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The Effect of Pharmaceutical Effluent on Physico-Chemical Properties and Plankton Diversity of Okun Stream, Ilorin, Kwara State, Nigeria

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ABSTRACT

Pharmaceutical effluents (PE) are liquid wastes produced during pharmaceutical manufacturing. These effluents can alter the physico-chemical characteristics (PSC) of water and can affect the plankton communities inhabiting such aquatic environments. The Okun Stream in Ilorin, Kwara State, Nigeria, receives PE from a pharmaceutical company. Three sampling locations—upstream, point of effluent discharge (POD), and downstream—spaced 500 meters apart, were sampled biweekly from January to June. Temperature, pH, and dissolved oxygen (DO) were measured *in situ*. Heavy metals (HM) were analyzed using standard digestion and laboratory methods. Plankton were collected using a plankton net and identified using standard taxonomic guidelines. PSC and HM data were analyzed using ANOVA. Principal component analysis (PCA) was used to examine correlations between PSC and HM across sampling locations. Canonical correlation analysis (CCA) was employed to explore the relationships between PSC, HM, and plankton communities. Plankton diversity indices were calculated, and Pearson's correlation coefficients were used to assess the relationships between PSC, HM, and plankton abundance. At the POD, DO levels were low (2.24 ± 0.87mg/ L). Across all sampling sites, iron (Fe) was the most abundant heavy metal, while lead (Pb) was the least. Among phytoplankton, Cyanophyceae were the most dominant, with relative abundances of 38.74, 38.4, and 43.68% at the upstream, POD, and downstream stations, respectively. Among zooplankton, Protozoans were most abundant, with values of 60.98, 61.65, and 63.12% at the respective stations. Zooplankton Shannon diversity indices of 0.6689 (upstream), 0.6657 (POD), and 0.7184 (downstream) indicate that the Okun Stream is heavily polluted. To mitigate pollution and protect aquatic life, the pharmaceutical company should implement proper effluent treatment before discharge. Additionally, local residents should reduce anthropogenic activities that contribute to waste inflow into the stream.

INTRODUCTION

Water is essential to all forms of life and plays a vital role in daily human activities **(Adeyemi-Ale *et al.*, 2014)**. Despite its importance, water remains one of the most poorly managed resources globally, with numerous pollutants entering various water bodies **(Fakayode, 2005)**. Major sources of water pollution include agricultural runoff, accidental oil spills, municipal waste, industrial discharge, and waste from free-range animals or migratory birds **(Adeyemi-Ale & Tijani, 2022)**. The improper disposal of waste continues to escalate environmental problems in Nigeria **(Adeyemi-Ale, 2014)**, and the discharge of industrial effluents, including pharmaceutical waste, into water bodies is a common practice in the country. Without adequate treatment, these effluents can alter the physico-chemical properties of receiving water bodies, thereby affecting the distribution and diversity of aquatic organisms inhabiting the stream **(Adeyemi-Ale, 2014)**.

Plankton are microscopic organisms that drift or float in water, as they are too small or weak to swim against currents **(Augustyn, 2023)**. They serve as the primary producers in aquatic ecosystems and are excellent indicators of water quality **(Kutama *et al.*, 2014)**. Plankton are crucial to the food chain, providing sustenance to larger animals and, indirectly, to humans—phytoplankton are consumed by zooplankton, which in turn are eaten by fish and mammals **(Augustyn, 2023)**. Due to their rapid growth and short life cycles, plankton can quickly reflect ecological and environmental changes. Thus, their species composition serves as a reliable indicator of water quality **(Khoirunnisaa & Junianto, 2021)**.

Pharmaceuticals are either natural or synthetic chemicals found in over-the-counter drugs, prescription medications, and veterinary treatments **(WHO, 2012)**. These include antibiotics, antianxiety, and antiepileptic drugs **(Xia-Lin *et al.*, 2017)**. Pharmaceuticals have significantly improved life expectancy and the quality of life for patients **(OECD, 2018)**. During drug production, residual by-products—known as pharmaceutical effluents—are generated. These are difficult to treat because they often contain recalcitrant substances such as pharmaceutical actives, as well as inorganic nutrients like phosphates, nitrates, and sulphates **(Kayode-Afolayan *et al.*, 2022)**. Heavy metals and metalloids, including Cr, Ni, Co, Cu, Cd, Pb, and As, are also present in pharmaceutical effluents and are highly toxic to aquatic life, even at very low concentrations **(James *et al.*, 2013; Alimba *et al.*, 2019; Hossen *et al.*, 2024)**.

A pharmaceutical industry is located near the Okun Stream in Ilorin, Kwara State, and discharges its effluents directly into the stream. This activity may alter the stream’s physico-chemical properties and affect its plankton community. Currently, there is limited information on the water quality and plankton diversity of Okun Stream. Therefore, this study provides baseline data on the impact of pharmaceutical effluents on the stream's water quality, as well as the diversity and abundance of its plankton populations.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Okun stream located in Ilorin West Local Government Area of Kwara State, Nigeria (Fig. 1). Okun stream lies between latitude $8^0$28" North of the Equator and Longitude $4^0$33" East of the Greenwich Meridian. There is a pharmaceutical industry located very close to Okun stream and it discharges its effluent directly into the stream. Activities around the upstream include farming, irrigation, soil packing for construction purposes, and bathing. The point of discharge refers to the location where the pipe releases pharmaceutical effluent directly into the stream (Plate 1), while the downstream site is characterized by minimal human activity. Each sampling point was approximately 500 meters apart.

