversity at station VII may be due to the acute drought conditions (Plate 3) and significant use of pesticides in the agricultural activities. Kasai and Hanazato (1995) reported that application of herbicides induced the decline in zooplankton density; similar trend with regard to fungicide has been recorded by Willis et al.(2004). High species diversity of rotifera has been recorded with the peaks of phytoplankton,this suggest that the increase in zooplankton production may be attributed to greater availability of food in the form of phytoplankton coupled with enabling temperature (Wadajo, 1982; Wadajo and Belay, 1984; Webber and Roff, 1995; Christou, 1998; Uyeet al., 2000). The Cladocera ranged from 25 to 55%, with a minimum at station V and maximum at station VII in the first year, while in second year, it ranged from 19 to 44% with minimum at station VI and maximum at station VII and VIII, respectively. The lower abundance and diversity of cladocerans found in the station VII may be explained as the grazing impact of planktivorous fishes (Wetzel, 1983; Arcifa et al., 1986; Basima et al., 2006; Jeppesen et al., 1998). However, Wetzel (2001) reported higher densities of cladocerans in littoral areas along with macrophytes.

Cladocerans feed on algae, small rotifers and copepod nauplii (Dodson et al., 2010). Rotifers and cladocerans strongly compete for the similar food resources (Kirk and

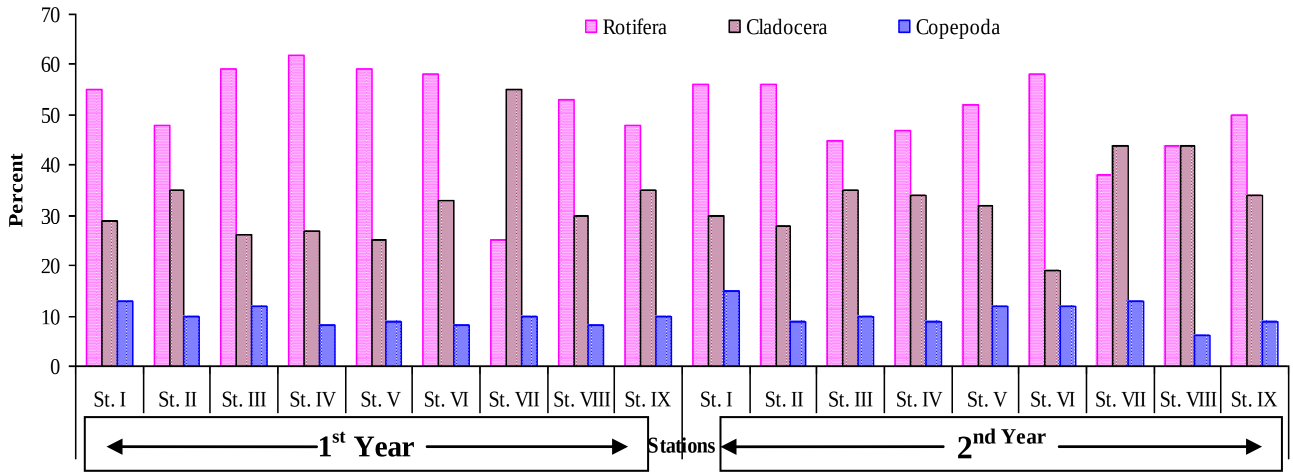


Figure 7. Percent contribution of Zooplankton groups at various stations.

Table 4. Zooplankton diversity indices of Bhoj Wetland (2008-10).

Station	Shannon diversity index		Simpson diversity index		Margalef diversity index		Pielou's evenness index	
	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Year
St. I	2.28	2.12	0.81	0.80	3.56	3.21	0.31	0.31
St. II	2.30	2.61	0.81	0.88	3.87	3.83	0.32	0.43
St. III	2.63	2.47	0.86	0.86	4.36	3.49	0.41	0.38
St. IV	2.39	2.58	0.82	0.88	4.37	3.65	0.29	0.41
St. V	2.27	2.20	0.84	0.81	3.81	2.75	0.3	0.36
St. VI	2.23	2.13	0.84	0.78	2.85	3.29	0.39	0.32
St. VII	2.21	2.39	0.81	0.87	2.88	2.52	0.46	0.68
St. VIII	2.68	2.58	0.87	0.85	4.46	5.39	0.36	0.25
St. IX	2.47	1.70	0.85	0.63	3.66	3.32	0.38	0.17

St.: station.

Gilbert, 1990). The large cladocerans limit the abundance ofrotifera and thus, are usually the more competitively dominant taxa (Kirk and Gilbert, 1990). When different taxa compete for the same limited food resources, some populations may experience a decline due to the competition for food, feeding capabilities and reproduction capacity (Kirk and Gilbert, 1990). This situation may lead to a dormant or resting stage for the zooplankton rather than its complete decline (Wetzel, 1983).

Zooplankton diversity indices

In the present investigation, Shannon -Wiener diversity index ranged between the values of 2.215 to 2.682 in the 1st year (2008-09). The highest diversity index was found to be 2.682 at station VIII and a lowest 2.215 at station VII (Table 4). While in the second year, the values of Shannon-Wiener diversity index ranged from 1.703 to 2.614. The highest value was 2.614 at station II and a lowest 1.703 at station IX, respectively (Table 4). In general, the index reveals that upper basin of Bhoj Wetland is more diverse. Wilhm and Dorris (1968) found that the value of index decline sharply in polluted zones of the lake.

Simpson diversity index varied between the values of 0.812 to 0.872. The minimum value of 0.812 was recorded at station II and a maximum of 0.872 at station VIII. While in the 2nd year of study, the diversity varied between 0.633 to 0.882 values. The minimum value was found to be 0.633 at station IX and a maximum of 0.882 at station IV, respectively (Table 4). Simpson's index of diversity showed that the index of diversity was significantly higher. The Simpson index (low value) indicates an increase in dominance of fewer species in Baigul water bodies (Mishra et al., 2010). The index value ranges from 0 and 1, the higher the index value, the higher the diversity.

The evenness components of diversity values were found to range 0.296 to 0.458. The lowest value of 0.296 was found at station IV and the highest value of 0.458 was found at station VII, respectively (Table 4). While in

Table 5. Percentage similarities (Sorenson's index) of Zooplankton (2008-09).

	Similarity matrix								
	St.I	**St.II**	**St. III**	**St.IV**	**St.V**	**St.VI**	**St.VII**	**St.VIII**	**St.IX**
St. I	*	49.42	49.15	77.37	64.15	71.83	21.59	46.01	47.98
St. II	*	*	61.83	57.56	66.43	64.62	40.91	46.15	60.30
St. III	*	*	*	50.17	51.69	54.97	42.70	37.21	52.52
St. IV	*	*	*	*	65.65	72.47	25.93	49.15	54.57
St. V	*	*	*	*	*	78.06	27.95	51.19	60.80
St. VI	*	*	*	*	*	*	27.41	52.75	62.08
St. VII	*	*	*	*	*	*	*	17.77	27.00
St. VIII	*	*	*	*	*	*	*	*	59.57
St. IX	*	*	*	*	*	*	*	*	*

St.: station.

the second year of investigation, the value varied between 0.171 and 0.682. The highest at station VII and a lowest at station IX was recorded.

The Margalef diversity index varied from 2.85 to 4.46 in the first year of study. The highest Margalef index was recorded at station VIII (4.46) and a minimum at station VI (2.85) while in the second year of study, the Margalef diversity index varied from 2.52 to 5.39. The minimum value (2.52) was recorded at station VII and the maximum (5.39) at station VIII (Table 4). Mukherjee (1997) reported that the higher species richness is characterized by larger food chain (Dumont, 1994).

Overall, Zooplankton species richness in the present study is quite high. The present study supports the idea that overall species richness is positively affected by the number of diverse habitats in the Bhoj Wetland. All the diversity indices (Shannon-weaver, Simpson diversity and Margalef diversity index) showed high diversity at station VIII (first year) and during the second year, Shannon diversity index was high at station II. Simpson diversity index was also high at same station and also at station IV. However, on the basis of Margalef diversity index, it was station VIII which recorded highest diversity. Applying evenness index of Pielou, the present values depict that the species are not evenly distributed.

Cluster analysis

Cluster analysis of Zooplankton

The Bray-Curtis Cluster analysis are shown in Table 5 and Figure 8. In the present study,for the similarity matrix, the highest value is 78.06 for station numbers V and VI and thus linked together at level 1. The second highest similarity value of 77.37% is between the stations I and IV, hence station I is linked to IV. Extreme differences in the zooplankton structure were detected between the sampling station VII and VIII. The zooplankton communities indicate similarities (Sorenson's Index) ranging from 17.77 to 78.06% during first year of the study period. Wetland exhibited higher zooplankton similarity, that is, >50% similarity in maximum instances (58.3%). This wetland, however, showed a relatively wide community similarity range (50-78.06%) during 2008 - 2009.

Bray-Curtis cluster analysis shows notable differences in annual cluster groupings in the sampled stations. In the second year of study, the Bray-Curtis cluster analysis indicated closer affinity in zooplankton between station III and IV and diverse composition of station VII and station VIII and IX communities during 2009 - 2010. During the second year,there was higher zooplankton similarity (4.75 - 68.42%). Majority of instances (25%) in the matrix however indicate similarities between 50-68.42% during 2009-2010 (Table 6 and Figure 9).

Wetlands are dynamic ecosystems, continually undergoing natural changes due to infilling with sediments and nutrients. They sustain all life and perform some useful functions in the maintenance of overall balance of nature (ecosystem). Rapid urbanisation, burgeoning human population and their various activities have contributed to the decline of quality and number of wetlands, also socio-economic value of the wetlands has decreased. Hence, it is imperative to focus on preservation of these endangered habitats to achieve ecological sustainability.Zooplankton community was represented by fivegroups namely: Cladocera, Rotifera, Copepoda, Ostracoda and Protozoa. The copepods and rotifera population were found maximum throughout the study period, whereas rotifer had maximum in number of diversity during both years of thestudy. Thus, the wetland plays a very important role inmaintaining the biodiversity. The presence of species indicative of eutrophic status condition in Bhoj wetland suggests that the ambient water has achieved a high trophic status on account of nutrient enrichment from its catchment area wherein varying types of anthropogenic activities were recorded as related to different socio-economic status of persons residing in its catchment area. It is concluded that the Bhoj wetland is under eutrophic state as a result of human stress in the

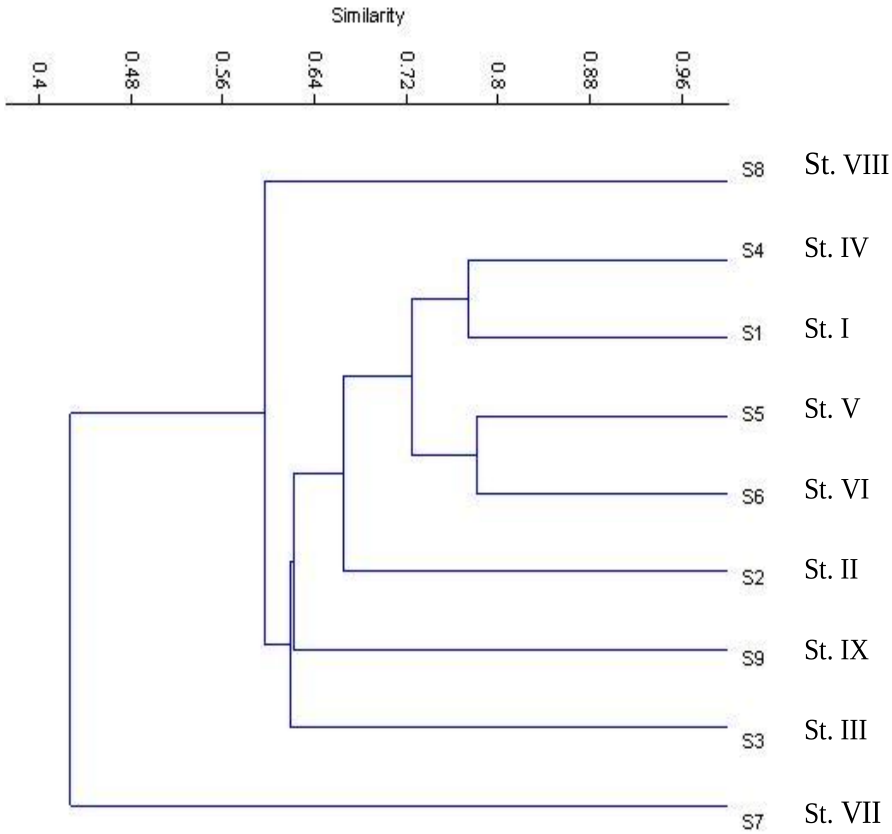


Figure 8. Bray-Curtis cluster analysis of Zooplankton of Bhoj Wetland (2008-09).

Table 6. Percentage similarities (Sorenson's index) of Zooplankton (2009-10).

	Similarity matrix								
	St.I	**St.II**	**St. III**	**St.IV**	**St.V**	**St.VI**	**St.VII**	**St.VIII**	**St.IX**
St. I	*	56.15	55.50	64.55	55.14	38.74	10.29	32.02	39.84
St. II	*	*	56.95	55.98	49.90	41.78	13.48	33.37	41.21
St. III	*	*	*	67.62	68.42	29.65	8.75	33.62	32.90
St. IV	*	*	*	*	59.48	31.61	9.30	44.99	39.81
St. V	*	*	*	*	*	25.89	6.92	37.80	37.05
St. VI	*	*	*	*	*	*	28.92	16.94	25.00
St. VII	*	*	*	*	*	*	*	4.75	9.07
St. VIII	*	*	*	*	*	*	*	*	32.32
St. IX	*	*	*	*	*	*	*	*	*

St.: station.

catchment area. Further, increase of plant nutrients in the wetland waters is deteriorating its ecological condition.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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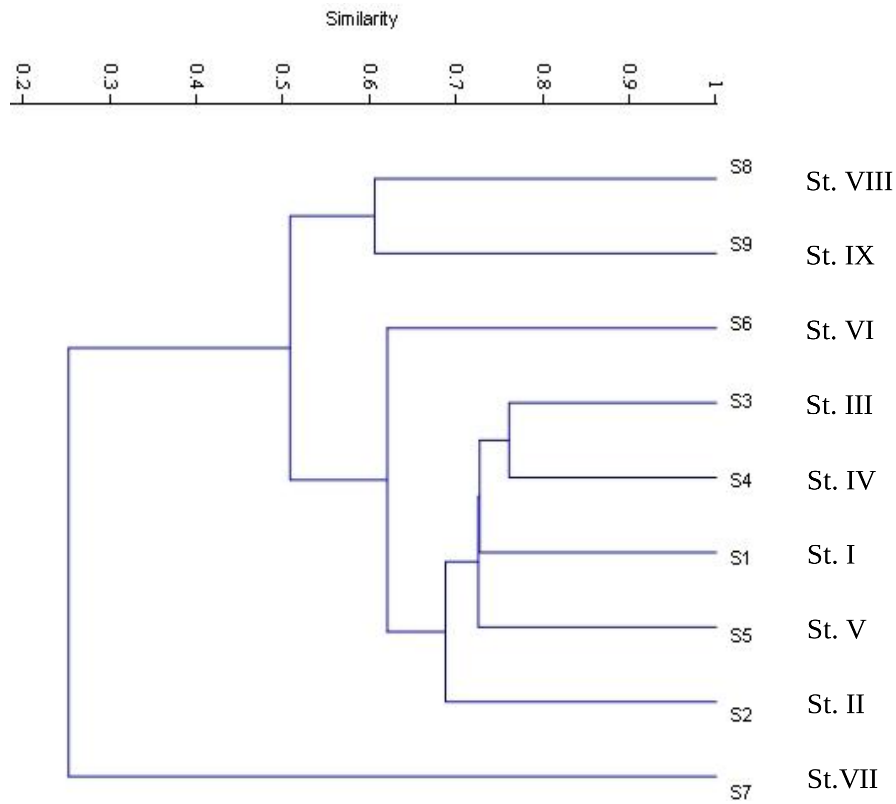

Figure 9. Bray-Curtis cluster analysis of Zooplankton of Bhoj Wetland (2009-10).

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Macro invertebrates fauna group and their relationship with environmental variables in River Benue at Makurdi, Benue State, Nigeria

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Indiscriminate dumping of wastes into rivers without control measures is widely practiced in the developing nations of the World as it is observed in River Benue at Makurdi. To ascertain the health status and integrity of River Benue at Makurdi, water samples and sediments were collected monthly from five different locations on the shoreline of the river for a period of two years (July 2011-June 2013). The physico-chemical quality of the water samples were examined using standard methods. The sediments were examined for the presence and absence of macro benthic fauna. The mean values were generally within the WHO and the Nigerian Standard for Drinking Water Quality accepted maximum limit except for colour and turbidity. The result of ANOVA for all the parameters was significant during the seasons (P<0.05), except for total dissolved solids (TDS), total suspended solids (TSS) and temperature (P>0.05). Across the locations temperature, bicarbonate, nitrate, sulphate, phosphate and copper were not significant (ANOVA, P>0.05). The result of the sediments showed that a total of 4,451 macro benthic fauna individuals comprising of 4 phyla and 21 taxa were obtained. Among this benthic fauna group, Athropoda had the highest population of 90.15%, Annelid 4.74%, Mollusca 3.39% and Platyhelminthes 1.7%. A significant relationship at some instances was noticed between benthic fauna group and some environmental variables indicating relationship between benthos and hydrochemistry of River Benue. It is recommended that the discharge of effluents and other waste into the River Benue should be controlled and enforced.