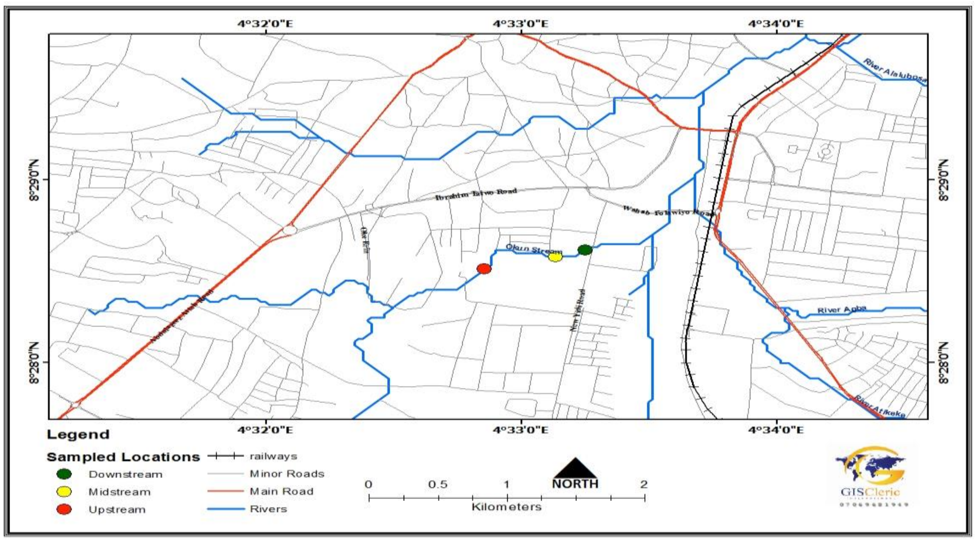

Fig. 1. Map of the study area showing the sampled locations

Plate 1. Point of discharge of pharmaceutical effluent into Okun stream

Collection of water and plankton samples

Water samples were collected from three sampling stations namely; the upstream, the point of pharmaceutical effluent discharge and the downstream from January to June. Water samples and plankton were collected bimonthly between the hours of 7:00 am and 9:00 am. The water samples for heavy metal analysis were collected in 500ml containers and were preserved with 0.75ml concentrated nitric acid (HNO_3) **(APHA/AWWA/WEF, 2012)**. The plankton samples were collected using plankton net (55µm mesh size) just below the surface water. Samples collected were immediately fixed with 4% formalin.

Measurement of physico-chemical variables of water

Temperature, pH and dissolved oxygen (DO) of the water were analyzed *in situ* with the use of mercury-in-glass thermometer, pH meter (Oakton waterproof instruments phlestr10) and DO meter model Acorn DO, respectively. The preserved water samples for heavy metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn and Pb) were analyzed in the laboratory using the Alpha Atomic Absorption Spectrophotometer after the nitric acid digestion method **(APHA/AWWA/WEF, 2012)**.

Identification of plankton

The preserved plankton samples were allowed to settle first and 1ml of the sample was drawn using a pipette and put into a Sedgewick rafter slide and then observed under the microscope. Keys provided by **Green (1960)**, **Whitford and Schaumer (1973)**, **Needham and Needham (1975)**, **Jeje and Fernando (1986, 1991)** and **APHA/AWWA/WEF (2012)** were used for the identification of plankton species. Their number in the 1ml sub-sample were examined, and the total number of cells per millilitre of phytoplankton for each sample were counted. Additionally, the total number of organisms per millilitre of zooplankton was also determined.

Data analysis

Statistical analysis

Physico-chemical analysis of water

The results of water analysis were subjected to one-way analysis of variance (ANOVA) to check for variations among the sampling stations. SPSS statistical software version 20 and Microsoft Excel Spreadsheet were used for data analysis and graphs.

Principal component analysis (PCA)

Principal Component Analysis (PCA) was carried out to check for the effects of physico-chemical variables and heavy metals at the three sampled points. This was analyzed with PAST 4.03.

Canonical correlation analysis (CCA)

Canonical correlation analysis (CCA) was done separately to analyze the relationships of physico-chemical variables and heavy metals with phytoplankton and also to analyze the relationships of physico-chemical variables with zooplankton. This was also done with PAST 4.0. CCA diagrams to determine the relationships between the physico-chemical variables and phytoplankton taxa and also the relationships between the physico-chemical variables and zooplankton taxa.

Biological analysis

The data were subjected to a statistical software program PAST 4.03 **(Hammer *et al.*, 2001)**. This software generated ten diversity indices: dominance (D), Simpson (1-D), Shannon (H), evenness (e^H/S), Brillouin, Menhinick, Margalef, Equitability (J), Fisher-alpha, and Berger-Parker.

Biodiversity indices were calculated to assess species richness, evenness, and dominance. According to **Hammer *et al.* (2001)**, dominance (D), also known as Simpson's Index of Dominance, is calculated as:

$$\mathbf{D = \Sigma(pi^2)}$$

where *pi* is the proportion of individuals in the *i*th species. Simpson's Diversity Index is then derived as:

Simpson's Index = 1 – D

Shannon's Index (also known as Shannon entropy) is a diversity index that considers both species abundance and evenness. Menhinick's Richness Index was used to measure species richness and is calculated as:

$$\mathbf{D_{mn} = S / \sqrt{N}}$$

where S is the number of taxa (species), and N is the total number of individuals in the sample.

Margalef's Richness Index is defined as:

$$\mathbf{D_{m}g = (S - 1) / \ln(N)}$$

where S is the number of taxa and N is the total number of individuals.

Equitability, or Pielou's Evenness Index, was calculated as:

$$\mathbf{E = H / \ln(S)}$$

where H is the Shannon diversity index and S is the total number of taxa.

Fisher's Alpha, a parameter of Fisher's logarithmic series model for diversity, was computed using the formula involving:

$$\mathbf{S = \alpha \times \ln(1 + N/\alpha)}$$

where S is the number of taxa, N is the total number of individuals, and α is Fisher's Alpha, which must be solved iteratively.

The Berger-Parker Dominance Index, as described by **Ajayan and Ajitkumar (2015)**, is calculated as:

$$\mathbf{d = N_{max} / N}$$

where N_{max} is the number of individuals in the most abundant species, and N is the total number of individuals in the sample.

Brillouin's Diversity Index, a suitable alternative to Shannon's Index particularly when complete censuses are used rather than random samples, was also computed **(Zaiontz, 2022)**.