Key words: River Benue, physico-chemical, macro benthic fauna, relationship.

INTRODUCTION

The health of the ecosystem is determined by the taxonomic composition of the community as well as its diversity (Idowu and Ugwumba, 2005). Benthic macro fauna are those organisms that live on or inside the deposit at the bottom of a water body (Barnes and Hughes, 1988; Idowu and Ugwumba, 2005). They are used to detect changes in the natural environment, monitor the presence of pollution and its effect on the ecosystem in which organisms' lives and, to monitor the progress of environmental cleanup (Otway and Gray, 1996;

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Nkwoji et al., 2010). They are used in testing water bodies for the presence of contaminants (Nkwojiet al., 2010). Studies have shown that there is entwining relationship between surface water quality and macro invertebrate diversity (Teferiet al., 2013). The physico-chemical parameters of lakes, ponds and rivers have considerable effect on the aquatic life. These parameters determine the productivity of a water body. Thus, a change in the physico-chemical aspect of a water body brings about a corresponding change in the relative composition and abundance of the organisms in that water (Adeyemiet al., 2009). All the same chemical and physical measurements used in evaluating water quality provide data that primarily reflect conditions that exists when the water sample was taken (Ikomiet al., 2005; Muralidharan et al., 2010). However, physico-chemical and biomonitoring are not mutually exclusive, an optimal limnological study involve both approach (Muralidharan et al., 2010). This is because the biological community gives an indication of past conditions as well as the current situation of the aquatic ecosystem (Nkwojiet al., 2010). Therefore, any negative effect caused by pollution in the community structure can in turn affect trophic relationship (Sharma and Chowdhary, 2011). An additional advantage of macro invertebrates' bio indicators is that they integrate stream conditions related to the flow and chemical characteristics as well as the cumulative impact of multiple potential contaminants (Yagowet al., 2006). Biological assessment is therefore a useful alternative for assessing the ecolo-gical quality of aquatic ecosystems since biological communities integrate the environmental effects of water chemistry, in addition to the physical and geo-morphological characteristic of rivers and lakes (Stevenson and Pan, 1999). As rural and urban communities in Nigeria and Makurdi in particular continue to rely on surface water sources and shallow wells for their water needs, it is important to know the quality of the water they use as a means of advancing their health in the face of grinding poverty (Akaahan et al., 2010). Apart from this, the water quality is also determination for the well-being of the fisheries resources which is of paramount importance. This research is aimed at complementing the previous work done on the quality of Nigerian inland waters of which River Benue is prominent for the sustenance of its flora and fauna composition as well as the benthic fauna in particular.

MATERIALS AND METHODS

Study area

The River Benue with its source from the Cameroonian mountains flows westwards into Nigeria. It is the second largest river in Nigeria and measures approximately 310,000 Ha. It is about 1.488 km in length with alluvia fertile flood plains on either banks (Welcomme, 1986). The Benue River flows through Makurdi and confluence with River Niger at Lokoja, the capital of Kogi state, Nigeria. Makurdi is the capital city of Benue state is located on Latitude 7°41' N and Longitude 8° 28' E. The size of the River Benue within Makurdi and major settlement runs through is approximately 671 m (Udo, 1981).

The rainfall seasons at Makurdi produces a river regime of peak flows from August to early October and low flow from December to April. The rainy season which last for seven months (April to October) has a mean annual rainfall ranging from 1200-2000 mm (Nyagba, 1995). High temperature values averaging 28-33°C are recorded in Makurdi throughout the year, most notable from March to April. Harmattan winds are accompanied with cooling effects mostly during the nights of December and January (Nyagba, 1995). All the same, the periodic dust plumes associated with this time of the year may encourage surface water pollution (Nyagba, 1995). Five stations were selected along the river course at Makurdi, Benue State for this study as follows(Figure 1):Site I(N07°^{0} 43.663ᑊE008° 35.427ᑊ): it is located behind Coca Cola PLC plant along Gboko road and it is approximate 1.5 km away from Site II. Site II (N07°43.615ᑊ E008° 35.300ᑊ): it is located directly behind Benue Brewery Plc along at Kilometer 5 along Gboko road. This site is impacted by the brewery effluents generated from the factory into the river.Site III (N07°43.649ᑊ E008° 35.302ᑊ): this site is located behind Mikap Nigeria Ltd, a rice processing factory along Gboko road. It is approximately 1 km away from Site II and 2.5 km away from Site I. This site receives effluents from the rice mill into the river. Site IV (N07°44.076ᑊ E008° 32.840ᑊ): this site is located behind Wurukum abattoir close the new bridge across the river.Abattoir waste is washed directly into this site. Farming and sand dredging also take place at this site on routine bases. Site V (N07°44.789ᑊE008° 30.624ᑊ): This site is located behind Wadata market along the river water course at Makurdi. Wastes from the heap refuse dumpsite behind the market are leached directly into the river.

Water sample collection and analysis

Water samples for physico-chemical analysis were collected at five different points from each of the five sampling locations. Fortnightly, routine sampling was conducted between 8:00 am and 12:00 noon on each sampling day. The water samples for biochemical oxygen demand analysis were collected in dark bottles of 1,500 ml capacity at the depth of 20 cm, while 1,500 mL (1.5 L) containers were used for collection of water samples for otherphysico-chemical parameters. Usually sampling bottles and containers were rinsed three times with river water at each sampling site before sample collection. The water sampler was rinsed for about six times at each sampling site before the collection of the samples. Each sample container was treated according to the analysis to be carried out on it on the field before they were transported to the laboratory. Surface water temperature, TDS, conductivity, pH, and DO were determined *in situ* on the field, while copper, nitrate, chloride, bicarbonate, sulphate, phosphate, TSS, turbidity, colour, BOD and COD were examined in the laboratory using standard methods (APHA, 1999).

Benthic fauna sample collection and analysis

Three successful hauls of benthic samples were taken from each station using a van Veen grab ($0.1m^2$) from an anchored boat with an out-board engine of 25 HP during the 24 months study period. The two shovels of the grab were held open by a small bar. The grab was then lowered into the river bed at the sampling sites. When the grab reaches the bottom of the river, the bar was automatically released. The two shovels of the grab sampler were closed tightly with sand and mud captured in it. The content of the grab were emptied into a polythene bags, labeled properly and taken to the laboratory for sorting and analysis. In the laboratory, the samples were sieved in order to remove fine sediments and any other extraneous material. Each of the sediment sample collected was washed three times in the laboratory through three sets of

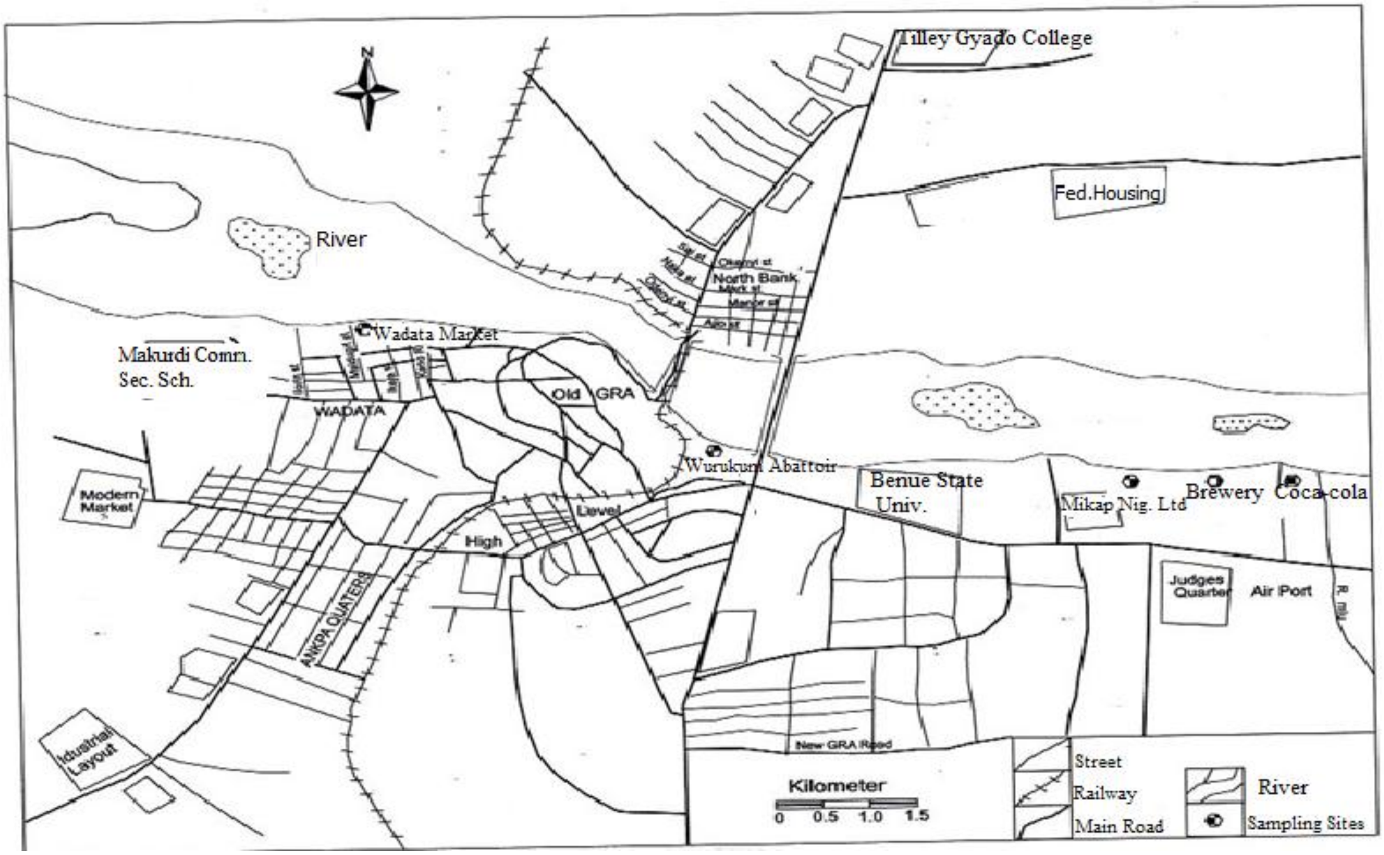

Figure 1. Map of Makurdi town showing sample site (Source: Ministry of Lands and Survey).

sieves, 1st 2 mm, then 1mm and finally 0.5mm mesh size sieves to collect the macro benthos in them (Esenowo and Ugwumba, 2010). The retained macro benthos were poured into bottles and labeled properly. The benthic fauna samples were then fixed with 4% formaldehyde. The washed and preserved sediments with benthic invertebrates were poured into a white enamel tray and sorted out. The sorting was made effective by adding moderate volume of water into container to improve visibility (George et al., 2009). Large benthic fauna were picked out using forceps while the smaller ones were pipetted out. The preserved animals were identified under light and stero dissecting microscope and counted. The identification was carried out using keys by Day (1967), Pennak (1978), Water and Rivers Commission, (2001) and Merrit and Cummins (1996).

Data analysis

Microsoft excel 2007 was used for graphical illustrations. Means and standard deviation were determined using SPSS version 20.ANOVA was determined to test the significant difference among meansof water quality parameters across stations and between seasons. Multiple linear correlation analysis was carried out on the water quality parameters and benthos to verify if there is any significant relationship between the water quality and benthic fauna composition of River Benue at the study sites.

RESULTS

The result presented in Table 1 is the mean and standard Deviation concentration of physico-chemical parameters in River Benue at Makurdi. The result showed that there was a significant difference in the mean concentration of the physico-chemical parameters across all the station (ANOVA -$P<0.05$) except for surface water temperature, bicarbonate ion, nitrate, sulphate, phosphate and copper that did not vary significantly across the stations (ANOVA-$P>0.05$). However across the station and season, there was significant difference between season and stations for COD and nitrate only.

Figure 2 shows the composition benthic fauna group along River Benue water course at Makurdi. The result indicates that the phylum Arthropoda recorded the highest population among other phyla across the locations during the period of this research. At Coca Cola, there was a slight difference between annelids, Mollusca and Platyhelminthes as was observed at the other four stations in Rivers Benue during the 24 months study period. The result presented in Table 2 indicates that correlation was significant between Arthropoda and turbidity, Platyhelminthes and turbidity, Arthropoda and bicarbonate, DO and Arthropoda; DO and Mollusca, DO and Platyhelminthes, copper and Arthropoda, copper and Mollusca, copper and platyhelminthes only at Coca-cola (Station I) location during the period of this study.

Data presented in Table 3 is the correlation between benthic fauna and physicochemical parameters was

Table 1. Mean concentration of physico-chemical parameters of River Benue at Makurdi.

Parameter	Unit	Sample station codes				
		I	II	III	IV	V
Conductivity	µS/cm	64.69±36.97	124.79±125.52	139.59±215.05	63.95±30.94	70.97±48.57
pH		6.95±0.86	6.90±0.74	6.49±0.87	6.46±0.84	6.33±0.59
TDS	mg/L	35.05±18.18	67.15±68.79	69.14±106.56	28.29±11.69	34.89±27.97
TSS	mg/L	41.00±25.42	87.56±57.39	87.09±91.17	52.17±51.58	44.25±49.75
Colour	TCU	244.54±128.53	393.01±175.73	344.28±157.89	208.07±113.63	192.60±143.79
Turbidity	NTU	46.89±26.66	91.38±56.54	83.47±65.83	49.12±47.22	44.53±44.28
Temperature	^{0}C	28.09±1.97	28.69±1.89	28.96±1.83	28.96±2.11	28.99±1.63
Bicarbonate	mg/L	122.52±57.20	185.61±126.59	182.69±178.63	121.98±59.13	126.66±69.85
Chloride	mg/L	145.19±109.98	173.07±71.27	169.72±82.86	117.44±59.46	138.56±83.32
Nitrate	mg/L	2.79±4.38	3.66±3.08	3.67±5.22	3.76±5.22	2.23±3.14
Sulphate	mg/L	10.95±15.84	16.40±18.98	17.24±15.21	12.55±11.84	10.41±9.84
Phosphate	mg/L	1.21±1.94	1.20±0.81	1.47±2.07	1.25±2.49	0.92±1.11
Copper	mg/L	0.16±0.27	0.31±0.34	0.24±0.28	0.12±0.27	0.11±0.09
DO	mg/L	4.47±2.18	3.28±2.30	3.09±1.71	4.37±2.41	4.42±2.57
BOD	mg/L	1.95±1.35	1.28±0.92	1.21±0.53	1.74±1.38	2.18±1.75
COD	mg/L	4.43±2.91	2.89±2.27	3.16±2.74	4.15±3.23	4.80±4.21

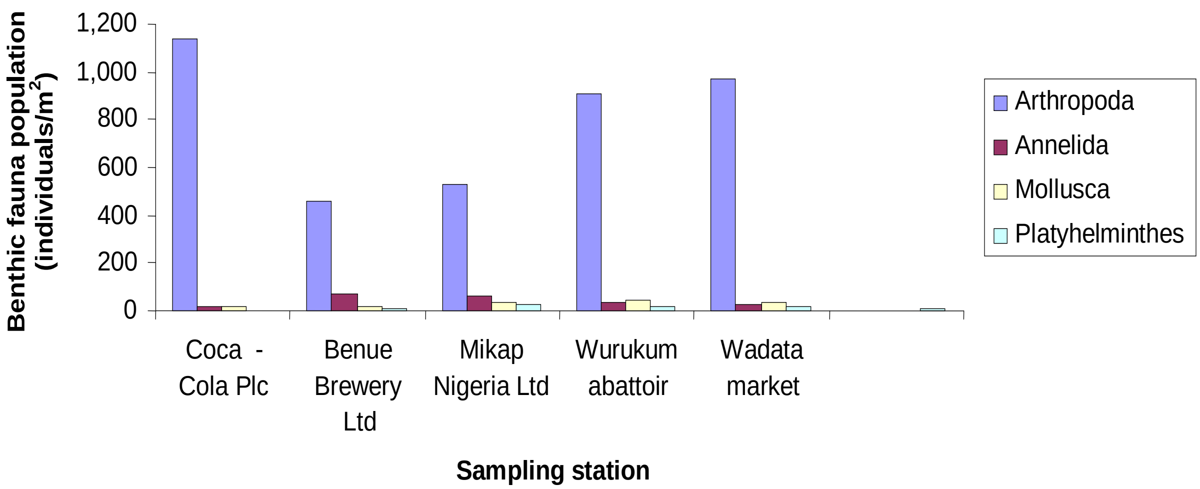

Figure 2. Benthic Fauna group population in River Benue at Makurdi.

deter-mined at Benue Brewery PLC (Station II) and significant with conductivity and Platyhelminthes, phosphate and Arthropoda and copper and Platyhelminthes only. The result in Table 4 showed that at Mikap Nigeria Ltd (Station III), correlation was significant between TDS and Annelida, TDS and Mollusca DO and Annelida, DO and Platyhelminthes only during the period of this study.

The result of correlation analysis between physic-chemical parameters and benthic fauna presented in Table 5 were significant between pH and Annelida, turbidity and Arthropoda, turbidity and Annelida, bicarbonate and Annelida and copper and Annelida at Wurukum abattoir (Station IV).