All formulas and procedures followed standard ecological methods. Published methods were used without modification unless otherwise noted. Sufficient detail is provided to ensure reproducibility of the diversity index calculations.

RESULTS AND DISCUSSION

1. The physico-chemical parameters and heavy metal concentrations of water samples from Okun stream

The results of the physico-chemical qualities, including heavy metals of the water samples from the three stations sampled are shown in Table (1).

The temperature and pH values across the sampling stations were within the stipulated environmental standards. The pH values ranged from neutral to slightly alkaline. However, the mean dissolved oxygen (DO) concentration at the point of discharge (2.24 ± 0.87mg/L) was considerably below the recommended minimum limit of 4.00mg/ L set by **NESREA (2012)**. At the downstream location, the DO value (3.50 ± 0.42mg/ L) was somewhat closer to the stipulated threshold but still fell below it. While the mean temperature and pH values did not differ significantly among the three sampling stations, the DO concentrations showed statistically significant differences across all sites. These variations in DO are likely attributed to the composition and nature of the pharmaceutical effluent being discharged into the stream **(Guo *et al.*, 2017)**.

Table 1. Mean and standard errors of means of physico-chemical parameters and heavy metal of Okun stream

Parameter	Upstream	Point of discharge	Downstream	NESREA LIMIT
Temperature (ºC)	22.61±0.37^{a}	23.14±0.29^{a}	22.39±0.15^{a}	Ambient
pH	7.81±0.06^{a}	7.88±0.07^{a}	7.75±0.08^{a}	6.5 - 8.5
DO (mg/L)	4.91±0.24^{a}	2.24±0.28^{b}	3.50±0.16^{c}	4.00
Al (mg/L)	0.08±0.01^{a}	0.10±0.01^{a}	0.09±0.01^{a}	0.2
Cr (mg/L)	0.03±0.01^{a}	0.06±0.04^{a}	0.03±0.01^{a}	0.5
Mn (mg/L)	0.07±0.01^{a}	0.13±0.03^{a}	0.09±0.01^{a}	-
Fe (mg/L)	0.88 ±0.19^{a}	1.54±0.56^{a}	1.29±0.56^{a}	0.5
Co (mg/L)	0.08±0.01^{a}	0.14±0.05^{a}	0.11±0.01^{a}	-
Ni (mg/L)	0.03±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}	0.1
Cu (mg/L)	0.04±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}	0.01
Zn (mg/L)	0.12±0.02^{a}	0.24±0.02^{a}	0.17±0.05^{a}	0.2
Pb (mg/L)	0.02±0.01^{a}	0.02±0.005^{a}	0.02±0.01^{a}	0.1

Means with similar superscripts show that they were not significantly different at $P< 0.05$.
NESREA – National Environmental Standards and Regulatory Enforcement Agency (2011) (Nigeria) maximum permissible limits for effluent discharges.

The mean values of iron and copper obtained from all the three stations exceeded the NESREA set limits of 0.5 and 0.01mg/ L, respectively, while it was only the mean value of zinc (0.24±0.02mg/ L) obtained at the upstream that slightly exceeded the set limit (0.2mg/ L), even though it was negligible. The order of concentrations of heavy metals analyzed in the water samples from the sampling stations were Fe > Zn > Mn > Al =Co > Ni = Cu > Cr > Pb at the upstream; Fe > Zn > Co > Al > Mn > Cu > Cr = Ni > Pb at the point of discharge; and Fe > Zn > Co > Al = Mn > Cr > Ni > Cu = Pb at the downstream. The most elevated metal level at all the sampling stations was that of Fe, followed by Zn, while the least was that of Pb.

2. Pearson correlation of physico-chemical variables

The most common way of measuring a linear correlation is the Pearson correlation coefficient **(Turney, 2024)**. It measures the strength between variables and relationships **(Kirch, 2008)**. The Pearson correlation of physico-chemical variables is shown in Table (2). Positive correlation was found between temperature and pH (r = 0.497; $P< 0.01$). Water temperature governs the availability of oxygen, while pH governs the chemical and biological process of water **(Kowalkowski *et al.*, 2006)**. Strong positive correlation was found between Cr and Pb (r = 0.783), Ni and Zn (r = 0.724), Ni and Pb (r = 0.725), and Zn and Pb (r = 0.766) also at significant level of 0.01. There was a significant correlation between DO and Cu (r = 0.334), Cr and Cu (r = 0.336), and negative correlation between pH and Cu (r = -0.330) at 0.05 significant level. The implication of the positive correlation of each pair is that as one variable is increasing, the concentration of the other is also increasing.

Table 2. Pearson's correlation coefficients of physico-chemical variables and metals of the sampled points of Okun stream

	Temp.	**pH**	**DO**	**Al**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zinc**	**Pb**
Temp.	1											
pH	.497**	1										
DO	-0.314	-0.327	1									
Al	0.072	-0.111	-0.089	1								
Cr	-0.063	0.059	-0.014	0.009	1							
Mn	0.299	0.036	0.294	-0.126	-0.132	1						
Fe	-0.198	-0.025	0.264	0.159	.584**	-0.275	1					
Co	-0.086	-0.294	-0.002	0.193	-0.211	-0.108	0.203	1				
Ni	-0.005	-0.032	0.311	0.216	.596**	0.030	.566**	0.046	1			
Cu	0.077	-.330*	.334*	0.257	.336*	0.071	0.302	0.075	.473**	1		
Zn	0.089	0.110	0.319	0.174	.575**	-0.131	.565**	0.012	.724**	.621**	1	
Pb	0.007	-0.056	0.225	0.160	.783**	0.003	.677**	0.012	.725**	.622**	.766**	1

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

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

3. Plankton composition

3.1. Phytoplankton community structure

There is overall water quality deterioration when there is intensive development of phytoplankton **(Piontek *et al.*, 2023)**. In this study, a total of 405,900 cells/ml of phytoplankton, represented by 31 species of phytoplankton were encountered. Twelve (12) species for each were those of Cyanophyceae (blue-green algae) and Chlorophyceae (green algae), while seven (7) species were detected for Bacillariophyceae (diatoms) (Table 3). The most dominant group was Cyanophyceae with 38.74, 38.4 and 43.68% at the upstream, point of discharge and downstream, respectively. Bacillariophyceae was the least dominant group at the upstream and point of discharge with 27.79 and 25.6%, respectively. The least dominant group at the downstream was Chlorophyceae (27.89%) (Fig. 2). The most abundant species in Cyanophyceae found at the upstream and downstream was *Nostoc planctonicum* with abundance percentages of 22.28 and 16.27%, respectively, while *Spirulina major* was the most abundant (18.06 %) Cyanophyceae at the point of discharge (Table 3).