Table 6 shows the result of the correlation between physico-chemical parameters at Wadata market (StationV) location of River Benue at Makurdi. The result showed that correlation was significant between TSS and Mollusca, turbidity and Arthropoda, turbidity and Mollusca, bicarbonate and Annelida, sulphate and Mollusca, phosphate and Annelida, and copper and Platyhelminthes only, during the period of this research.

During this present investigation, turbidity was determined to correlate negatively with Arthropoda and Platyhelminthes benthic fauna group at Coca-colalocation. Similarly, turbidity was observed to correlate negatively with Arthropoda and positively with Annelida

Table 2. Correlation between environmental variables and Benthic fauna Group at Station I.

Parameter	Arthropoda		Annelida		Mollusca		Platyhelminthes		Sample size(N)
	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	
Conductivity	−0.18	0.39	−0.33	0.11	−0.26	0.21	0.02	0.92	24
pH	0.24	0.26	0.05	0.83	−0.07	0.76	0.16	0.45	24
TDS	−0.07	0.76	−0.28	0.21	−0.19	0.35	0.03	0.87	24
TSS	−0.08	0.71	0.17	0.43	0.05	0.83	0.11	0.59	24
Colour	−0.25	0.24	0.05	0.80	−0.14	0.50	0.19	0.38	24
Turbidity	−0.43**	0.04	0.01	0.96	−0.16	0.44	−0.43**	0.03	24
Temperature	−0.04	0.85	−0.34	0.10	−0.14	0.52	−0.14	0.52	24
Bicarbonate	0.46**	0.02	−0.09	0.66	0.12	0.56	0.37	0.07	24
Chloride	0.15	0.48	0.18	0.38	0.05	0.82	−0.17	0.41	24
DO	−0.57**	0.004	−0.19	0.37	−0.004**	0.98	−0.56**	0.005	24
BOD	−0.39	0.06	−0.21	0.33	0.007	0.75	−0.35	0.09	24
COD	−0.41	0.05	−0.15	0.48	−0.15	0.49	−0.38	0.07	24
Nitrate	−0.27	0.19	0.22	0.31	−0.16	0.45	−0.21	0.33	24
Sulphate	−0.21	0.32	−0.01	0.96	−0.06	0.79	−0.36	0.08	24
Phosphate	0.26	0.22	0.29	0.17	0.16	0.44	−0.05	0.83	24
Copper	0.02	0.47*	0.19	0.36	0.54**	0.007	0.03	0.44*	24

**Correlation is significant at the 0.01 level (2- tailed); * Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation between environmental variables and Benthic fauna Group at Station II.

Parameter	Arthropoda		Annelida		Mollusca		Platyhelminthes		Sample size(N)
	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	
Conductivity	−0.36	0.08	0.21	0.24	0.67	0.04	0.61**	0.001	24
pH	−0.13	0.54	0.25	0.23	0.03	0.88	0.23	0.28	24
TDS	0.29	0.17	−0.27	0.20	0.27	0.21	0.59**	0.003	24
TSS	−0.13	0.53	−0.03	0.91	0.06	0.76	0.04	0.42*	24
Colour	0.22	0.29	−0.05	0.81	0.24	0.26	0.05	0.41*	24
Turbidity	0.00	0.99	−0.21	0.31	−0.02	0.92	0.33	0.11	24
Temperature	0.38	0.07	0.06	0.78	0.08	0.72	0.37	0.71	24
Bicarbonate	0.36	0.09	−0.14	0.51	0.15	0.47	0.13	0.55	24
Chloride	0.05	0.83	0.28	0.19	0.11	0.60	0.05	0.83	24
DO	−0.32	0.12	0.08	0.71	−0.15	0.48	−0.23	0.29	24
BOD	−0.27	0.21	0.08	0.72	0.02	0.92	−0.12	0.58	24
COD	−0.22	−0.11	0.11	0.62	0.04	0.84	−0.09	0.66	24
Nitrate	−0.16	0.44	−0.18	0.40	0.03	0.89	−0.14	0.50	24
Sulphate	−0.15	0.46	0.13	0.53	−0.04	0.85	0.43	0.87	24
Phosphate	0.04	0.43*	0.39	0.18	0.99	0.001	0.87	0.03	24
Copper	0.59	0.12	0.82	0.05	0.22	0.30	0.04	0.43*	24

** Correlation is significant at the 0.01 level (2- tailed)* Correlation is significant at the 0.05 level (2-tailed).

at Wurukum abattoir. At Wadata market location, turbidity was determined to correlate positively with Arthropoda and Mollusca during the 24 months study period. The negative significant correlation of benthic fauna group observed at Coca-cola and Wurukum abattoir might be due to the unidentified interactions of certain factors operating at these areas.The locations where no significant correlations were observed may be attributed to the fact that at these locations, the environmental factors were interacting with the benthic community. The finding of this work agrees with the work of Ishaq and Khan (2013) that reported a negative significant relationship between turbidity and benthic fauna groups in River Yamun. Significant positive correlations were

Table 4. Correlation between environmental variables and Benthic fauna Group at Station III.

Parameter	Arthropoda		Annelida		Mollusca		Platyhelminthes		Sample size(N)
	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	
Conductivity	−0.24	0.25	0.08	0.70	−0.13	0.54	0.11	0.62	24
pH	−0.16	0.46	0.09	0.71	0.19	0.36	0.08	0.70	24
TDS	−0.12	0.57	0.77**	0.56	0.80**	0.84	0.40	0.05	24
TSS	0.18	0.40	0.12	0.52	0.22	0.30	0.09	0.67	24
Colour	0.09	0.68	0.06	0.79	0.29	0.17	0.11	0.60	24
Turbidity	−0.11	0.61	−0.26	0.21	0.05	0.81	0.11	0.59	24
Temperature	−0.23	0.28	0.07	0.74	−0.21	0.31	0.12	0.57	24
Bicarbonate	0.13	0.54	0.39	0.05	−0.01	0.96	0.32	0.13	24
Chloride	0.36	0.08	0.21	0.22	0.31	0.14	0.07	0.76	24
DO	−0.31	0.13	0.02	−0.48*	−0.09	0.65	0.13	0.43*	24
BOD	−0.04	0.82	−0.32	0.12	0.05	0.81	−0.21	0.32	24
COD	−0.01	0.95	−0.17	0.42	0.03	0.88	−0.13	0.53	24
Nitrate	−0.05	0.81	−0.08	0.69	0.21	0.33	−0.05	0.81	24
Sulphate	−0.15	0.48	−0.27	0.19	−0.11	0.62	−0.18	0.40	24
Phosphate	0.08	0.71	−0.10	0.63	0.03	0.88	0.10	0.64	24
Copper	0.22	0.31	0.30	0.15	0.27	0.19	0.30	0.15	24

** Correlation is significant at the 0.01 level (2- tailed); * Correlation is significant at the 0.05 level (2-tailed).

Table 5. Correlation between environmental variables and Benthic fauna Group at Station IV.

Parameter	Arthropoda		Annelida		Mollusca		Platyhelminthes		Sample Size(N)
	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	
Conductivity	0.19	0.36	0.33	0.11	0.16	0.44	0.11	0.60	24
pH	0.12	0.57	0.05	0.41*	0.21	0.33	0.21	0.33	24
TDS	0.11	0.61	0.19	0.37	0.08	0.69	−0.26	0.22	24
TSS	−0.26	0.21	0.30	0.15	−0.06	0.77	0.30	0.15	24
Colour	−0.12	0.57	−0.27	−0.19	−0.02	0.93	0.05	0.80	24
Turbidity	0.04	−0.43*	0.04	0.41*	0.18	0.41	0.18	0.41	24
Temperature	0.02	0.91	−0.05	0.80	0.09	0.69	−0.20	0.35	24
Bicarbonate	0.05	0.83	0.02	0.47*	0.04	0.87	0.07	0.74	24
Chloride	0.24	0.26	0.29	0.16	0.17	0.42	0.09	0.76	24
DO	0.03	0.89	0.20	0.34	0.36	0.08	0.06	0.79	24
BOD	−0.05	−0.82	−0.03	0.89	−0.12	0.55	0.05	0.82	24
COD	0.08	0.69	0.07	0.75	0.08	0.72	0.05	0.80	24
Nitrate	−0.32	0.13	0.22	0.29	0.13	0.55	−0.04	0.84	24
Sulphate	−0.17	0.42	0.33	0.12	−0.11	0.58	−0.20	0.30	24
Phosphate	0.11	0.62	0.35	0.09	0.31	0.13	0.06	0.78	24
Copper	0.34	0.10	0.13	0.50*	0.24	0.26	0.03	0.88	24

** Correlation is significant at the 0.01 level (2- tailed); * Correlation is significant at the 0.05 level (2-tailed).

noticed between bicarbonate and Arthropoda benthic fauna and Annelida at Coca-cola and Wurukum abattoir, respectively, during the present study. This result disagrees with the result of an earlier study that reported a negative significant correlation between bicarbonate and Annelida and Mollusca (Sharma and Chowdhary, 2011). However, this finding agrees with the result of a study in River Tawi India that reported positive significant correlations between bicarbonate and Arthropoda and Annelida (Mohan et al., 2013). Correlation coefficient for DO was found to be negative with Arthropoda, Mollusca and Platyhelminthes at Coca-cola during the present study. A similar result was obtained at Mikap Nigeria Ltd location where DO correlated negatively with Annelida and positively with Platyhelminthes.However significant correlation in DO was not observed in the surface

Table 6. Correlation between environmental variables and Benthic fauna Group at Station V.

Parameter	Arthropoda		Annelida		Mollusca		Platyhelminthes		Sample Size(N)
	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	
Conductivity	−0.19	0.36	−0.03	0.88	0.12	0.56	0.02	0.93	24
pH	−0.03	0.88	0.03	0.89	0.20	0.35	0.21	0.33	24
TDS	−0.19	0.38	−0.18	0.41	0.06	0.77	0.19	0.37	24
TSS	−0.25	0.24	−0.37	0.08	0.04	0.43*	−0.04	0.85	24
Colour	−0.08	0.72	−0.32	0.13	−0.30	0.15	0.03	0.87	24
Turbidity	0.02	0.46*	−0.26	0.21	0.56**	0.05	0.12	0.58	24
Temperature	−0.05	0.80	−0.06	0.79	−0.07	0.76	0.19	0.38	24
Bicarbonate	−0.11	0.61	0.04	0.42*	−0.14	0.51	0.18	0.41	24
Chloride	0.07	0.76	0.06	0.79	0.40	0.05	−0.01	0.95	24
DO	−0.22	0.29	−0.05	0.79	−0.14	0.53	−0.03	0.89	24
BOD	0.02	0.91	0.05	0.81	0.04	0.86	−0.007	−0.97	24
COD	−0.01	0.95	0.04	0.36	0.09	0.68	−0.05	0.94	24
Nitrate	−0.28	0.17	0.12	0.56	0.36	0.08	−0.05	0.83	24
Sulphate	−0.08	0.69	0.07	0.75	0.03	−0.44*	−0.11	0.60	24
Phosphate	0.14	0.52	0.01	0.50*	0.23	0.25	−0.27	0.21	24
Copper	−0.13	0.53	0.01	0.96	0.97	0.007	0.01	0.49*	24

**Correlation is significant at the 0.01 level (2- tailed); *Correlation is significant at the 0.05 level (2-tailed).

waters at Benue brewery, Wurukum abattoir and Wadata market locations. This may be due factors contributing to the correlation of DO and benthic fauna. The result of this study disagrees with the result of an earlier study in Himalayan River that did not show any significant correlation between DO and Annelida, Arthropoda, and Mollusca (Sharma and Chowdhary, 2011). All the same, this result agrees with the findings ofan earlier study that reported positive and negative significant correlations between DO and benthic fauna (Mohan et al., 2013). Similarly, Chowdhary et al. (2013) reported a significant correlation between DO and Arthropoda, Mollusca and Annelida across the study stations in River Tawi within the vicinity of Jammu city India. This result is in agreement with the findings of this present investigation. A significant positive correlation was noticed between copper and Arthropoda, Mollusca and Platyhelminthes at Coca-cola locations during this study. A similar result was observed at Wurukum abattoir location where copper positively correlated with Annelida. All the same, no significant correlation was observed at Benue Brewery; Mikap Nigeria Ltd and Wadata market. This would be that other factors and not copper is impacting on the benthic fauna at these locations. Across the locations except at Benue Brewery, there was a positive correlation between conductivity and Platyhelminthes benthic fauna in River Benue. This result may be due other factors responsible for the benthic fauna group population, as a result of lack of correlations between benthic fauna and conductivity at these locations. This result disagrees with the result of an earlier study that showed a positive significant correlation between conductivity and Arthropoda (Ishaq and Khan, 2013).A strong positive significant correlation was observed between total dissolved solids (TDS) and Platyhelminthes at Benue Brewery. Similarly, strong positive significant correlation was noticed between TDS and Annelida and Mollusca at Mikap Nigeria Ltd. No significant correlation was observed between TDS and benthic fauna at the other three locations. The lack of significant correlations between TDS and benthic fauna at these locations may be attributed to other unidentified factors interacting with benthic fauna at these locations and not TDS. This finding disagrees with the result of a study that showed a negative significant correlation between TDS and Arthropoda (Ishaq and Khan, 2013).A positive significant correlation was observed between total suspended solids (TSS) and Platyhelminthes and between TSS and Mollusca respectively at Benue brewery and Wadata market locations of River Benue during this study. All the other locations did not record a significant relationship with TSS and benthic fauna group. Ishaq and Khan (2013) reported a significant negative correlation between TSS and Arthropoda in their study that disagrees with the result of this research. Bilotta and Brazier (2008) reported that an elevated TSS in surface waters is associated with benthic drift. This is evident in the positive correlation between TSS and benthic fauna during the course of this study. The colour of the waters was observed to positively correlate significantly with Platyhelminthes at Benue brewery location only, throughout the study period. There was no significant correlation at all the other locations. The poor colour of effluents discharged into the river Benue at this location may be responsible for the significant relationship.

Phosphate during this present investigation was observed to positively correlate significantly with Arthropoda at Benue Brewery and Annelida at Wadata market. This result disagrees with the finding of studies that reported negative significant correlation between phosphate and benthic fauna in Rivers (Ishaq and Khan, 2013; Mohanet al., 2013). Correlation coefficient for pH was observed to be positively significant with Annelida at Wurukum abattoir only throughout the 24 months period of this research. However, such a clear tendency in correlations of pH and benthic locations at River Benue was not noticed in the other locations. The significant correlation showed that the pH of the water was interacting with Annelida at Wadata market. This result disagrees with the finding of a study that reported pH not to be significantly correlating with any benthic group in River Tawi India (Sharma and Chowdhary, 2013). All the same, the findings of this research agree with the result of an earlier study that reported a significant positive correlation between pH and Annelida in a River system in India (Mohan et al., 2013). Throughout the period of this research, sulphate was observed to be negatively correlated with Mollusca at Wadata market. This assertion agrees with the report of a study that showed a negative significant correlation between sulphate and Mollusca (Mohan et al., 2013).

However, during this present study, surface water temperature, chloride, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and nitrate did not correlate significantly with any of the benthic fauna group throughout the study period. All the same, other studies reported significant correlation between surface water temperature, nitrate, chloride, BOD, COD and benthic fauna group (Ishaq and Khan, 2013; Mohan et al., 2013). The result of this study agrees with the findings of an earlier study that reported no significant correlation between chloride and benthic fauna group (Annelida, Arthropoda and Mollusca) in Himalayan River India (Sharma and Chowdhary, 2011). The lack of significant correlation between surface water temperature, chloride, nitrate, BOD, COD and benthic fauna during this study may be due to other environmental variables interacting with the benthic fauna groups.

Conclusion

Correlation studies of benthic fauna diversity with environmental variables revealed that relationship between benthic fauna and physico-chemical parameters are highly complex and mostly controlled by unidentified interaction of different factors.All the groups (Arthropoda, Annelida, Mollusca and Platyhelminths) of benthic fauna were noticed to correlate positively with certain Environmental parameters, while other benthic fauna correlated negatively with environmental parameters. However no correlation was observed between the environmental parameters and Annelida at Stations I and II throughout the period. Similarly, Mollusca did not correlate with any environmental parameter at Stations II and IV during the study period. More so, Platyhelmiths did not show any correlation with any of the environmental parameters during the time of the study. Those that were negatively correlated may be utilized as indicator species or groups for identifying the ecological status of the River Benue. However, there was no significant correlation between benthic fauna and surface water temperature, chloride, BOD, COD and nitrate throughout the period of this study. The result of this study showed that, River Benue at Makurdi is polluted along its course. This poses a health risk to humans who rely on the river as the primary source of domestic water supply without adequate treatment and the aquatic biota.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Assessment of indigenous *Bradyrhizobia* spp. population levels in low phosphorus soils of southern Cameroon using cowpea (*Vigna unguiculata* L. Walp) as trap crop

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This study used three cowpea (*Vigna unguiculata* L. Walp) varieties, (DschMMBr, Vyuniebe and 58-77) as trap crops to estimate the population of indigenous *Bradyrhizobia* spp. Soil samples were collected in two sites (Nkoemvone and Nkometou) of Southern Cameroon known to accommodate acid soils with low phosphorus (P) levels, and the population of the indigenous rhizobia was determined using the most probable number (MPN) plant infection technique. The results of the MPN counts indicated that the total *Bradyrhizobia* population in Nkoemvone was between 1.0 and 5.8 × 10^5 cells per gram of soil sample while in Nkometou, it was between 5.8 x10^3 and 1.0 x10^4 cells per gram of soil sample. Using the cowpea variety DschMMBr as trap crop, the *Bradyrhizobium* spp. population estimate was 5.8 x10^3 and 1.0 x 10^5 cells per gram of soil sample, in Nkometou and Nkoemvone, respectively. When the trap crop was changed to Vyuniebe, the population estimate remained the same in Nkometou but substantially increased (3.1 x 10^5 cells per gram of soil sample) in Nkoemvone. Using the variety 58-77 in Nkometou, a *Bradyrhizobium* spp. population size of 1.0 x10^4 cells per gram of soil sample was estimated whereas this population was 5.8 x 10^5 in Nkoemvone. Overall, population sizes of rhizobia using the three varieties were higher in Nkoemvone soil than in Nkometou soil. The cowpea variety 58-77 seemed to have high nitrogen fixation potentials as it formed nodules more than the two other varieties in both soils. The levels of *Bradyrhizobia* populations observed in the two sites were adequate to give satisfactory results on nodulation and nitrogen fixation. This suggests that cowpea production in southern Cameroon does not require inoculation.