Table 3. The relative abundance of phytoplankton species encountered in Okun stream, Ilorin

Point of collection	Phytoplankton taxa	Species	No of cells/ml	% Abundance
Upstream	Cyanophyceae	*Anabaena circularis*	3300	5.43
		Aphanocapsa delicatissima	7260	11.96
		Calothrix adscendens	3960	6.52
		Coelastrum sphaericum	5940	9.78
		Microcystis aeruginosa	5610	9.24
		Nostoc planctonicum	13530	22.28
		Oscillatoria tenuis	11880	19.57
		Phormidium mucicola	2640	4.35
		Raphidiopis curvata	660	1.09
		Spirulina major	5940	9.78
	Bacillariophyceae	*Chaetoceros affine*	1980	4.55
		Coscinodiscus centralis	1980	4.55
		Mastigloia sp.	7920	18.18
		Navicula muralis	9900	22.73
		Navicula perotti	9240	21.21
		Nitzschia sigmoidea	990	2.27
		Synedra fasciculata	11550	26.52
	Chlorophyceae	*Ankistrodemus falcatus*	5940	11.32
		Cladophora glomerata	6600	12.58
		Closterium setaceum	7260	13.84
		Oocystis eremosphaeria	4620	8.81
		Pediatstrum duplex	990	1.89
		Scenedesmus quadricauda	5280	10.06
		Spirogyra sp.	21780	41.51
Point of Discharge	Cyanophyceae	*Anabaena circularis*	2640	5.56
		Aphanocapsa delicatissima	6930	14.58

		Calothrix adscendens	1980	4.17
		Coelosphaerium Kuetzingianum	6930	14.59
		Microcystis aeruginosa	6270	13.19
		Nostoc planctonicum	990	2.08
		Oscillatoria tenuis	5280	11.11
		Phormidium mucicola	7920	16.67
		Spirulina major	8580	18.06
	Bacillariophyceae	*Chaetoceros affine*	660	2.08
		Mastigloia .	4950	15.63
		Navicula muralis	5610	17.71
		Navicula perotti	1980	6.25
		Nitzschia sigmoidea	7590	23.96
		Synedra fasciculata	10890	34.38
	Chlorophyceae	*Ankistridesmus falcatus*	2970	6.67
		Cladophora glomerata	7590	17.04
		Closterium setaceum	6600	14.81
		Pediatstrum duplex	4620	10.37
		Scenedesmus quadricauda	1980	4.44
		Spirogyra sp.	16500	37.04
		Spirotaenia condensate	660	1.48
		Staurastrum limneticum	3630	8.15
Downstream	Cyanophyceae	*Anabaena circularis*	7590	13.86
		Aphanocapsa delicatissima	6930	12.65
		Aphanizomenon flos-aquae	990	1.81
		Calothrix adscendens	990	1.81
		Coelosphaerium Kuetzingianum	5280	9.64
		Microcystis aeruginosa	3300	6.02
		Nostoc planctonicum	8910	16.27
		Oscillatoria tenuis	5940	10.84
		Phormidium mucicola	7260	13.25

		Spirulina major	7590	13.86
	Bacillariophyceae	*Chaetoceros affine*	1650	4.63
		Coscinodiscus centralis	3960	11.11
		Mastigloia sp.	5610	15.74
		Navicula muralis	6270	17.59
		Navicula perotti	6270	17.59
		Nitzschia sigmoidea	4950	13.89
		Synedra fasciculata	6930	19.44
	Chlorophyceae	*Chaetophora* sp.	1320	3.77
		Cladophora glomerata	2310	6.60
		Closterium setaceum	1320	3.77
		Oocystis eremosphaeria	990	2.83
		Pediatstrum duplex	660	1.89
		Scenedesmus quadricauda	1650	4.72
		Spirogyra sp.	17160	49.06
		Spirotaenia condensate	1980	5.66
		Staurastrum limneticum	5280	15.09
		Xanthidium fasciculatum	2310	6.06