Key words: *Bradyrhizobia* spp., *Vigna unguiculata*, nodulation, most probable number, low P soils, Southern Cameroon.

INTRODUCTION

In the soil, microbes constitute a large portion of the biodiversity (Fortin et al., 2008) and their activity can influence a number of important ecosystem processes, including nitrogen and carbon cycling (Fortin et al., 2008; van der Heijden et al., 2008), soil formation (Rillig and Mummey, 2006), plants nutrient acquisition and productivity (Dommergues et al., 1999; Sene et al., 2010). Interactions between plants and microbes are particularly important since plants represent the main pathway through which element that severely limits microbial growth, enters the soil (Kahindi et al., 1997; van der Heijden et al., 2006; van der Heijden et al., 2008).

Among plant-microbe interactions, the legume-rhizobia symbiosis that converts nitrogen gas (N_2) into ammonia is probably the best studied (Kahindi et al., 1997; Mwend et al., 2011). Legume-nodulating rhizobia play a great role in maintaining soil fertility (Kahindi et al., 1997). However, effective nitrogen fixation in legumes depends on many factors (Voisin et al., 2003, 2007) including the presence of effective and abundant rhizobia in the soil (Giller, 2001; FAO, 1984). These rhizobia can either be indigenous or applied as inoculum.

Legume hosts differ in the range of partners with which they form symbioses. Some legumes nodulate with a restricted number of rhizobial strains or species while others nodulate with a wide range of fast- and slow-growing rhizobia (Maingi et al., 2006). In addition, factors such as high soil temperature (Giller, 2001), nutrient deficiencies (Beck and Munns, 1984; Watkin et al., 1997; O'Hara, 2001), low levels of soil moisture (Boonkerd and Weaver, 1982), low pH (<5.5), low clay and organic matter (Dudeja and Khurana, 1989; De Mallaro and Izaguirre, 1994) adversely affect rhizobial survival. Consequently, soils varying in their fertility status will respond differently to rhizobial inoculation.

On the other hand, legume hosts also differ in their response to inoculation. For instance, studies have shown that cowpea (*Vigna unguiculata* L.Walp) is a very promiscuous legume host as it has rarely been found to respond to inoculation unless when grown in a soil where the conditions are not conducive for the survival of rhizobia (Ahmad et al., 1981; RangaRao et al., 1985; Giller, 2001). Moreover, from a practical point of view, the use of inoculants is also cumbersome and difficult to exploit by farmers (Hornetz et al., 2000; Kaleem, 2002) who face problems in acquiring and storing inoculants because cooling facilities are not readily available. As a result, cowpea crops as are grown by farmers in Africa and receive no inoculants and little or no commercial nitrogen fertilizer.

To reduce the need for inoculation, legume varieties that can be nodulated by indigenous rhizobia have to be selected for farmer use. As a prerequisite of this selection process, an assessment of the indigenous rhizobial population levels in low phosphorus soils of southern Cameroon using cowpea as trap crop was undertaken: (1) to measure the soil richness of cowpea-nodulating rhizobia in the humid forest zone of Cameroon and (2) to determine the impact of pH and soil P levels on the abundance of this B*radyrhizobia* population and (3) to identify the best variety to use as a trap crop.

MATERIALS AND METHODS

Study site description

Soil samples used in this experiment originated from two sites. The first site is located at the Institute of Agricultural Research for Development (IRAD) experimental station in Nkoemvone, in the South region of Cameroon. The second site belonging to a farmer is located at Nkometou, one of the benchmark villages of the International Institute of Tropical Agricultural (IITA), the central region of Cameroon. Both regions are part of the humid forest zone of Cameroon.

The agro-ecological characteristics of the two locations are presented in Table 1. The IRAD experimental field at Nkoemvone has a past history of trials involving maize (*Zea mays* L.), cowpea and mucuna (*Mucuna pruriens* L.) rotation. The rotatory trails ended in 2004 and since then the field has been cultivated with maize or left un fallow. The dominant species while on fallow was the elephant grass (*Pennisetum purpureum* Schumach). The farmer's field at Nkometou was left unfallowed for 6 years with siam weed (*Chromolena odorata* (L.) R. King & H. Robinson) as domi-nate species; then cassava (*Manihot esculenta* Crantz) and groun-dnuts (*Arachis hypogaea*Linn.) cultivation followed for 2 years (March 2010- December 2011); then maize for two seasons (April - July and September – December) in 2012. Soil samples were collected in December 2012 after the maize was harvested.

Soil sampling

Each study site was divided into 10 sub-sections based on the vegetation, topography and cropping and tillage practices (compact and non compact soils). Ten soil sub-samples were collected from each sub-section in the two study sites, Nkoemvone in the south and Nkometou in the center region. Prior to sampling, surface debris were removed. After clearance of debris from the surface, the soil core was removed at 30 cm depth with a soil auger (Abaidoo et al., 2002). The 10 samples from each sub-section were then bulked, homogenized, sieved (<2 mm), and divided into two parts. Two composite samples were then produced from bulked samples from all the 10 subsections. One composite sample was air-dried for chemical analyses in the biochemical laboratory in the International Institute of tropical Agriculture (IITA) and the second was stored at 4°C in clean paper bags for microbiological analyses.

Soil analysis

Soil pH (Table 2) was determined in water on 1:1 soil/water ratio (IITA, 1982). Organic carbon was determined by chromic acid digestion (Heanes, 1984) and total N was determined using auto-analyser (Bremner and Mulvaney, 1982). Available P was deter-mined using Mehlich-3 extraction method (Mehlich, 1984). Cation exchange capacity was determined by saturation with 1 N ammo-nium acetate and extraction of ammonium with 2 M potassium chloride (TSBF, 1993). Exchangeable acidity was determined by titration method after extraction with 1 N KCl (Anderson and Ingram, 1993) while ECEC was determined by summation of exchangeable cations and exchangeable acidity. Soil particle size (Table 3) analysis was done by the hydrometer method (Bouyoucos, 1951).

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Table 1. The summary of agro-ecological characteristics of the two locations.

Parameter	Nkometou	Nkoemvone
*AEZ	Zone V	Zone V
Longitude	11°15' E	11º20' E
Latitude	3°62'N	2º90'N
Altitude	700(masl)	560(masl)
Climate	Equatorial	Equatorial
Annual Rainfal1	1600 mm	1692,2 mm
Rainfall pattern	Bimodal	Bimodal
Temperature:		
Mean Minimum	19.2°C	19.7°C
Mean Maximum	26.6°C	29.1°C
Dry months:		
Long	Mid DEC-Mid MAR	Mid DEC-Mid MAR
Short	Mid JUN-Mid AUG	Mid JUN-Mid AUG
Rainy season:		
First	Late March –Early June	Late March –Early June
Second	Early Sept – Early Dec	Early Sept – Early Dec
Vegetation	Degraded Tropical Rainforest	Tropical RainForest
Soil type:		
FAO	Ferric Acrisols	Ferric Acrisols
USDA	Rhodic Kandiudult	RhodicKandiudult

*AEZ = Agro-ecological zones of Cameroon; Zone V = humid forest with bimodal rainfall; masl = meters above sea level. Adapted from ICRAF, 1993

Table 2. Soil chemical properties of the study sites.

Characteristic	Site	
	Nkoemvone	Nkometou
Soil depth	0-20cm	0-20cm
pH (water)	4.52	5.49
Ca cmol(+) /kg	0.58	1.92
Mg cmol(+) /kg	0.32	1.30
K cmol(+) /kg	0.18	0.13
Na cmol(+) /kg	0.28	0.29
Al cmol(+) /kg	2.33	0.40
CEC cmol(+) /kg	8.07	7.27
P ppm or ug/g	7.51	3.07
Mn ppm or ug/g	1.26	123.20
Fe ppm or ug/g	106.00	65.70
Org C (%)	1.73	1.69
Total N (%)	0.12	0.12
C/N	13.92	14.32
Oxalate extractable Fe (ppm or ug/g)	1976.60	1418.91

Sterilization and pre-germination of seeds

Seeds of cowpea varieties, DschMMBr, Vyuniebe and 58-77 were used. DschMMBr and Vyuniebe are local varieties grown in the western and northern regions of Cameroon, respectively, while the variety 58-77 was provided by the Senegalese Agricultural

Table 3. Soil physical properties of the study sites.

Site	Sand	Clay	Silt	textural class	bulk density	*Field capacity*
	%	%	%		Mg/m^3	cm^3 water/cm^3 soil
Nkoemvone	42.25	46.38	11.37	Clay	1.29	0.360
Nkometou	53.32	34.26	12.42	Sandy clay loam	1.35	0.290

Research Institute *(*ISRA*).* These varieties were chosen because they nodulated well with soils from both sites in a previous experiment (Atemkeng, unpublished data). Cowpea seeds were collected, pre-screened and purified in pots. Seeds of good viability (with a germination percentage higher than 80%), undamaged and of uniform colour and size were selected (Maingi et al., 1999). One hundred seeds of each variety were surface sterilized by immersing them into a 3% solution of sodium hypochlorite for 5-10 min. The solution of sodium hypochlorite was prepared by adding 10 parts of commercial bleach (5.25% sodium hypochlorite) to 7.5 parts of water. The seeds were rinsed 8 times with sterile distilled water after surface sterilization. They were then soaked in clean sterile distilled water and allowed to imbibe it for one hour. They were transferred aseptically to 2% water agar plates with a spoon-shaped spatula. Twenty five seeds were placed in each plate. The plates with the seeds were incubated upside down at 2°C to enable the radicles to grow away from the water agar. The incubation period was four days. Seedlings whose radicles attained a length of 1-2 cm after the incubation period were considered ready for transferring to glass tubes.

Plant growth medium and inoculation

The growth medium, used in this study was a mixture of sterile black soil and sand (3:1) sterilized in an autoclave at 121°C for 15 min. Five nitrogen-free stock solutions was prepared as described by Beck et al. (1993). For each litre of full-strength plant growth solution, 0.5 ml was added from each of the five stock solutions. The pH of the solution was adjusted to 6.8 using NaOH (1.0 M) or HCL (1.0 M). All solutions were sterilized by autoclaving at 121°C for 15 min. To prepare the inocula, whole soil inocula were diluted to 10^{-1} by suspending 10 g of each soil sample in 90 ml of sterile water and then shaking for 15 to 20 min with a wrist shaker. Serial dilutions were made to give from 10^{-1} to 10^{-10}. An aliquot of 1 ml of diluent was used to inoculate pre-germinated, surface-disinfected cowpea seedlings grown in glass tubes. Four tubes were inoculated with each dilution. Uninoculated control plants were included for each dilution to determine if cross contamination of tubes occurred. The growth tubes in rackholders were transferred to a growth chamber in the Regional Biocontrol Laboratory at IRAD Yaounde, Cameroon where the temperature was maintained at 30°C with a 14-h photoperiod provided by fluorescent lighting. Sterile water was added as required through sterile straws. The racks were positioned 60 cm apart in the growth chamber in a completely randomized design. Application and regular checking of levels of nitrogen-free nutrient solution was done on daily basis to ensure that the seedlings were adequately moistened.

Plant infection counts

The rhizobial populations in each soil were estimated using the most probable number technique as described by Somasegaran and Hoben (1994). Cowpea was used as the trap host to check for the abundance of indigenous cross nodulating *Bradyrhizobium* spp. Data were collected 8 weeks after planting. At harvest, the stems of the plants were cut at the level of the growth medium. The plants in the glass tubes were scored for the presence or absence of nodules. The roots were carefully washed using a gentle stream of water to remove sand, taking care not to destroy the roots and nodules. Nodulation was observed (+, for nodulation or - for no nodulation) and the number of nodulated (+) plants units was recorded beside each dilution. The presence of a single nodule in a tube was considered a positive score. The total number of nodulated units was obtained by summing up the nodulated units at each dilution level. Uninoculated controls were used to check for sterile conditions.

The MPN was calculated from the most likely number (m) obtained from the MPN tables according to the formula:

$$MPN = (m \times d)/v$$

where: *m* is the most likely number from MPN tables, *d* is the lowest dilution in the series and *v* is the aliquot used for inoculation (Somasegaran and Hoben, 1994).

RESULTS AND DISCUSSION

The most probable number technique based on plant infection count is commonly used to estimate numbers of rhizobia in soil or to determine the quality of inoculants produced in sterile conditions (Beck et al., 1993; Somasegaran and Hoben, 1994). Empirical models have been used to describe the response to inoculation of legumes (Thies et al., 1991). These models indicate that population density as estimated by the MPN- plant infection assay is one of the primary factors determining the magnitude of legume response to indigenous soil rhizobia. This is one of the main reasons why the *Bradyrhizobia* populations had to be determined in the two field sites before evaluating cowpea for nitrogen fixation related traits.

In this study, the cowpea varieties used formed nodules following inoculation with serial dilutions of soils from the two study sites. However, there were variations in the number of nodulated units per variety and per study site. The estimated total *Bradyrhizobium* spp. population in Nkoemvone (Table 4) soil ranged between 1.0 and 5.8×10^5 cells per gram of soil sample while the population size at Nkometou (Table 5) was between 5.8×10^3 and 1.0×10^4 cells per gram of soil sample. Using the cowpea variety DschMMBr as trap crop, the *Bradyrhizobium* spp population estimated was 5.8×10^3 and 1.0×10^5 cells per gram of soil sample, respectively, in Nkometou and Nkoemvone soils. The population estimate was the same in Nkometou but increased in Nkoemvone when the trap

Table 4. Nodulated units planted with three cowpea varieties with inocula from Nkoemvone soil.

Serial dilution	Number of nodulated units by each variety		
	Dsch MMBr	Vyu niebe	58-77
10^{-1}	4	4	4
10^{-2}	4	4	4
10^{-3}	3	4	4
10^{-4}	3	3	3
10^{-5}	2	2	3
10^{-6}	2	2	2
10^{-7}	1	1	1
10^{-8}	0	1	1
10^{-9}	0	0	0
10^{-10}	0	0	0
control	0	0	0
Total	19	21	22
MPN (cells per gram of soil sample)	$1.0 X 10^5$	$3.1 X 10^5$	$5.8 X 10^5$

Number of replications, n = 4; dilution steps, s = 10; number of nodulated units, (+) = 19; 21; and 22. Lowest dilution in the series, d = 10^{-1}; v = 1 ml.

Table 5. Nodulated units planted with three cowpea varieties with inocula from Nkometou soil.

Serial dilution	Number of nodulated units by each variety		
	Dsch MMBr	Vyu niebe	58-77
10^{-1}	4	4	4
10^{-2}	3	4	4
10^{-3}	2	2	3
10^{-4}	2	2	2
10^{-5}	2	1	1
10^{-6}	1	1	1
10^{-7}	0	0	0
10^{-8}	0	0	0
10^{-9}	0	0	0
10^{-10}	0	0	0
control	0	0	0
Total	14	14	15
MPN (cells per gram of soil sample)	5.8×10^3	5.8×10^3	1.0×10^4

Number of replications, n = 4; dilution steps, s = 10; number of nodulated units, (+) = 14; 14; 15. Lowest dilution in the series, d = 10^{-1}; v = 1 ml.

crop was changed to Vyuniebe. A population of 5.8×10^3 and 3.1×10^5cells per gram of soil sample was recorded in Nkometou and Nkoemvone, respectively. The cowpea variety, 58-77 seemed to have higher nitrogen fixation potentials as it formed nodules more than the two other varieties in both soils. Indeed, using this variety, a *Bradyrhizobium* spp population size of 1.0×10^4 cells per gram of soil sample was estimated in Nkometou as compared to 5.8×10^5 in Nkoemvone. Similar high rhizobia populations of 4.9×10^2, 3.5×10^3 and 4.3×10^4 cells g-1 of soil were recorded from three soils in West Africa (Ahmad et al., 1981). However, the cropping history of the soils was not provided.

Generally, population sizes of rhizobia using the three varieties were higher in Nkoemvone soil than in Nkometou soil. These results showed that the levels of *Bradyrhizobia* populations recorded in the two study sites were adequate to give satisfactory outputs on nodulation and nitrogen fixation without inoculation. This is in agreement with earlier findings by Nambiar et al.(1983)

where it was shown that most cultivated tropical soils have a rhizobial population of more than 100 rhizobia cells per gram of soil capable of nodulating the legumes grown on such soils. More so, in soils where naturalized rhizobial populations are high (>10^3 rhizobium bacteria per g soil), introduction of new strains can be difficult and often unsuccessful (Thies et al., 1991; Brockwell et al., 1995).

The Nkoemvone site had higher *Bradyrhizobia* populations in all the categories than the Nkometou site. This difference could be linked to the cropping history of both sites as well as the soil physico-chemical properties especially the pH and soil P level. Both soils had been cultivated with legumes in the past. However, while cowpea had been cultivated at Nkoemvone, the site at Nkometou had only been cultivated with groundnut that is known to be nodulated only by a subgroup of *Bradyrhizobium* spp. (Yousef et al., 1987). Legumes serve to maintain rhizobia in the soil through rhizosphere effects and senescence of nodules. Studies by Woomer et al. (1988) and Yousef et al. (1987) highlighted the effects of legumes on rhizobial populations. They reported that groundnut rhizobia which prefer a pH ranging from 7.6 to 8.1, are not favored by increasing soil organic carbon over 1%. In our study, soil samples collected in Nkometou displayed low pH (5.49) and organic C content above 1% (1.69%). This implies that the survival of *Bradyrhizobium* spp. that nodulate groundnut in Nkometou was hampered as is evident by the lower soil rhizobia population estimated. Soil P in Nkometou was also very low (3.07 ppm) as compared to Nkoemvone (7.51 ppm). Since cowpea is more tolerant to phosphorus deficiency than are most grain legumes (Alkama et al., 2008), the growth, nodulation and rhizobia survival in previous experiments with cowpea in Nkoemvone may have been less affected as was groundnuts in Nkometou soil with very low P. The fact that cowpea could be nodulated by soil dilutions from both sites corroborated the idea that cowpea is a promiscuous legume, and rhizobia are facultative symbionts which in the sapro-phytic state, are independent of their host legumes (Woomer et al., 1988).

Conclusion

Bradyrhizobium spp. also referred to as cowpea miscellany was detected in the soils from the two study sites. However, the numbers varied per site and depended on the trap crop used. Good infectivity was observed using the cowpea variety 58-77 in this study. This implies that this variety may have high nitrogen fixation potential and can be cultivated in southern Cameroon to improve soil fertility. From this study, the rhizobia population from both sites was above 10^3 cells per gram of soil. This strongly suggests that cowpea production in southern Cameroon does not require inoculation. The low soil pH and P status can even aggravate the negative effects of inoculating cowpea in this zone. This knowledge of indigenous bradyrhizobia populations in Southern Cameroon will be very valuable for developing strategies to improve biological nitrogen fixation (BNF) for increasing cowpea yields at low costs since inoculation is proven not to be necessary.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Impacts of different drains on the seawater quality of El-Mex bay (Alexandria, Egypt)

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El Mex Bay area received domestic, industrial and agricultural waste water from the surrounding area. The need to pay much attention to the potential effects of anthropogenic inputs upon the hydrochemical characteristics of the bay is an emergency. Several water samples have been analyzed for physico-chemical characteristics during 2010/2011: Seven samples from El Mex bay and 4 samples from drains. The study revealed that pH, salinity, dissolved oxygen, oxidizable organic matter, biological oxygen demand, and chlorophylls-a,b,c, were in the ranges 7.16-8.97; 0.3–42.04; ND–22.26 mg/L; 0.4–112.00 mg O_2 /L; 3.23–111.29 mg/L; 0.11–241.91 µg/L; 0.03-242.78 µg/L and 0.01-239.55 µg/L, respectively. The increasing organic supply introduced into the studied restricted area characterized by its relative slow rate of self-purification results in elevation of OOM compared to previous studies. The ranges of nutrients (µM/L) were: 0.15-2815.85, 0.28–22.85, 0.2–58.42, 0.25-30.45 and 0.11-40.23 for ammonia, nitrite, nitrate, reactive phosphate and reactive silicate, respectively. Principle Component Analysis (PCA) which was used to develop water quality index (WQI) revealed that El-Dekhila Head and El Qalaa drain were the most polluted sampling sites that resulted from the disposal of industrial, agricultural and domestic wastes. Depending on DIN/DIP ratio of seawater for El-Mex Bay and adjacent drains the majority of the investigated area sums to be P-limited.

Key words: Seawater quality,El-Mex bay, drains, Alexandria, Egypt, nutrient salts, hydrographic characteristics.

INTRODUCTION

The strategic action plan for Mediterranean (EEAA, 2009) has identified several "hot spots and sensitive areas" on the northern coast of Egypt, which for several decades have been experiencing a continuous increase in population, development and environmental degradation (Shreadah et al., 2006, 2011; Shobier et al., 2011; Shadia et al., 2012; Abdel Ghani et al., 2013). El-Mex Bay which is located west of Alexandria City is an industrial zone. It receives huge amounts of untreated industrial wastes as a consequence of growing heavy industries (petrochemicals, pulp metal planting, industrial dyes, and textiles) and the uncontrolled disposal of the resulting wastewater transports

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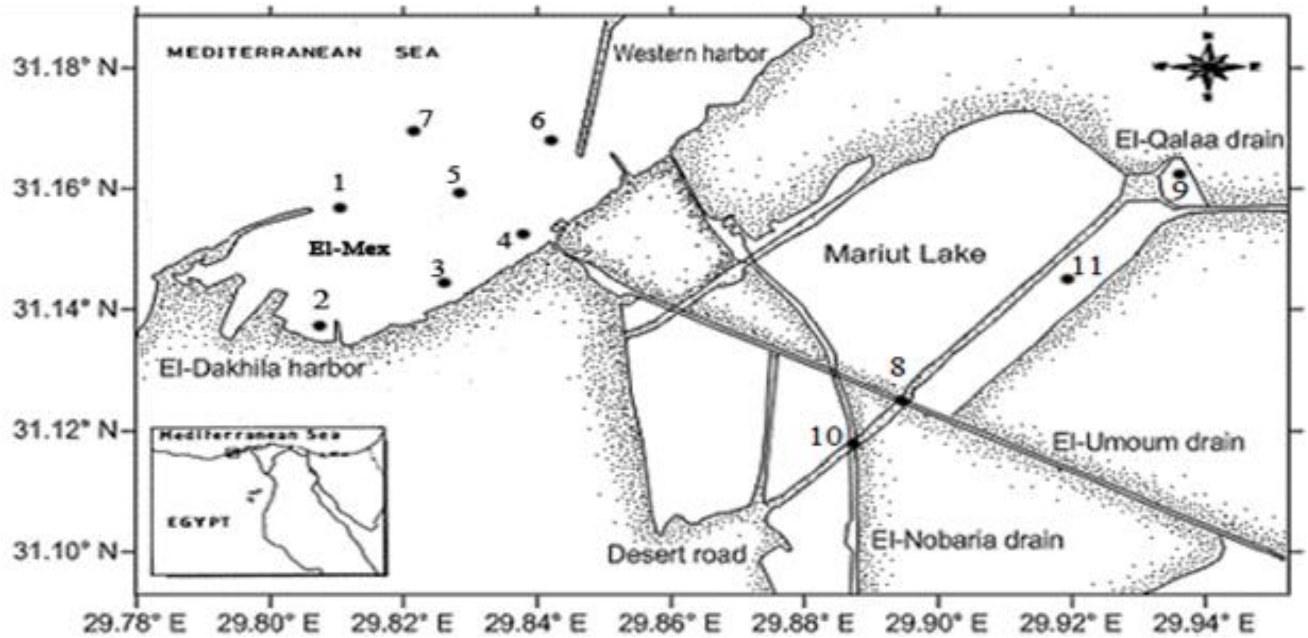

Figure 1. El-Mex Bay area, sampling locations.

the agricultural water from El-Beheira Governorate as well as mixed wastes from the Lake Mariut and discharges them into El-Mex Bay via El Mex pumping station in southern part of the bay. It receives a heavy load of wastewater (2.4×10^9 m^3/year) both directly to the sea from industrial outfalls and indirectly from Lake Mariut via El-Mex pumping station (Shriadah and Emara, 1992a, b; Emara et al., 1992; Said et al., 1994). The physico-chemical characteristics of El-Mex area have been studied by many authors (Shriadah and Emara, 1996; Emara et al., 1992; Said et al., 1994; Tayel et al., 1996; Mahmoud et al., 2005; Shreadah et al., 2006a, b; Zakaria et al., 2007; Abdallah, 2008; Nassim et al. 2010; El Zokm et al., 2012; Fathy et al., 2012; Okbah et al., 2013; Hamdy et al., 2014). Degradation of water quality due to land-based pollution is a serious problem in the Mediterranean coastal areas. The countries of the Mediterranean Sea basin face a variety of shared environmental problems that are trans-boundary in nature (EEAA, 2009). Two millions cubic meter of partially untreated sewage wastes (treatment efficiency less than 30%) are directly disposed to El Umoum drain before the pumping station. El Nubaria sailing cannel is a fresh water cannel of Nile water tells 30 km south of Alexandria where many new industries dispose their water in this cannel and lead to its pollution before combined with the main basin of Lake Mariut. Two openings off El Mex Bay at El Umoum drain are far with 1.92 and 1.82 km from El Mex Bay. Principal component analysis is successfully applied to sort out hydrographical and chemical from commonly collected water quality data to establish spatial and temporal variations in water quality (Olobaniyi and Owoyemi, 2006).

The present study aimed to follow the changes of physico-chemical characteristics and study impacts of nutrients salts come from uncontrolled land-based discharge (industrial, agricultural and domestic to El Mex Bay to assess water quality.

Study area

El-Mex bay is one of the main fishing grounds of Alexandria located between longitude 29º 47.1′ to 29º 50.4′ E and latitude 31º 7.5′ to 31º 9′ N (Figure 1). It represent a shallow sheltered Estuary west of Alexandria, extends for about 15 km between El-Agamy headland to the west to the Western Harbor to the east and from the coast to a depth of about 30 m. The Bay has a mean depth of 10 m. Its surface area is about 19.4 km^2 and its volume is 190.3 x 10^6 m^3 and the rate of waste water added to the basin via El -Umoum drain is 2452.7×10^6 m^3/y (Shriadah and Emara 1992 a,b; Said et al., 1994,; Fahmey et al., 1997; Shriadah et al., 2006a,b; Shaltout et al., 2008; Fathy et al., 2012). El-Mex Bay is dominated by two types of currents. El Mex Bay is classified as a microtidal estuary (to~30 cm) and coastal currents drive water masses eastward at an average velocity of 0.5 knots. The wave height on the inner shelf reaches 1.5-2 m in winter (Stanley et al., 2006). Seven stations are chosen within the Bay to cover the whole Bay specially area around industrial activities and 4 samples from the drains.

MATERIALS AND METHODS

Water temperatures were measured *in situ* to the nearest 0.1°C by using ordinary thermometer, and also transparency was measured *in situ* using secchi disc. Salinity of samples was determined by measuring the electrical conductivity using an inductive salinometer (Thermo; Orion 150A+). The pH- value was measured by using bench type (JENWAY, 3410 Electrochemistry Analyzer pH-meter). The method used to determine oxygen and biological oxygen demand is the common Winkler method, modified by Carritt and Carpenter (1966). Determination of oxidizable organic matter was carried out according to the method described by FAO (1975). Chlorophyll-a,b and c were determine using 0.45 µm membrane filter according to Strickland and Parsons (1975). Nutrient salts were spectrophotometrically determined using a double beam spectrophotometer (UV VIS-SPEKOL® 1300/1500 single beam), according to the methods mentioned by FAO (1975).

Residence time

It is calculated according to El-Gindy et al. (1986) as follows:

$$\text{Residence time (t)} = \frac{V\,(Si - So)}{Si \times QR}$$

So is the salinity in surface layer, Si is the salinity in subsurface layer; V, the volume of the water in the bay; QR is the rate of fresh water added to the basin.

Evaluation of water quality index

Lohani and Todino (1984) utilized principal components technique to provide a quick analytical method for the water quality index (WQI) proposed as given by the following formula (Davis, 1986):

$$WQI = \sum_{n=1}^{n} (\lambda_n / \sum \lambda) \times PCn$$

For PC Assessment model, n is the number of effective components, λ_n, are the Eigen values of the effective components, $\Sigma\lambda$ sum of the Eigen values and PCn are the principal component factor scores. For the evaluation of hot spot sites, principal component factor scores and WQI corresponds to more polluted site evaluated. High values of principal component factor scores mean that this site is from hotspots.

RESULTS AND DISCUSSION

Hydrographical conditions

Water temperature

The maximum temperature was attained in autumn reaching 30.9°C at El-Qalaa, while the minimum temperature was about 15.0°C in winter at El-Umoum. The seasonal averages preceded also as normal trend as expected; it increased from winter (19.00°C) through spring (19.53°C) reaching maximum level during summer (28.50°C) and decreased slightly again during autumn (27.07°C) (Table 2a and 2b). Hamedy et al. (2014), Okbah et al. (2013), Nessim et al. (2010) and Zakaria et al. (2007) recorded temperature ranges compatible to the present study which are 18.1-29.1, 18- 31,15.1-29.9 and 14.5 - 31.0°C in El Mex Bay water. Temperature changes according to many variables such as season, day time, depth, tide, wind, current, water inflow and turbidity (Whaby and Shriadah, 1984).

Salinity

In the present study, salinity is used as indicator to reflect changes resulting from the mixing of fresh and seawaters. Salinity of El-Mex Bay was mostly affected by the amount of drainage water of El-Umoum drain and the rate of exchange with the adjoining open sea (Shriadah and Emara 1992a, b, Said et al. 1994; Fahmey et al., 1997; Shriadah et al., 2006a, b; Shaltout 2008; Fathy et al., 2012; Okbah et al., 2013). As illustrated in Table 2a and 2b, the lowest salinity (0.30) was recorded during spring, while autumn represented the highest salinity (7.30) for drains with an average for the 4 seasons of 3.75. Nessim et al. (2010) recorded salinity range 4.67-6.44 and average value of 5.79 for drains in El Mex area. Salinity is measured in surface and subsurface layers for El- Mex Bay to calculate the residence time in the bay. As shown in Table 1a, salinity increased with increasing depth and the bay is classified into two water masses overlapping each other. Water mass with salinity >35 is identified to occupy most of the bay bottom area. Regionally, the average values of salinity, in general, showed noticeable local variations according to the distance of the different sites from the effluents whereas the annual salinity averages increased seawards and started with 1.68 at El-Qalaa drain to 4.53 at El-Umoum drain reaching maximum level at EL-Mex Bay (St.1-7) 30.76. Based on the distribution of surface salinity in the investigated area salinity gradient identification; stations 8, 9, 10 and11 characterized by Mixed land drainage type L with salinity < 10.0 in all seasons. For El Mex Bay in spring 2010, three types of water are recorded due to high water circulation and high water current; Diluted sea water (D) with a salinity range from 30.00 to 38.50 station (1) and Mediterranean Sea water (S) of salinity > 38.50 stations (2-7) beside L type. For autumn 2010, four types are recorded; Mixed water (M) salinity of 10.0 to 30.0 at stations 5 and 6. D type, were characterized by stations 1, 2, 3, 4 and 7 beside L type. For winter 2011, four types are obtained; M type for stations 1, 2 and 3; D type for stations 4 and 6 and S type for stations 5 and 7 beside L type. For summer 2011, M type at stations 1,2,3,4 and 5, D type were characterized by equations 6 and 7 beside L type. The data recorded in the present study is in accordance with those of Said et al. (1994); Mahmoud et al. (2005); Zakaria et al. (2007) and Okbah et al. (2013).

Table 1. List of Stations, site code and different sampling locations.