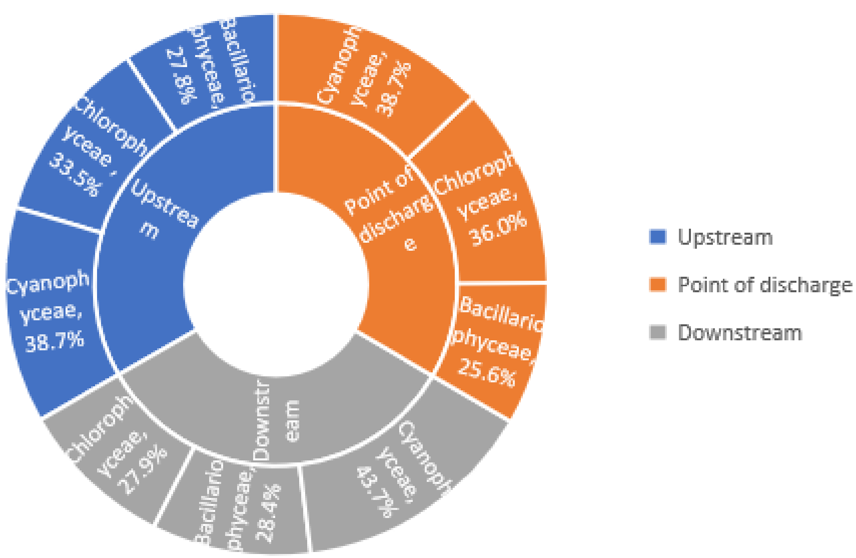

Fig. 2. Composition percentage of phytoplankton during the sampling period

Cyanophyceae, also known as Cyanobacteria, are known to proliferate in nutrient-polluted water bodies, often resulting in harmful algal blooms **(Piontek *et al.*, 2023)**. The dominance of Cyanophyceae observed in this study indicates a gradual decline in water quality. Their high abundance, not only at the point of effluent discharge but also IN upstream and downstream locations, may be attributed to anthropogenic activities such as agricultural runoff, bathing, and other informal uses of the stream for domestic purposes. Similarly, **Atobatele *et al.* (2007)** reported Cyanophyceae as the most dominant phytoplankton in the Ogunpa Stream, which receives untreated domestic sewage in Ibadan, Nigeria. Cyanophyceae were also recorded as the most abundant phytoplankton at various points along the Osere Stream, which receives detergent effluents **(Adeyemi-Ale *et al.*, 2014)**. Likewise, **Babatunde *et al.* (2014)** found Cyanophyceae to be the dominant phytoplankton in the lentic segment of the Kudiddiffi-Kubanni Stream. While, **Anago *et al.* (2013)** reported their dominance in Awba Lake, Ibadan, attributing these occurrences to anthropogenic influences.

Pathogenic species of Cyanobacteria are capable of producing thermophilic and thermotolerant toxins, including biologically active neurotoxins. These compounds are potentially harmful due to their ability to metabolize organic matter, thereby posing serious health risks to the environment, animals, and humans **(Velariani *et al.*, 2024)**. The presence of *Spirulina* sp. at the point of discharge in Okun Stream may be linked to its large-scale production for nutraceutical and cosmetic purposes, which are among the products manufactured by the pharmaceutical company responsible for the effluent discharge **(Converti *et al.*, 2006; Gami *et al.*, 2011; Yuan *et al.*, 2018)**. In contrast, **Begum and Khanam (2009)** found Bacillariophyceae to be the dominant phytoplankton group in a river in Bangladesh receiving pharmaceutical effluents, suggesting geographical and effluent-composition differences may influence phytoplankton community structure.

3.2. Zooplankton community structure

In an aquatic environment, zooplankton communities act as the vital link between the phytoplankton and the whole food chain **(Ogamba *et al*., 2005)**. Table (4) shows the zooplankton taxa encountered at the sampling stations. A total of 144,540 zooplankton/ml were encountered at the three stations throughout the sampling period. The population of zooplankton at the upstream (156,750 zooplankton/ml) was the highest, while that of point of discharge (123,750 zooplankton/ml) was the least. The population of 125,400 zooplankton/ml was encountered at the downstream. Protozoa and Rotifer were two groups of zooplankton encountered at the three sampling stations. However, crustacea was found only at the downstream. The only species of crustacea found at the downstream was *Daphnia* sp. Fourteen species of zooplankton were encountered altogether in the three sampling locations. Protozoans were the most abundant group at each of the sampling points. Their percentage abundances were 60.98, 61.65, and 63.12% at the upstream, point of discharge and downstream respectively (Fig. 3). *Vorticella mayerii* was the most abundant species of Protozoa at the upstream and the point of discharge with percentage abundances of 25 and 30.49%, respectively. *Euglypha tuberculata* (21.35%) was the most abundant Protozoa at the downstream.

Table 4. The relative abundance of zooplankton species encountered in Okun stream, Ilorin

Point of collection	**Zooplankton group**	**Species**	**No of ml**	**% Abundance**
Upstream	Protozoan	*Arcella costata*	2970	15.00
		Askenasia volvox	4950	9.00
		Carchesium sp.	1320	4.00
		Euglypha tuberculata	5940	18.00
		Spirostomum sp.	8910	27.00
		Tokophrya sp.	660	2.00
		Vorticella mayerii	8250	25.00
	Rotifer	*Camptocercus* sp.	3300	15.63
		Keratella tropica	12210	57.81
		Lepadella patella	5610	26.56
Point of Discharge	Protozoan	*Arcella costata*	660	2.44
		Askenasia volvox	990	3.66
		Carchesium sp.	990	3.66
		Colpoda sp.	990	3.66
		Didinium bolbianii	4620	17.07
		Epistylis sp.	5280	19.51
		Euglypha tuberculata	990	3.66
		Spirostomum sp.	2310	8.54

		Tokophrya sp.	1980	7.32
		Vorticella mayerii	8250	30.49
	Rotifer	*Camptocercus* sp.	3300	19.61
		Keratella tropica	6600	39.22
		Lepadella patella	6930	41.18
Downstream	Protozoan	*Arcella costata*	1650	5.62
		Askenasia volvox	1320	4.49
		Carchesium sp.	3630	12.36
		Colpoda sp.	660	2.25
		Epistylis sp.	990	3.37
		Euglypha tuberculata	6270	21.35
		Spirostomum sp.	5940	20.33
		Tokophrya sp.	2970	10.11
		Vorticella mayerii	5940	20.22
	Rotifer	*Keratella tropica*	7260	44.00
		Lepadella patella	3630	22.00
		Camptocercus sp.	5610	34.00
	Crustacean	*Daphnia* sp.	660	100.00

Protozoans are excellent tools to assess both pollution and toxicity **(Nicolau *et al*., 2001)**. They respond quickly to changes in the environment and many of them have tolerance to extreme environmental conditions **(El-Tohamy *et al.*, 2024)**. Protozoans were the most abundant zooplankton group recorded by **Davies and Otene (2009)** in their study of Minichinda Stream in Port Harcourt, Rivers State, Nigeria—an area heavily impacted by anthropogenic activities. *Vorticella* species are considered excellent bioindicators of ecosystem health due to their widespread presence in freshwater environments and their sensitivity to water quality changes **(Gendron, 2018)**. According **to Perez-Uz *et al.* (2010)**, *Vorticella* spp. tend to thrive in aquatic habitats characterized by high levels of organic matter and limited nitrogen cycling capacity. The abundance of *Vorticella mayeri* observed at the upstream and point of discharge stations in this study could be linked to both the human activities occurring around the upstream area and the organic-rich nature of the pharmaceutical effluent. Similarly, *Vorticella mayeri* was also reported downstream of the Osere Stream, which receives detergent effluent in Ilorin **(Adeyemi-Ale *et al.*, 2014)**.

Euglypha tuberculata, another protozoan species identified in this study, is known for its wide environmental tolerance and ability to survive in diverse aquatic habitats **(Mostafa *et al.*, 2023)**.

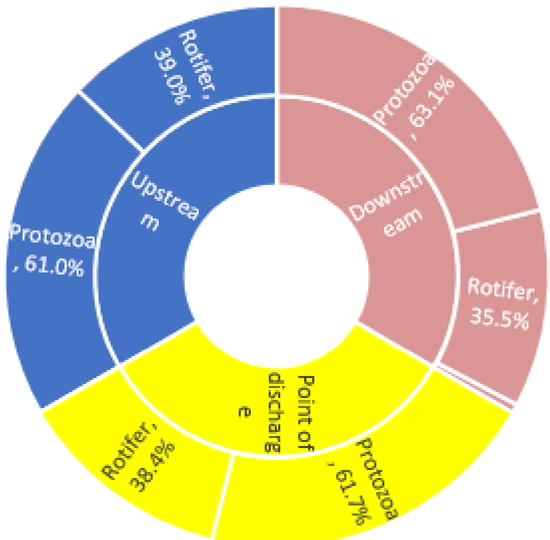

Fig. 3. Percentage composition of zooplankton during the sampling period

3.3. Principal component analysis (PCA of physico-chemical qualities and sampled points

The PCA of physico-chemical qualities of the three sampled locations showed that Cr, Co, Pb and Ni were higher at the downstream than upstream, while Fe, Zn, Do and Mn were higher at the upstream. At the point of discharge, temperature, Cu and pH were inversely proportional to the concentration of Cr, Cu, pH, Ni, Fe, Zn, Mn and DO at both downstream and upstream (Fig. 4).

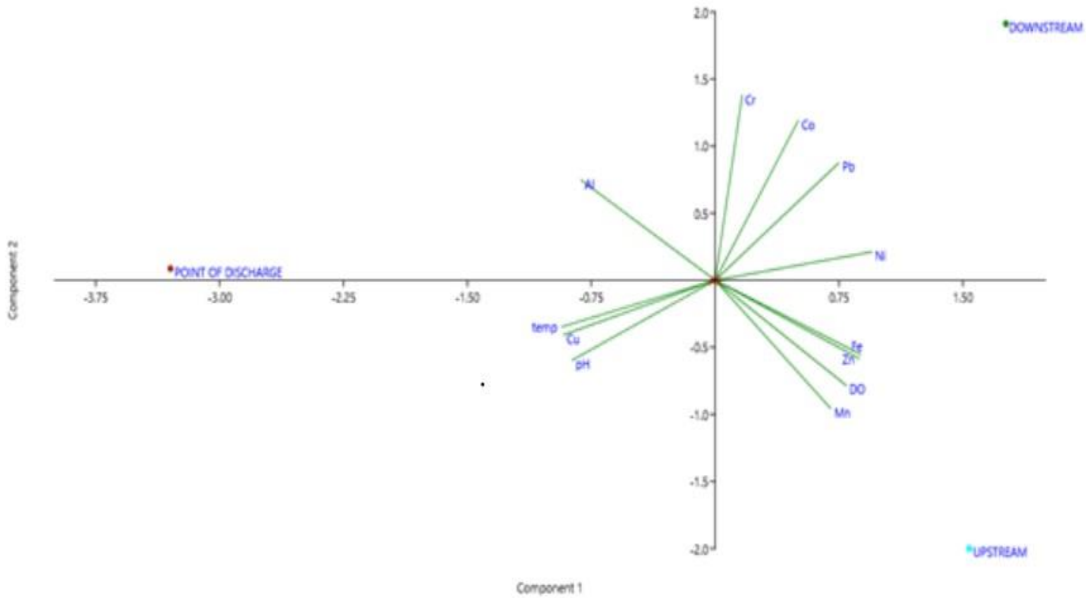

Fig. 4. PCA plots of physico-chemical qualities and sampled points

3.4. The relationships between plankton abundance and physico-chemical variables

The Canonical correspondence analysis (CCA) ordination diagram showed the relationship existing between phytoplankton and the physico-chemical variables. The following variable, Al, temp., Cu and pH showed to have a negative effect on the abundance of Cyanophyceae, while Cr, Cu, Pb, Ni, Fe, Zn, DO and Mn showed to have a positive effect on the abundance of Cyanophyceae. Meanwhile, Bacillariophyceae abundance increased with increasing values with the following variables, Ni, Fe, Zn, DO, Mn but decreased with increasing values in Al, temp, Cu and pH (Fig. 5).

The CCA ordination diagram showed the relationship existing between zooplankton and the physico-chemical variables. Decrease in pH and Cu values positively affected *Spirostomum* sp. (Spi), *Askenasia volvox* (Asv), *Arcela costata* (Arc) and *Euglypha tuberculate* (Eut) while increase in Mn, DO, Zn, Fe, Ni and Pb values showed negative effect on their abundance. Increase in Cr and Co positively affected the abundance of *Daphnia* sp. (Das), *Colopoda* sp. (Cos), *Carchesium* sp. (Cas) and *Tokophry* sp. (Tos) and they were negatively affected with increasing values of temperature, pH and Cu (Fig. 6).