Name	Site Code	Latitude	Longitude
El-Dekhila Head	1	31° 09' 16.1"	29° 48' 17.4"
El-Hexane Factory (cooling station)	2	31° 08' 34.1"	29° 48' 18.2"
Petrochemicals company	3	31° 08' 17.7"	29° 48' 44.4"
In front of El-Umum Drain	4	31° 09' 18.3"	29° 50' 20.9"
Far 500 m from station 4	5	31° 09' 51"	29° 47' 59"
In front of Western Harbour	6	31° 09' 53.6"	27° 50' 47.1"
Far 1200 m from Station 4	7	31° 10' 58.2"	23° 50' 33.0"
El-Umum Drain (Near Shore)	8	31° 09'18.2"	29° 55'37.7"
El-Qalaa Drain (Near Shore)	9	31° 09'2.88"	29° 55'36.48"
El-Noubaria Drain (Near Shore)	10	31° 07'19.2"	29° 53'52.8"
Marriott Lake (Near Shore)	11	31° 07'19.2"	29° 53'52.8"

Residence time of El Mex Bay

The residence time of El-Mex Bay water in the present study varied seasonally as following 1.4 day in spring 2010, 5.62 day in Autumn 2010, 5.33 day in winter 2011 and 12.48 day in summer 2011. El Gindy et al. (1986) recorded residence time value of 1.2 day; however Shaltout (2008) recoded residence time that ranged from 8.6 to 13.3 days. This variation may be attributed to physical barriers.

pH-Value

Hydrogen ion concentration plays an important role in many of the life processes. Living organisms are very dependent on, and sensitive to pH. As shown in Table 2a, during the period of study, maximum readings of pH-values at waste water stations were observed during winter at Marriutt (8.58) while the lowest pH-value of 7.16 was measured during autumn at El-Qalaa. The pH-value of seawater at El-Mex Bay ranged between 8.97 (summer) at station 1 and 7.36 (winter) at station 3. Most of data lies at alkaline side (>7- <9). Slight variations in the pH values were observed by comparing the present pH values with those of the previous studies (Mahmoud et al., 2005; Nessim et al., 2010; Fathy et al., 2012; Okbah et al., 2013). This coincided principally with the relative increase or decrease in the quantity and quality of the discharged effluents. The pH values of the drain waters showed a dependence on the nature of the effluents conveyed from each drain. The lower values may be associated with high organic matter content in the sewage (Whaby and Shreadah, 1984). The pH-level depends on many characteristic parameters including; temperature, alkalinity, dissolved oxygen, organic matter, chlorophylls as well as minor and major constituents. It seems that the relative high pH-value during summer in waste waters could be attributed to the phytoplankton bloom. The highest pH-value observed during summer at El-Mex Bay stations, was accompanied by an increase in dissolved oxygen (Said et al., 1994; Fahmey et al., 1997). The variability of pH values in the surface water of El Mex Bay was explained to 55% by DO followed by organic matter (Tayel et al., 1996).

Water transparency

The transparency values was affected by domestic, agriculture effluents and suspended matter and floating substances (Whaby and Shreadah, 1984). McCave (1989) pointed out that since water clarity determines several factors affecting water ecosystem, the increase in water clarity may help in explaining define response among oligotrophic, mesotrophic, eutrophic and hyper-trophic water bodies to nutrient abatement measures. The regional and seasonal variations of secchi depth were fluctuated between 5.00 cm during winter at El-Qalaa to 100.00 cm during summer at Mariut Lake for drains. With respect to El-Mex Bay, the minimum transparency of 30.00 cm was investigated during summer (St.4) while, winter season gave the maximum level of 370.00 cm (St.7), with an average for the 4 seasons of 119.29 cm. Lowest Secchi values were observed in El-Mex drains during summer 2011, while El-Mex Bay showed a relatively higher Secchi values. This could be attributed to quantity and quality of the discharged water into El-Mex drains that can be related to the presence of greater amount of particulate materials, including phytoplankton and the effect of discharged water (Said et al., 1994, Fahmey et al., 1997, Shriadah et al., 2006a,b, Shaltout, 2008; Fathy et al., 2012). Fathy et al. (2012) reported transparency range of 28-85 cm for El Ummum and El

Table 2a. Seasonal variations of hydrographical parameters at El-Mex Bay (2010-2011).

Season	Stations	Temp. (°C)	Salinity		pH	Transp. (cm)	DO mg/L	BOD mgL	OOM mgO/L	Chl-a	Chl-b	Chl-c
			Surface	sub							µgL	
Spring (2010)	1	21	32.6	42.3	7.4	140	9.26	20.17	34.4	4.9	ND	ND
	2	20	38.6	43	8.05	150	4.95	12.41	24	16.42	0.51	8.26
	3	19.5	39	35.9	8.25	50	5.76	14.77	5.6	5.23	1.6	9.26
	4	19	42.3	39.6	7.95	50	7.89	16.06	12.4	24.98	1.3	2.28
	5	19	38.8	42.5	8.25	150	7.14	16.54	0	5.85	1.42	8.76
	6	19.2	40.2	43.1	8.3	250	7.87	16.7	10.4	1.63	0.03	1.03
	7	19	40.4	41.8	8.35	250	8.45	22.76	4	1.49	0.22	1.02
	Av	19.53	39.13	41.17	8.08	148.57	7.33	17.06	12.97	8.64	0.85	5.1
Autumn (2010)	1	27	36.4	39.3	8.37	140	3.32	9.78	1.2	53.18	12.41	18.2
	2	27	32.2	39	8.03	45	2.84	8.32	1.2	8.55	1.34	12.14
	3	27	32.7	38	8.03	90	2.84	7.5	1.2	15.39	5.7	16.12
	4	27	34.3	37	8.27	125	4.62	13.83	4.4	42.78	4.91	39.26
	5	26.5	22.3	33.2	7.76	75	1.79	4.48	10.4	6.93	5.15	22.2
	6	27	19.6	38	7.6	110	1.7	4.77	4	21.16	6.06	9.07
	7	28	38	39.2	8.31	150	6.08	18.05	23.6	57.92	6.02	5.27
	Av (1-7)	27.07	30.21	37.67	8.05	105	3.31	9.53	6.57	29.42	5.94	17.46
Winter (2011)	1	19	29.4	40	8.2	70	5.03	25.14	16	108.75	13.16	137
	2	20	24.3	39.7	7.8	70	4.05	20.27	10.4	24.19	242.78	ND
	3	19	24.4	39.3	7.36	70	3.9	16.25	18.4	55.24	24.83	168.64
	4	20	31.7	39	7.85	60	7.3	20.37	0	241.91	107.99	ND
	5	18	39.3	40.1	8.43	180	10.22	20.44	3.2	128.04	14.43	159.62
	6	19	36.8	40	8.3	160	9.58	28.55	24.8	152.99	66.12	283.91
	7	18	40	40	8.5	370	9.18	29.79	10.4	40.03	25.83	153.98
	Av	19	32.27	39.73	8.06	140	7.04	22.97	11.89	107.31	70.74	180.63
Summer (2011)	1	29	20.1	38.5	8.97	135	22.26	111.29	16	91.53	2.54	86.63
	2	29	24.1	38.4	8.29	65	6.45	27.42	12	22.34	107.21	ND
	3	28.5	15.1	38.3	7.75	50	0.65	3.23	20.8	47.22	4.49	98.26

Table 2a. Seasonal variations of hydrographical parameters at El-Mex Bay (2010-2011).

	Stations	Temp. (°C)	Salinity		pH	Transp. (cm)	DO mg/L	BOD mg/L	OOM mgO/L	Chl-a	Chl-b	Chl-c
			Surface	sub							µg/L	
Season	4	28.5	14.5	37.7	7.84	30	ND	ND	9.6	202.57	36.65	ND
	5	28	28.8	38.4	8.27	105	6.45	32.26	8	107.75	3.42	101.14
	6	28.5	30.7	38	8.33	100	6.45	32.26	1.6	129.85	14.92	169.61
	7	28	36.7	38.3	8.34	100	3.87	19.35	5.6	34.48	5.97	88.46
	Av	28.5	21.43	38.23	8.26	83.57	6.59	37.63	10.51	90.82	25.03	108.82
Average for 4 seasons	1	24	29.63	40.03	8.24	121.25	9.97	41.59	16.9	64.59	7.03	60.46
	2	24	29.8	40.03	8.04	82.5	4.57	17.1	11.9	17.88	87.96	5.1
	3	23.5	27.8	37.88	7.85	65	3.29	10.44	11.5	30.77	9.15	73.07
	4	23.63	28.2	30.83	7.98	66.25	4.95	16.75	6.6	128.06	37.71	10.39
	5	22.88	32.3	38.55	8.18	127.5	6.4	18.43	5.4	62.14	6.1	72.93
	6	23.43	31.83	39.78	8.13	155	6.4	20.57	10.2	76.41	21.78	115.91
	7	23.25	38.5	39.83	8.38	217.5	6.9	22.49	10.9	33.48	9.51	62.18
	Av	23.53	30.76	38.13	8.11	119.29	6.07	21.8	10.49	59.05	25.61	57.15

Nubaria drains at the period of 2010/2011.

Dissolved oxygen (DO)

Dissolved oxygen is frequently the key parameter in determining the extent and kinds of life in a water body. The distribution of DO in waste water attained its minimum average of 2.02 mg/L (St.8) during spring, tended to increase during summer, 5.48 mg/land during winter, 6.13 mg/L reaching a maximum level of 7.14 mg/L (St.10) in autumn, with an average for the 4 seasons of 4.57 mg/L(Table 2a). The amount of DO in seawater station fluctuated between a minimum of 0.65 mg/L (St.3) and a maximum of 22.26 mg/L (St.1) which was recorded during summer, with an average for the 4 seasons of 6.07 mg/L (Table 2a).

The smallest values were detected in the drains that ranged from ND to 7.14 mg/L (Table 2b); coincided with the consumption of oxygen in the decomposition of organic matter (Whaby and Shreadah, 1984; Said et al., 1994; Tayel et al.; 1996; Fahmey et al., 1997). DO concentrations of the present study are compared to previous studies in El Mex area by Hamdy et al. (2014); Okbah et al. (2013); Shredeah et al. (2013) with reported ranges of 4.4-14.6, 1.17-8.45, 1.11-4.66. Shreadah (2006a, b), Mahmoud et al. (2005) and Tayel et al. (1996) reported average values of 4.29, 12.3 and 6.81 mg /L respectively.

Biological oxygen demand (BOD)

The amount of dissolved oxygen which is consumed during organic matter degradation, gave a general picture of allochthonous organic load (Whaby and Shriadah, 1984). Table2a demonstrates that seasonal variations of BOD occurred at El-Mex Bay stations whereas the maximum average of 111.29 mg/Land minimum ND were observed during summer at stations 1 and 4 respectively, with an average for the 4 seasons of 21.80 mg/L for El Mex Bay (Table 2a). A range of seasonal variations at waste water sites was reported 11.98 – 36.9 mg/L at stations 11 and 9 respectively (Table 2b). Generally, when BOD levels are high, there is a decline in DO

Table 2b. Seasonal variations of hydrographical parameters at drains (2010-2011).

Season	Stations	Temperature (°C)	Salinity	pH	Transp. (cm)	DO (mg/L)	BOD (mgL)	OOM (mgO/L)	Chl-a (µg/L)	Chl-b (µg/L)	Chl-c (µg/L)
Spring	8	23.6	2.8	8.01	15	2.02	12.6	14.84	7.98	0.65	0.65
	9	25	1.1	7.53	15	ND	28.8	58.2	7.72	1.75	1.17
	10	23.6	0.3	8.41	15	ND	20.35	12.2	0.92	0.01	0.01
	11	23.6	2.8	8.56	20	3.2	11.98	ND	9.22	0.92	0.92
Autumn	8	28.9	6.2	7.57	25	6.82	27.4	14.4	6.24	1.76	1.76
	9	30.9	2.6	7.16	10	ND	30.5	40.8	1.87	1.81	1.81
	10	28.9	7.3	7.55	50	7.14	29.98	7.2	6.47	8.53	8.53
	11	29	6	7.69	25	6.98	26.89	15.2	4.68	1.32	1.32
Winter	8	15	4.2	8.16	30	6.13	24.19	8	35.71	5	6.21
	9	18	1.7	8.02	5	ND	36.9	112	9.17	10.64	19.7
	10	16	4.2	8	30	3.55	17.69	40	1.43	42.16	5.45
	11	17	5.4	8.58	50	6.13	25.81	2.4	ND	3.75	ND
Summer	8	28	4.9	8.15	30	5.48	24.19	11.2	186.83	ND	ND
	9	29	1.3	7.63	5	ND	29.8	8	29.3	8.18	8.18
	10	28	3.2	7.94	50	2.9	13.42	52.8	ND	ND	ND
	11	28	1.6	7.79	100	ND	23.85	27.2	ND	ND	ND
Average for 4 seasons	8	23.88	4.53	7.97	19.03	5.11	22.1	12.11	59.19	1.85	2.87
	9	25.73	1.68	7.59	7.53	ND	31.5	54.75	12.02	5.6	7.72
	10	24.13	3.75	7.98	26.71	4.53	20.36	28.05	2.2	12.68	4.66
	11	24.4	3.95	8.16	34.86	4.08	22.13	14.93	3.47	1.5	1.12

levels. This is because the demand for oxygen by the bacteria is high and they are taking oxygen from the oxygen dissolved in the water (Whaby and Shreadah, 1984). If there is no organic waste present in the water, there would not be as many bacteria present to decompose it and thus the BOD will tend to be lower and the DO level will tend to be higher. The present study recorded values higher than that recorded by Hamdy et al. 2014 (1.1-5.7 mg/ L) in El Mex region. Fathy et al. (2012) recorded a range of 4.84-30.56 mg/L for El Nubaria and El Umum drains for winter and summer region in the period 2010 to 2011.

Oxidizable organic matter (OOM)

Organic matter plays a major role in aquatic systems. It affects biogeochemical processes, nutrient cycling, biological availability, and chemical transport and interactions (Cole, 1979). Oxidizable organic matter has been used as basic water quality parameter to assess organic pollution. Organic matter can come from allocthonous source: which are brought to aquatic system by river runoff, with smaller atmospheric transport (Chester, 2000) or autochthonous sources such as excretion of organic matter are driven by living organisms and decomposition of dead organisms and detritus via long chain of decom-position processes (Duursma, 1961). The seasonal varia-tion of OOM occurred at drains where the maximum of 112.00 mgO_2/L and minimum of ND were observed during winter at station 9 and spring 2010 at station 11, respectively (Table 2b).

A range of seasonal values at El-Mex Bay stations was reported (0-34.4 mgO_2/L), with an average for the 4 seasons of 10.49 mgO_2/L, the values of OOM at waste water stations were nearly doubled that at seawater site. The increasing organic supply introduced into the restricted studied area under relative slow rate of self-purification (Shriadah and Emara 1992a,b, Said et al. 1994, Fahmey et al. 1997, Shriadah et al. 2006a,b, Shaltout 2008, Fathy et al. 2012) results in elevation of OOM content at El-Mex Bay (Table 2a). OOM concentrations of the present study are comparable with those of Okbah et al. (2013); Shreadah et al. (2013); Nessim et al. (2010) with ranges of 0.96-8.4, 5.2-38.4 and 5.44-14.56 mgO_2/L respectively.

The type of wastewater discharge can be determined according to BOD/ OOM (ECPH, 1975). If it is 1:1, it is characteristic of well purified water; the biodegradable compounds have a ratio of ≤ 2:1 while that of 2:1-4:1 is specific for crude domestic sewage, carbohydrates and proteins enriched wastes (food processing wastes) have ratios equal or greater than those for sewage. In the present study, the annual mean BOD/OOM ratios is amounted to 1.95:1, this may indicate that most of the sewage wastes reaching the investigated region had a biodegradable character.

Chlorophylls-a, b and c

Chl-a

It is considered as the main pigment that can be used for the determination of phytoplankton biomass (Carlson, 1977). The levels of Chl-a in El-Mex Bay showed wide variations (Table 2a). These were fluctuated between 1.49 µM/L in spring 2010 at station 7 and 241.91 µM/L in winter 2011 at station 4. The values of the chlorophyll-a in El-Mex drains were fluctuated between ND in summer at drains 10 and 11 and 186.83 µM/L in summer at drain 8 (Table 2b). The present study recorded that range of Chl-a exceed that recorded by Faragallah et al. 2009 in Eastern Harbour (0.41-78.68 µM/L) and that recorded by Emara et al. (1992) in El Mex Bay (0.00-5.56 µM/L).

In general, the high values of Chlorophyll-concentrations of the present study are higher than those of Shams-El Din and Dorgham (2007) for Abu Qir Bay which reported values of 14.9 µg/L in the investigated area reflect signs of eutrophication which is used as an estimation of the standing phytoplankton crop.

Chl-b

It is a form of chlorophyll. It helps in photosynthesis by absorbing light energy. It is more soluble than chlorophyll *a* in polar solvents because of its carbonyl group. The levels of Chl-b in El- Mex Bay showed wide variations. They fluctuated between 0.03 µM/L in spring 2010 at station 6 (In front of the Western Harbour) and 242.78 µM/L in winter 2011 at station 2 [El- Hexane Factory (cooling station)] may due to water temperature beside the wastes Table 2a. The average values of chlorophyll-b in El-Mex drains were fluctuated between ND at drains 8,10 and 11 in summer 2011 and 42.16 µM/L in winter El-Nubaria drain Table 2b .

Chl-c

It is found in certain marine algae, such as diatoms and dinoflagellates. The levels of Chl-c in El-Mex Bay showed wide variations (Table 2a). These were fluctuated between 0.54 µM/L in spring 2010 at station 5 (Far 500 m from El-Umoum Drain) and 283.91 µM/L in winter 2011 at station 6 [In front of Western Harbour]. The regional mean values of Chl-c were increased in waste water, at which it is directly in front of the Western Harbour beside El-Umum drain. The average values of the chlorophyll-c in El-Mex drains fluctuated between 0.01 µM/L in spring at drain 10 and 19.70 µM/L in winter at El Qalaa drain (Table 2b).

Nutrient salts

Nutrient salts are considered as very important compounds essential for the living organisms in natural waters. These are chemical substances used for maintenance and growths of biota, which are critical for survival of organisms. Essential nutrients, N and P are of particular concern in aquatic systems, because their availability can limit the growth of aquatic. Nutrients represent the fertility of water, on which primary productivity and, ultimately fish production depend on it. Moreover, the amount of autotrophic producers is a function of the nutrient concentrations. This relation has often been considered as the main determinant of the dynamics of aquatic systems and persisting presence of algae in great numbers Cloern (2001).