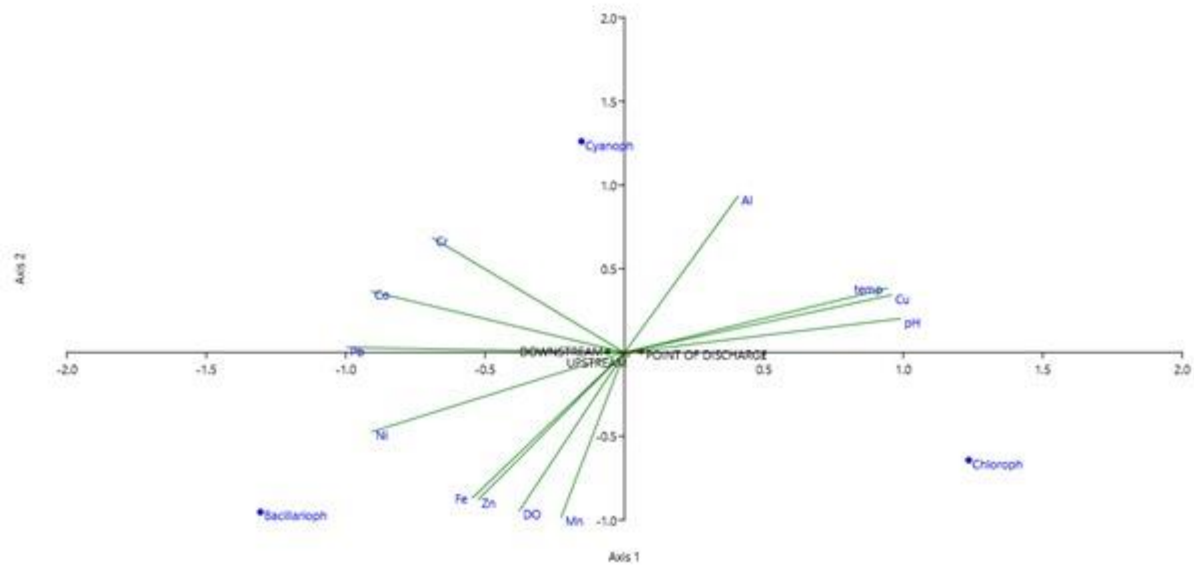

Fig. 5. CCA plot of phytoplankton and environmental variables

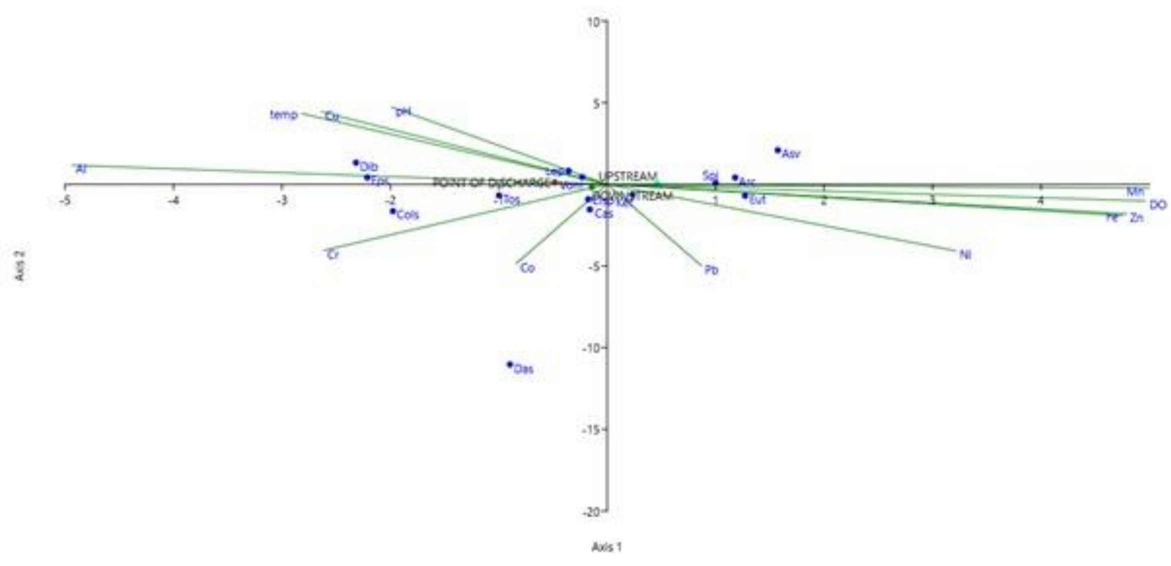

Fig. 6. CCA plot of zooplankton and environmental variables

3.5. Relationships between physico-chemical variables and plankton taxa

The Pearson correlation coefficients (r) of the physico-chemical variables and both the phytoplankton and zooplankton taxa of Okun stream are shown in Table (5). Significant influences were shown by some physicochemical variables on some plankton taxa. Moderate positive correlations were observed between some physico-chemical variables of water and some phytoplankton and zooplankton. For instance, at $P < 0.01$, DO correlated with Rotifer (r = 0.513); chromium correlated with Chlorophyceae (r = 0.529) and crustacea (r = 0.568); iron correlated with Cyanophyceae (r = 0.456) and Chlorophyceae (r = 0.479); nickel correlated with Cyanophyceae (r = 0.435), Chlorophyceae (r = 0.522), Rotifer (r = 0.478) and Crustacea (r = 0.491); Zn correlated with Chlorophyceae (r = 0.441), Protozoa (r = 0.431) and Rotifer (r = 0.501); and lead correlated with Chlorophyceae (r = 0.462).