Inorganic nitrogen species (NH_4-N, NO_2-N and NO_3-N)

Dissolved inorganic nitrogen concentrations DIN (the sum of ammonia, nitrite and nitrate) in the bay water were relatively high. Generally, the high levels of DIN were affected directly by the discharged water from the drains, which were containing high amounts of agricultural fertilizers.

Ammonia ($NH4^+$-N): Ammonia is a highly variable para-meter quickly produced and processed by the bacterial decomposition of organic matter (ammonification), the production of NH_4^+ as an excretory product, especially from zooplankton and its production by bacterial reduction of NO_2^- and NO_3^- (nitrogen-immobilization). In principle, NH_4^+ salts are the form of N preferred by algae and only when NH_4^+ concentrations are depleted to < 0.0021mg/l, NO_3^- and NO_2^- will be utilized (Unesco , FAO, 1988). Ammonia is discharged into water bodies through industrial wastes and as a product of municipal or community wastes as well as agricultural wastes where ammonia containing fertilizers are used.

Ammonium nitrogen exists in aqueous solution either as ammonium ion ($NH4^+$) or as ammonia (NH_3) depending on the pH of the solution, in accordance with the following equilibrium reaction:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

At pH above 7, the equilibrium is displaced to the left. At pH< 7, $NH4^+$ is predominant.

Ammonia is the majority of dissolved inorganic nitrogen (DIN) for both waste and sea waters. It represented a ratio of 3.14 to 98.86% from DIN respectively (Table 3a and b; Figures 3 to 4). Seasonal and regional distribution of ammonia is illustrated in Table 3a and b. The high concentration of ammonia at waste water stations is derived from decomposition of a huge amount of organic matter, domestic, agriculture and industrial effluents. The striking increase in the amount of ammonia observed at El Qalaa was accompanied by anoxic condition, high OOM and low DO content. Almost all the combined inorganic nitrogen in anoxic waters was found as ammonia ions, while NO_2 disappeared and NO_3 ions were in a relative low concentration (Riley and Chester, 1971). El-Umoum site had much lower ammonia content in accordance with Nessim et al. (2010) which recorded concentration range of 14.40 to 61.4 µM/L during 2007/2008. With respect to El-Mex Bay (St.4) during summer represented the highest level (233.30 µM/L) while spring gave the lowest one (0.15 µM/L), with an average for the 4 seasons of 74.29 µM/L. Mahmoud et al. (2005) recorded average concentration of 227.35 µM/L in El Mex region during 2003/2004. El-Mex water is enriched with ammonia during spring-summer period. The high ammonia concentration in these seasons could be attributed to waste water discharge through El-Umoum Drain as well as planktonic animals excretions.

Nitrite: It is a minor constituent of DIN which consist of 5.2 to 11.8% for waste and sea waters, respectively and is characterized as intermediate compound which could be derived either from the oxidation of ammonia or reduction of nitrate and can be removed from solution during nitrogen assimilation by phytoplankton. Regionally, absence of nitrite is accompanied by an increase in ammonia at Mariut station during spring. The maximum concentration of nitrite during summer period may be attributed to the excretion of extracellular nitrite by phytoplankton (Riley and Chester, 1971). It may also influence the distribution of nitrite within the surface layers of natural waters (Hutchinson, 1957). High averages reported during spring at waste and sea water locations may be due to allochthonous inputs by El-Umoum drain, in addition to autochthonous source resulting from decaying of organic matter and oxidation processes (Figures 3 to 4). Seasonal variations in nitrite concentration in waste water appeared to range from 0.28 µM/Lduring winter at El-Qalaa drain to 20.25 µM/L during summer at El-Nubaria drain, with an average for the 4 seasons of 5.11 µM/L (Table 3b). With respect to El-Mex Bay, nitrite showed a maximum concentration of 22.85 µM/L and a minimum of 0.3 µM/L during spring and summer, respectively, with an average for the 4 seasons of 4.74 µM/L (Table 3a). Compared the data in present study with previous studies, Nessim et al. (2010) recorded a range from 7.4 to 11.1 µM/Lfor waste water and 1.8 to 11.3 for El Mex Bay. Mahmoud et al. (2005) recorded average value of 14.32 µM/Lfor El Mex area.

Nitrate: Sillen (1961) denoted that nitrate is considered to be the only stable oxidation level in the presence of oxygen in water. It comprises about 1.75 to 17.4% of DIN in waste and sea waters, respectively (Table 2 and Figure 2). The decrease of nitrate in this study at El-Mex bay stations during summer could be attributed to two factors; the first is the assimilation by plants and the second is denitrification (i.e. the reduction of nitrate to nitrite before releasing N_2O or N_2- molecules (Hutchinson, 1957) . Nitrate concentrations in El Mex Bay showing arrange of ND to 56.22 at stations 5 and 6 in summer and Autumn, respectively. Emara et al. (1995) reported Nitrate concentration of 7.33 ± 4.4 µM/L. While, for waste water it ranged from 0.46 µM/L at El- Umum to 58.42 µM/L at El- in Qalaa drain in Autumn at Summer seasons respectively. Autumn exhibited relative higher values greater than any other season ,this result is in agreement with those of Nessim et al. (2010) in El Mex area during 2007/2008 which recorded range of 6.3-21 µM/Land average value of 13.3 µM/L. Much higher average value of NO_3 (82.75 µM/L) was recorded by Mahmoud et al. (2005) in El Mex *region in 2003/2004. Based on the estimations of El Gindy et al. (1986); Nitrate flux to the sea at El Mex Bay was 878 kilograms.*

Reactive phosphorus (PO_4)

Phosphorus is an essential and sometimes limiting fac

Table 3a. Nutrient salts (µM/L) concentration at El-Mex area (2010-2011).

Seasons	Stations	NH_3/N	NO_2/N	NO_3/N	DIN	PO_4/P	SiO_4/Si
Spring (2010)	1	171.25	0.63	1.17	173.04	5.04	19.94
	2	32.8	2.43	1.3	36.52	1.58	11.51
	3	39.8	4.53	0.21	44.53	30.45	12.03
	4	13	22.85	6.05	41.9	11.59	30.6
	5	ND	2.83	1.96	4.79	2.56	8.55
	6	13.2	1.95	4.43	19.58	1.05	4.22
	7	0.15	0.73	1.91	2.78	1.61	5.07
	av	45.03	5.13	2.43	46.16	7.7	13.13
Autumn (2010)	1	10.3	0.68	8.4	19.37	0.98	4.11
	2	58.05	3.55	8.01	69.61	1.44	32.78
	3	55.4	3.13	47.95	106.47	1.47	27.57
	4	26.5	2.5	10.1	39.1	1.33	14.84
	5	111.1	12.73	49.49	173.32	0.88	88.73
	6	119.9	6	56.22	182.12	3.92	99.86
	7	31.9	2.4	17.98	52.28	1.23	13.76
	av	59.02	4.43	28.31	91.75	1.61	40.23
Winter 2011	1	0.5	7.6	7.8	15.9	5.85	16.13
	2	2.2	11.75	9.17	23.12	7.56	38.59
	3	3.6	12.18	6.98	22.75	8.33	38.59
	4	8.65	5.08	8.51	22.23	2.87	10.92
	5	ND	0.5	0.96	1.46	2.1	ND
	6	ND	2.03	8.11	10.14	2.17	3.29
	7	ND	0.73	4.93	5.66	2.52	0.48
	av	3.74	5.69	6.64	14.46	4.49	18
Summer (2011)	1	113.1	7.35	6.24	128.25	0.7	1.22
	2	170.9	6.73	6.7	186.79	2.45	33.63
	3	216.9	0.63	0.58	224.5	8.86	57.79
	4	233.3	0.3	0.2	242.11	12.25	50.39
	5	126.3	2	ND	129.26	2.21	23.16
	6	75.1	2.93	0.31	86.14	1.79	16.84
	7	96.5	6.15	1.4	107.58	1.4	30.12
	av	147.44	3.73	2.57	157.8	4.24	30.45
average foe the 4 seasons	1	73.79	4.06	5.9	84.14	3.14	14.08
	2	65.99	6.11	6.29	79.01	3.26	30.37
	3	78.93	5.11	13.93	99.56	12.28	29.19
	4	70.36	7.68	6.21	86.33	7.01	16.82
	5	118.7	4.51	13.1	77.2	1.93	48.64
	6	69.4	3.23	17.27	74.49	2.23	27.67
	7	42.85	2.5	6.56	42.08	1.69	4.95
	av	74.29	4.74	9.89	77.55	4.51	24.53

nutrient for maintenance of aquatic life. When it found in large content, it produces eutrophication condition which is considered as a potential pollutant. The high enrichment of wastewater with phosphate during winter-spring period is mainly attributed to the allochthonous huge amount of domestic and drainage effluents enriched with phosphate and other fertilizers discharge into this area (Said et al., 1994; Fahmey et al., 1997). The release

Table 3b. Nutrient Salts (µM/L) concentrations in El Mex drains 2010-2011.

Seasons	Stations	NH_3/N	NO_2/N	NO_3/N	DIN	PO_4/P	SiO_4/Si
	8	65.1	12.33	9.88	87.3	4.83	77.59
	9	726.25	0.63	2.65	729.53	26.95	49.84
Spring (2010)	10	89.6	1.38	1.59	92.57	0.84	6.18
	11	53.5	0.33	20.06	73.88	5.11	111
	av	233.61	3.66	8.55	245.82	9.43	61.15
	8	63.4	1.08	41.18	105.65	2.63	111
	9	1820	1.93	58.42	1880.34	9.59	99.86
Autumn (2010)	10	193.4	8.38	28.56	230.33	0.77	111
	11	87.6	1.3	39.83	128.73	1.16	111
	av	541.1	3.17	42	586.26	3.54	108.22
	8	196.4	9.88	28.37	234.65	18.31	2.15
	9	2815.85	0.28	3.76	2819.88	18.06	16.28
Winter 2011	10	207	16.08	25.14	248.22	21.67	0.63
	11	228.4	1.25	12.58	242.23	0.7	1.37
	av	861.91	6.87	17.46	886.24	14.68	5.11
	8	4.85	2.4	0.48	7.73	0.11	103.75
	9	45.4	2.75	31.75	79.9	29.05	189.44
Summer (2011)	10	ND	20.25	29.37	49.62	4.45	73.41
	11	42.9	1.5	0.35	44.75	26.67	2.15
	av	31.05	6.73	15.49	45.5	15.07	92.19
	8	82.44	6.42	19.98	108.83	6.47	73.62
	9	1351.88	1.39	24.14	1377.41	20.91	88.86
average for the 4 seasons	10	163.33	11.52	21.16	155.18	6.93	47.8
	11	103.1	1.09	18.21	122.4	8.41	56.38
	av	425.19	5.11	20.87	440.96	10.68	66.66

of large amount of phosphate during decaying and oxidation of organic substances was investigated at El-Qalaa location during summer-spring period coincided with oxygen depletion which agreed with the study of Riley and Chester (1971). At El-Mex Bay area, high consumption, as expected, in phosphate ion by marine flora was observed during spring blooming. A clear maximum was recorded during summer season since most of organic load was decayed. El-Mex Bay water, influenced by land-based sources, is still too high in phosphorus content if compared with open seawater. The waste and bay waters exhibited average phosphate concentration of 10.68 and 4.51 µM/L respectively reflected an eutrophication condition (UNEP, 1988). The average for the 4 seasons of PO4 in waste water and El Mex Bay in this study is lower than average that measured by Nessim et al. (2010) and Mahmoud et al. (2005) which are 14.0 and 19.69 µM/Lfor waste water and El Mex Bay water respectively.

Silicate (SiO_4): Seasonal and regional variation of silicate are illustrated in Tables 3a and 3b.Significant changes in silicate content were demonstrated at waste water ranging from 2.15 during Winter to 189.44 µM/L during Summer with an average for the 4 seasons of 66.66 µM/L (Table 3b). The waste water at the area of study exhibits silicate content four times greater or more (on, average) than that at seawater Figure 3 .Silicate concentration in El Mex Bay water varied from ND in winter to 99.86 in autumn with an annual averageof 24.53 µM/L (Table 3a). Coastal waters have, in general, quite high silicate contents, since they are affected by land-based sources (Riley and Chester, 1971). During Autumn-summer period, El-Mex Bay water was characterized by high silicate content >50 µM/L and low salinity value <40 as a function of discharged waters. The concentration of silicate during spring-winter period is relatively low owing to its consumption by diatoms and radiolarian. Nessim et al. (2010) recorded values that ranged from33.7 to 221.9

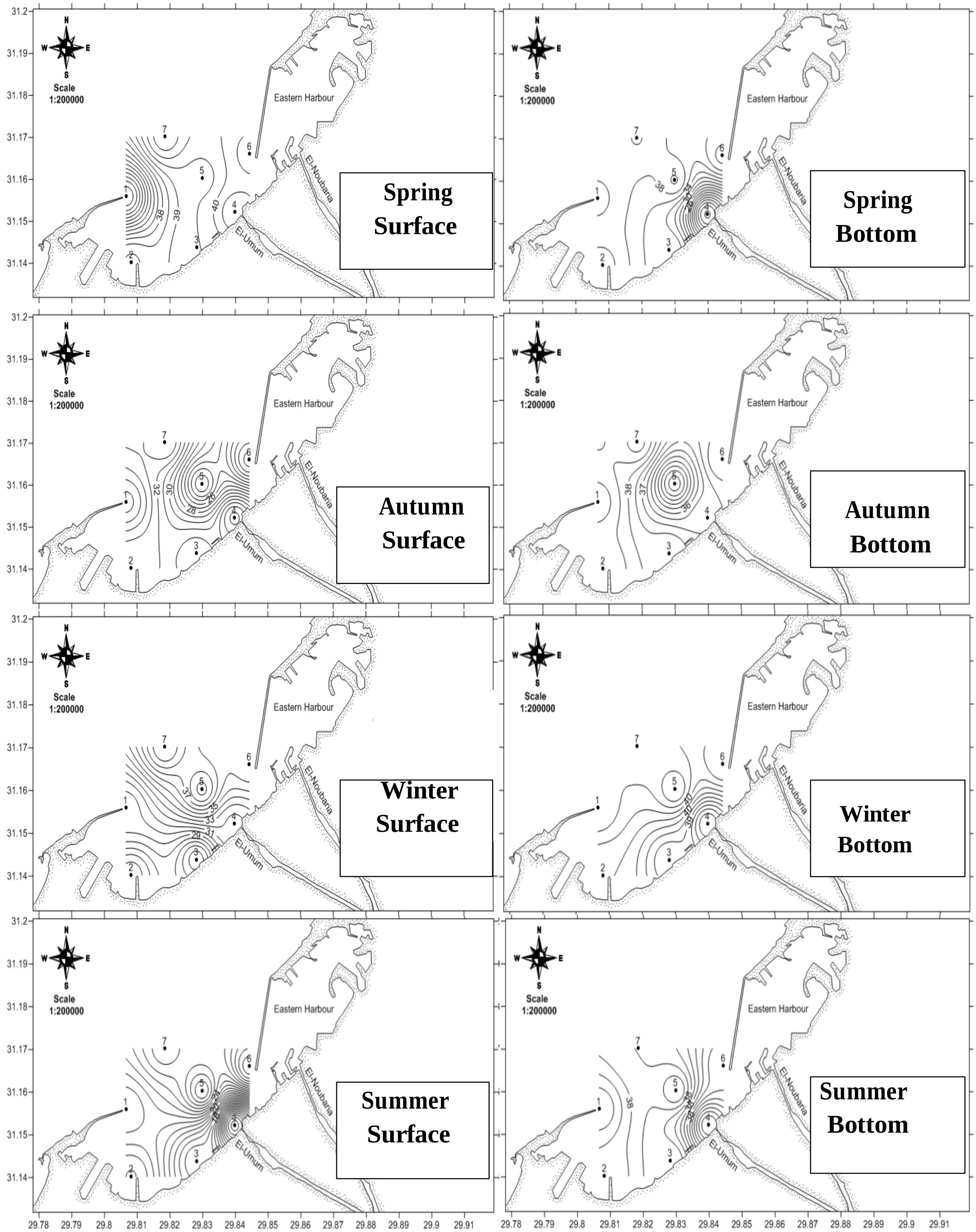

Figure 2. Horizontal distribution of salinity at surface and bottom water in El-Mex Bay.