Table 5. Correlations (r) between the physico-chemical variables and plankton taxa of Okun stream, Ilorin

Plankton taxa/ Physicochemical parameters	**Temp.**	**pH**	**DO**	**Al**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Pb**
Cyanophyceae	-0.124	-0.317	0.314	0.033	0.324	-0.082	0.456^{**}	0.197	0.435^{**}	0.272	0.380^{*}	0.311
Bacillariophyceae	0.109	-0.159	0.322	-0.248	0.061	0.202	0.029	0.072	0.153	0.073	0.163	0.003
Chlorophyceae	-0.022	-0.239	0.161	0.229	0.529^{**}	-0.016	0.479^{**}	0.266	0.522^{**}	0.266	0.441^{**}	0.462^{**}
Protozoa	-0.102	-0.237	0.291	-0.127	0.346^{*}	-0.009	0.335^{*}	0.174	0.351^{*}	0.233	0.431^{**}	0.399^{*}
Rotifer	-0.085	-0.287	0.513^{**}	0.065	0.075	0.012	0.175	0.351^{*}	0.478^{**}	0.532^{**}	$0..501^{**}$	0.262
Crustacea	-0.002	0.124	0.029	0.082	0.568^{**}	-0.010	0.320	-0.145	0.491^{**}	0.097	0.250	0.339^{*}

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

The positive correlation between Rotifers and dissolved oxygen (DO) suggests that Rotifer populations tend to increase as DO levels rise. Similar findings were reported in Ologe Lagoon, Lagos, Nigeria **(Clarke *et al.*, 2013)**, and the Backshore Wetland of Expo Garden, Shanghai **(Yin *et al.*, 2018**). Due to their sensitivity to environmental changes, the species composition of Rotifers serves as a useful indicator of water quality **(Yin *et al.*, 2018)**. A similar correlation was also observed between iron and Cyanobacteria **(Rosales & Rollon, 2011)**. Cyanobacteria, known for their adaptability, respond readily to variations in environmental factors such as light, temperature, and exposure to metal ions; iron, in particular, plays a role in promoting their growth.

At a significance level of $P < 0.05$, weak positive correlations were observed between Protozoa and the metals chromium (r = 0.346), iron (r = 0.335), nickel (r = 0.351), and lead (r = 0.399). In addition, cobalt and nickel showed weak correlations with Rotifers and Protozoa, respectively (r = 0.351). Zinc also correlated weakly with Cyanophyceae (r = 0.380), while lead showed another weak correlation with Protozoa (r

= 0.339). In contrast, **Ibrahim and Abullahi (2008)** reported a negative correlation between lead and zooplankton in the Challawa River, Kano State, Nigeria.

3.6. Diversity indices of plankton

The three sampling locations showed similar phytoplankton diversity, as reflected in the values of Simpson's dominance index (D) (Table 6). Despite the close values, the downstream station exhibited the highest phytoplankton diversity. In contrast, the Simpson's index for zooplankton indicated that the point of discharge was the most diverse among the three sites.

Shannon's diversity index is a widely used metric for assessing both species abundance and richness. According to **Hammer *et al.* (2001)**, it ranges from 0 (indicating a single dominant taxon) to higher values representing greater diversity. It also serves as a proxy for pollution status: index values below 1 indicate heavy pollution, values between 1 and 3 suggest moderate pollution, and values above 3 signify clean water **(William & Dorris, 1968; Ajayan & Ajitkumar, 2015; Al-Taee & Mahmoud, 2023; Anyanwu *et al.*, 2023)**. Based on this classification, the Shannon indices for phytoplankton—1.09 (upstream), 1.084 (point of discharge), and 1.075 (downstream)—suggest that the Okun Stream is moderately polluted. However, the Shannon indices for zooplankton—0.6689 (upstream), 0.6657 (point of discharge), and 0.7184 (downstream)—fall below 1, indicating that the stream is heavily polluted in terms of zooplankton diversity. As noted by **Nolan and Callahan (2005)**, higher Shannon values reflect greater species diversity. Therefore, among phytoplankton, the upstream site was the most diverse, while among zooplankton, the downstream site showed the greatest diversity (Table 6).

Species richness was assessed using both Menhinick and Margalef indices. The upstream station recorded the lowest phytoplankton richness, with values of 0.007577 (Menhinick) and 0.1672 (Margalef). For zooplankton, the point of discharge showed the lowest richness, with corresponding values of 0.008528 and 0.1706.

Equitability (or evenness) was also evaluated using Simpson's index, expressed as a proportion of its maximum value, with results ranging from 0 to 1 (where 1 indicates perfect evenness). The phytoplankton equitability values—0.9918 (upstream), 0.9868 (point of discharge), and 0.9789 (downstream)—suggest a relatively even distribution of species across all stations. In contrast, the zooplankton equitability value at the downstream site (0.6539) indicates uneven species distribution.

Table 6. Diversity indices of plankton in Okun Stream

Diversity indices	Upstream		Point of Discharge		Downstream	
	Phytoplankton	**Zooplankton**	**Phyto**	**Zoopl**	**Phyto**	**Zoopl**
Dominance_D	0.3393	0.5241	0.3426	0.5272	0.3494	0.5244
Simpson_1-D	0.6607	0.4759	0.6574	0.4728	0.6506	0.4756
Shannon_H	1.09	0.6689	1.084	0.6657	1.075	0.7184
Evenness_e^H/S	0.991	0.976	0.9856	0.973	0.9771	0.6837
Brillouin	1.089	0.6688	1.084	0.6656	1.075	0.7182
Menhinick	0.007577	0.008597	0.008528	0.009547	0.008472	0.01391
Margalef	0.1672	0.09175	0.1706	0.09355	0.1704	0.1861
Equitability_J	0.9918	0.965	0.9868	0.9604	0.9789	0.6539
Fisher_alpha	0.2228	0.1568	0.2271	0.1597	0.2269	0.247
Berger-Parker	0.3874	0.6098	0.384	0.6165	0.4368	0.6312

Note: Phytp is phytoplankton
Zoopl is zooplankton

CONCLUSION

The combination of anthropogenic activities around Okun Stream and the discharge of untreated pharmaceutical effluents likely contributed to the reduced abundance of zooplankton, indicating that the stream is experiencing pollution stress. The low dissolved oxygen levels, along with the dominance of pollution-indicator phytoplankton such as Cyanophyceae, further confirm the deteriorating water quality.

To mitigate these impacts, the pharmaceutical company must implement proper effluent treatment protocols before discharging waste into the stream. Additionally, local residents should be discouraged from engaging in activities that contribute to waste influx, such as indiscriminate dumping and unsustainable land use practices near the stream. These measures are essential for preserving the aquatic fauna and maintaining the ecological integrity of the Okun Stream.

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