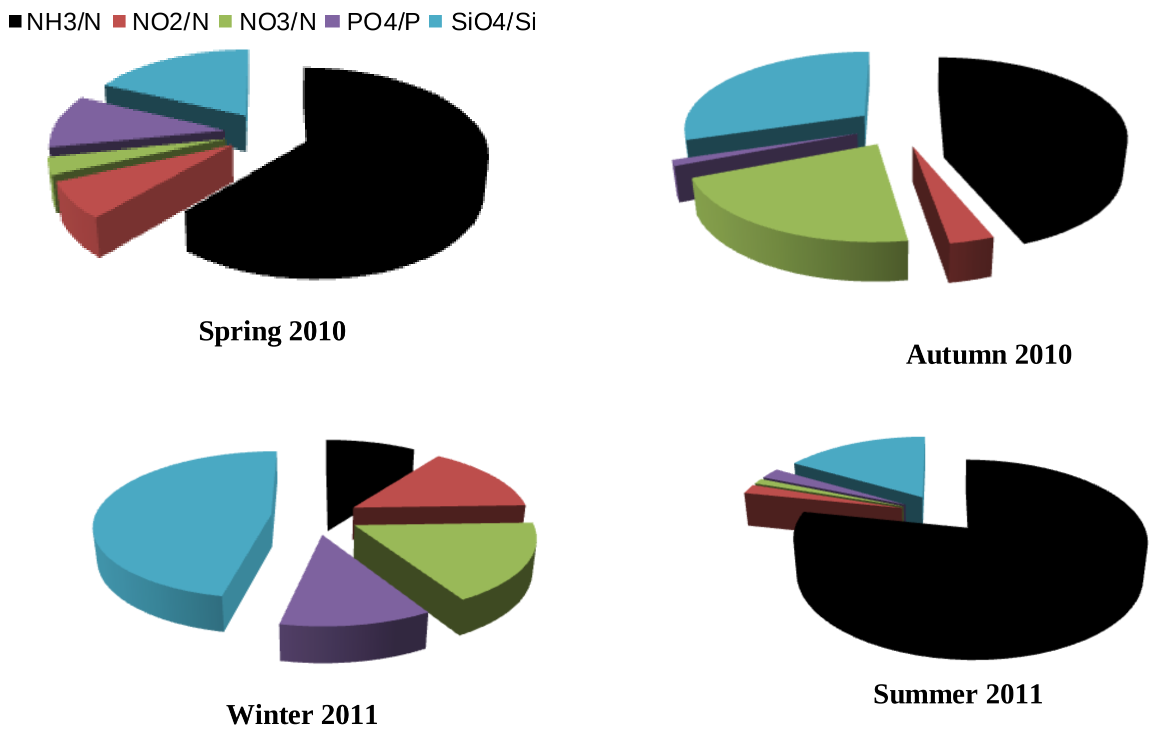

Figure 3. Average Values of Seasonal Variations of nutrient salts at El Mex Bay (2010-2011).

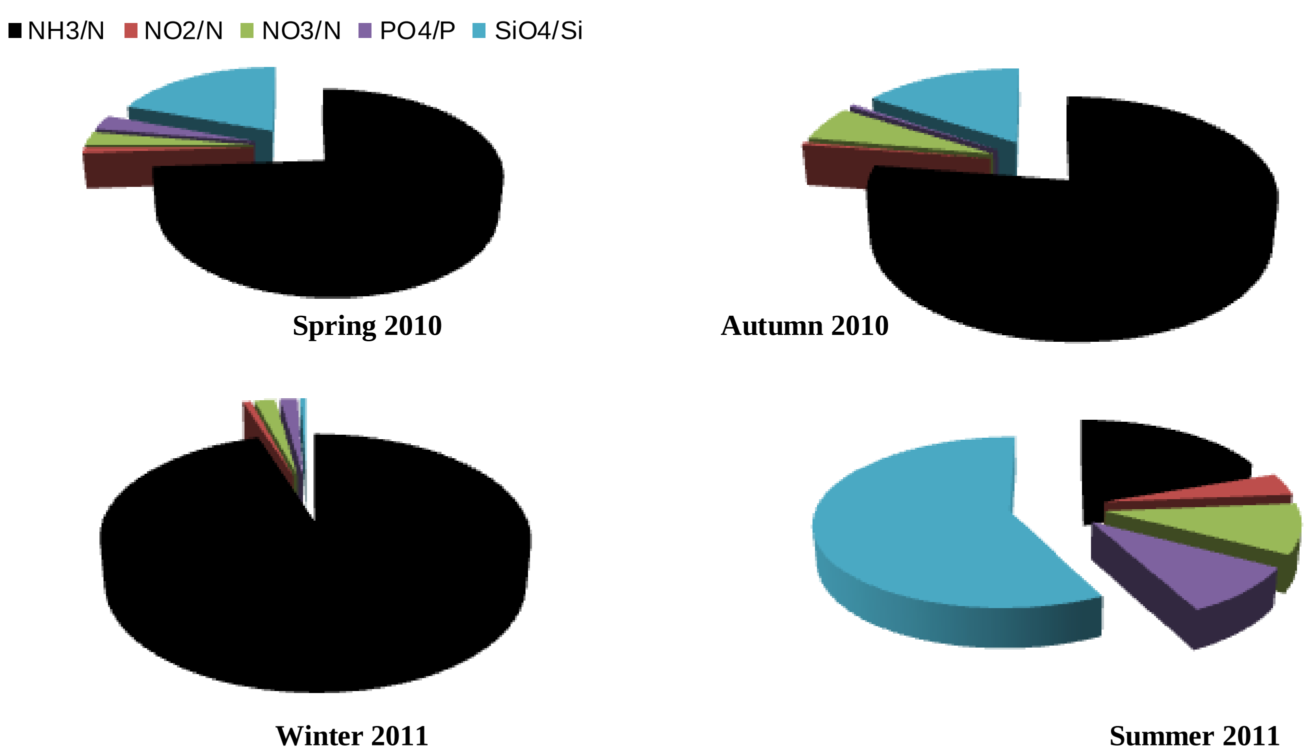

Figure 4. Average Values of Seasonal Variations of nutrient salts at drains (2010-2011).

for waste water and from 14.5 to 59.6 for sea water. Mahmoud et al. (2005) recorded average silicate content of 140.69 µM/L for El Mex bay water.

Dissolved inorganic nitrogen (DIN) and PO_4-P are the main forms of N and P that are readily bioavailable for the growth of phytoplankton. The DIN/DIP ratio in the different regions of El-Mex Bay during study period is illustrated in Figure 5. The average ratios are higher at station 5 near El-Umoum drain in winter (108.38) and the lowest (2.53) was observed at station 1 in winter. In drains the higher values were observed as 380.63 in summer of drain 8 and the lower was 2.38 in the same season of drain 9. The average for the 4 seasons of N/ P ratios for whole area is 27.29. The higher ratios than that reported by Redfield's ratio with value of 16:1 revealed high nitrogen content in comparison with that of phosphorous. The values of N/P ratio could be related to allocthonous conditions (Whaby and Shriadah, 1984; Okbah, 2005). Smith (1979) found that phytoplankton yield depends mainly on N/P ratio; ratio > 15-17 was indicating that phosphorus was the critical controlling factor, from < 9-10: 1 indicates that the yield varied with nitrogen and > 21 shows that phosphorus was the primary controlling factor.

Water budget

According to Abdallah (2007), the annual water budget flux into the Mediterranean sea at El-Mex Bay through El-Mex pump station is 6.98 x 10 6 m^3/d , El-Umum is 4.2x10 ^{6} m^3/d , Al-Qalaa is 7.5x10^5 m^3/d, El-Nubaria is 1.54x10 ^{6} m^3/d and Mariut Lake 3.5x10^5 m^3/d. We can calculate from the present averages the annul salts budget (metric ton/year) reflux into the bay area as follows: for El-Umum drain: ammonia 284.39; nitrite 9.84; nitrate 26.84; phosphate 307.30 and silicate 112.86. For El-Qalaa: ammonia 44.93; nitrite 0.34; nitrate 6.43; phosphate 120.35 and silicate 20.92. For El-Nubaria: ammonia 83.44; nitrite 6.28; nitrate 11.67; phosphate 117.10 and silicate 20.92. In Mariut Lake: ammonia 14.26; nitrite 0.13; nitrate 14.46; phosphate 28.25 and silicate 3.66 ton/ year.

Water quality standard limits of surface water parameters according to criteria produced by different local and international authorities and organizations are used to identify the water quality in El Mex bay area as shown in Table 4. The data declared that for many parameters the values recorded in the present study are within the range of good quality except for ammonia, Chl-a and BOD which exceeded in some stations the recommended values by different authorities and organizations.

Principal component analysis (PCA)

It is applied for multivariate data derived from the water quality parameters analysis of 11 water samples for 4 seasons in El-Mex Bay and surrounded drains. The output data revealed that four factors (PC1- PC4) affected El-Mex water quality, association and sources, with cumulative covariance of 84.46%. Varimax rotated components matrix is given in Table 5 to give an overview on the nature of loading among the parameters. PC1, PC2, PC3 and PC4 have covariance of 51.19, 13.89, 10.73 and 8.65% respectively. PC1 represented loading of salinity (0.947), transp. (0.812), Chl-c (0.744) and chlorinity (0.947) associated with negative loading to NO_3 (-0.730). This factor refers to important role of salinity in the study area. This result is in accordance with those of Mahmoud et al. (2005), Zakaria et al. (2007) and Okbah et al. (2013). PC1may be affected by anthropogenic source of nitrate like fertilizers, industry, sewage and sludge. PC2 represented loading for temperature (0.700), OOM (0.763), NH_3 (0.893) and phos-phate (0.872). This factor indicated that at higher tem-perature, OOM, NH_3 and PO4 amount was higher. PC3 represented loading for DIN/DIP (0.800 associated with negative loading of Chl-b µg/L (-0.708). PC4 represented high loading for BOD (0.972). Since, BOD is a measure of the oxygen used by microorganisms to decompose wastes it revealed a large quantity of organic waste in the study area.

Extraction method

This included principal component analysis; Rotation method: Varimax with Kaiser Normalization; marked loadings are >0.70; rotation converged in7 iterations; N= 11; Bold numbers positive loading, and italic number negative loading.

Evaluation of water quality index (WQI)

For the evaluation of hot spot stations, high values of principal component factor scores mean that this station is from hotspots. According to WQI, El-Dekhila Head and El Qalaa drain are two hot spots in this study area. According to PCA data the origins of pollution corresponding to the hot spots are summarized in Table 6.

Conclusion

The effect of wastewater on seawater was discussed through the water budges of nutrients from 4 drains into El Mex Bay. Based on the distribution of surface salinity in the investigated area, salinity gradient identified 4water types due to water circulation and high water currents. The residence time of El-Mex Bay water in the present study varied from 1.4 to 12.48 day .The present study throws light on the difference between the data of sea water and waste water compared to the previous studies. A noticeable variation in ammonia levels with striking

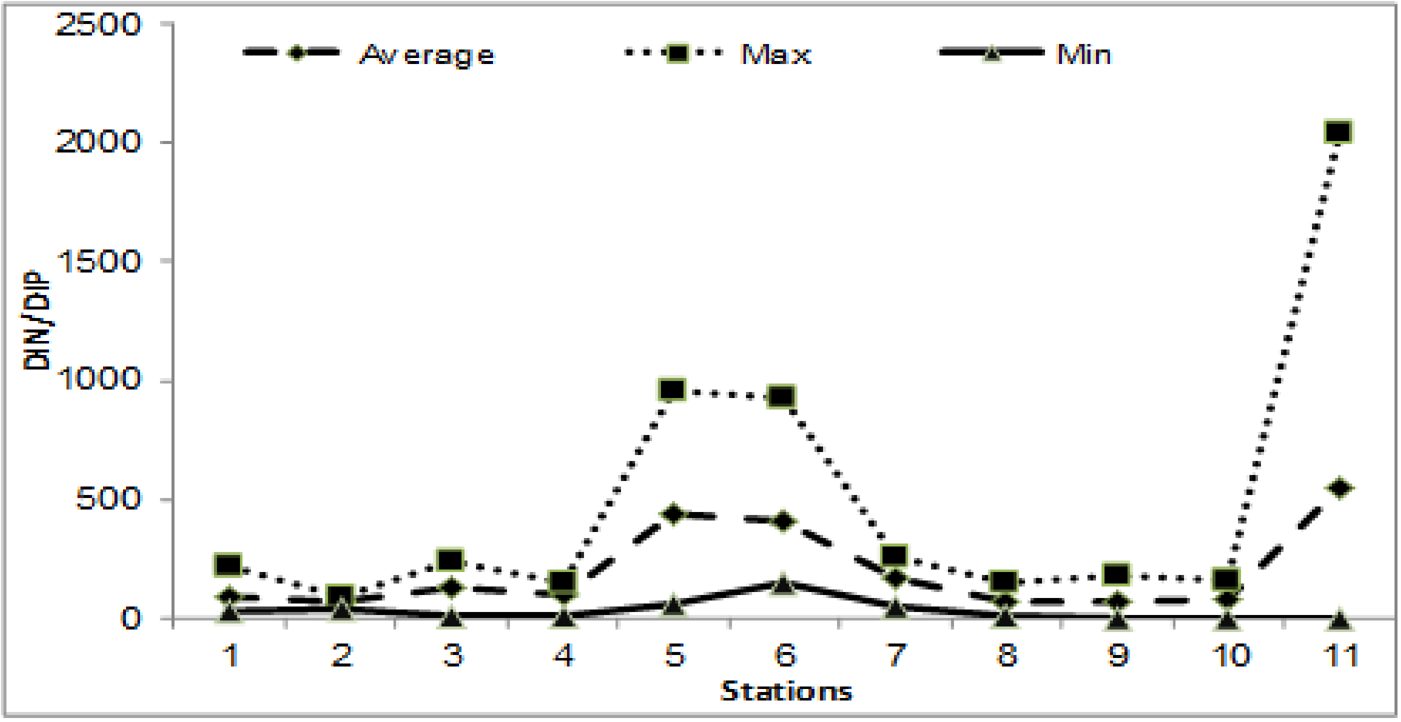

Figure 5. DIN/DIP distribution against sample stations.

Table 4. Water quality standard limits for surface water parameters according to criteria produced by different local and international authorities and organizations.

Parameter	WHO	EPA	Egyptian Standards	Portuguese Decree-law no 263/98	Present study	
	1973	1982	1994		S.W	W.W
Phosphate (mg/L)	10	-	5	0.4	0.02-1.89	0.01-1.8
Nitrate (mg/L)	0.05	-	40	25	0.01-1.57	0.01-1.64
Ammonia (mg/L)	0.5	-	3	0.05	0.05-2.06	0.07-39.42
Chl-a (µg/L)	20	-	-	-	ND-241.91	
DO(mg/L) (mg/L)	0.17	5	≥4	-	ND-22.26	
Transparency (cm)	10	-	-	-	5-370 cm	
pH	6-8.5	6.5-9.00	6.00-9.00	6.5-8.5	7.36-8.97	
Temperature (°C)	-	16-32	10>normal	-	15.0-30.9	
Salinity	-	-	2 normal	-	19.6-42.3	0.3-7.3
BOD (mg/L)	-	-	≤60	-	4.48-111.29	
COD (mg/L)	-	-	100	-	NM	

increase at El Qalaa drain as a result of domestic sewage was due to the reduction of NO_3 to NH_4-N via a denitrify-cation process. The large increase in nutrient loading has led to the impairment of many water bodies. This includes the eutrophication of water bodies that can lead to dissolved-oxygen depletion, species shifts, and fish kills. The high values of N/P ratio may be a result suggesting that phosphorus is the most limiting factor for the growth of phytoplankton. Principle component analysis (PCA) which was used to develop water quality index (WQI) revealed that El-Dekhila Head and El Qalaa drains were the most polluted sampling sites which resulted from the disposal of industrial, agricultural and domestic wastes. Ammonia, Chl-a and BOD according to different authorities and organizations exceed recommend values. Development of strategies for waste water treatment

Table 5. Varimax rotated component matrix of physico chemical parameters and nutrient salts in El Mex Bay and surrounded drains.

Parameter	PC1	PC2	PC3	PC4
Temperature (°C)	-0.521	0.700	-0.108	0.393
Salinity	**0.947**	-0.284	-0.063	-0.099
pH	0.387	*-0.815*	0.226	0.214
Transparency (cm)	**0.812**	-0.378	0.232	0.149
DO (mg/L)	0.437	*-0.741*	0.045	0.467
BOD (ml/L)	-0.057	0.080	0.089	**0.972**
OOM (mgO/L)	-0.416	**0.763**	-0.015	0.409
Chl-a (µg/L)	0.482	-0.237	-0.024	-0.113
Chl-b (µg/L)	0.301	0.008	-0.709	-0.258
Chl-c (µg/L)	**0.744**	-0.150	0.456	-0.013
NH_3 (µM/L)	-0.233	**0.893**	0.095	0.281
NO_2 (µM/L)	-0.298	-0.353	-0.673	-0.268
NO_3 (µM/L)	*-0.730*	0.391	0.395	-0.117
PO_4 (µM/L)	-0.391	**0.872**	0.029	-0.011
SiO_4 (µM/L)	*-0.745*	0.482	0.235	-0.038
DIN/DIP	0.019	-0.267	**0.800**	-0.254
Chlorinity	**0.947**	-0.284	-0.063	-0.099
Covariance %	51.19	13.89	10.73	8.65
CV %	51.19	65.08	75.81	84.46

Table 6. Principal component factor scores and water quality index (WQI) of water samples in study area.

Hot spot	PC1	PC2	PC3	PC4	WQI	Parameter
El-Dekhila Head (St.1)	0.63	-0.688	-0.265	**2.36**	0.477	According to high value of PC4 the effective parameter is BOD (41.59 mg/L)
El Qalaa drain	-0.532	**2.74**	0.27	0.88	0.253	According to high value of PC2 the effective parameters are Temp (25.73°C), OOM (54.75 mgO/L),NH_3 (1351.88 µ M/L) and PO_4 (20.91 µ M/L)

PC= principal component factor score; WQI=Water Quality Index; Bold number indicates high effect of factor scores (>0.7).

must be evaluated. Alternatively, environmentally acceptable, biocide should be strongly supported.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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