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Response of Zooplankton Community in the Nile River to Contaminants from Waste Water Treatment Plants? With a Special Reference to Caffeine and Zinc at Assiut Government, Egypt

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ABSTRACT

The negative impact of point sources of pollution such as effluents from wastewater treatment plants (WWTPs) is a significant problem for freshwater ecosystems. Zooplankton can be used as indicators of the ecological status in rivers. Therefore, this research aimed to investigate the effects of contaminants resulting from WWTPs discharges on zooplankton community in the River Nile, Assiut, Egypt. Four different collecting sites have been selected to achieve the present study. The collecting samples of water, sediment and plankton were carried out in the summer (31st of July 2022) and the winter (2nd of February 2023). A total of 30 zooplankton taxa have been recorded; the maximum number of zooplankton structure was exposed by Rotifers (43.33%) followed by Meroplankton (20%), Protozoa (16.67%), Cladocera (13.33%), and least by Copepoda (6.67%). In this study, some species of zooplankton disappeared or become rare in the plankton structure in S (WWTPs drain) and J (where WWTPs mixed with River water) sites compered to R (upstream before the WWTPs discharge) and A (downstream after the WWTPs discharge) sites. Overall, the zooplankton structure of the river showed a diverse spatial distribution due to the influence of WWTPs discharge. This study highlights unprecedented information to understand how caffeine, Zn and some other physicochemical parameters may interact with aquatic environment, using the zooplankton commonly found in the aquatic habitat. Several WWTP treatment systems must be implemented or improved to reduce the discharge of contaminants from these point sources into the Nile River.

INTRODUCTION

Freshwater rivers offer humans with a variety of ecosystem facilities, such as water supply, fish production, biogeochemical cycles, energy and recreation, nevertheless numerous human activities, including wastewater discharges and WWTPs, pose a threat to them [1, 2, 3]. River ecosystems are frequently impacted by pollution from point

sources, such as wastewater treatment plants (WWTPs) [4, 5], particularly in urban area [2, 3].

Any alteration in environmental factors and water quality disturbs animals living in the water ecosystems. In most cases, its effects cause damage to the natural biological communities as well as to individual species and population [6]. Species gatherings in aquatic environments redirect interactions between organisms and the abiotic ecological factors, as well as among organisms [7]. Plankton species are one of the main valued bioindicators of environmental conditions meanwhile they are ecological indicators of many environmental conditions [8]. Zooplankton represents the major components of aquatic invertebrate fauna and main groups of plankton that are extremely sensitive to environmental differences.

Zooplankton are heterotrophic, microscopic animals and they mark a good assemblage of minute floating animals that form the food webs in any aquatic ecosystems. Due to their drifting nature, shorter life span, large density, high species diversity, and different tolerance to pressure, they are used as a bioindicators for monitoring the water quality, trophic status, and pollution level of water bodies [9, 10]. Zooplankton communities are cosmopolitan in nature, thus changes in their community strcutre can provide status on how influence as disturb the aquatic ecosystem.

Wastewater treatment plants (WWTPs) are measured a key input path for micropollutants into aquatic ecosystems [11, 12]. Biological data, together with physical and chemical data represent an important tool to evaluate water quality in rivers. Therefore, the aim of the present work is to study the changes in abundance and diversity of zooplankton community according to the discharge of WWT in the Nile River. Additionally, the composition, distribution, and species diversity of invertebrates in aquatic ecosystems depend mainly on seasonal variations [13]. Thus, in the present study, two extreme seasons were selected for sampling: summer and winter. Choosing these two seasons may have contributed to a covering of more distinct variations in invertebrate community structure, which could be associated with winter and summer periods.

MATERIALS AND METHODS

The study area and sampling sites

The water, sediment and plankton samples have been collected in triplicates from four sites (Figure 1) on 31st of July 2022 (summer) and 2nd of February ٢٠٢٣ (winter) from Assiut city, Egypt (27º 14′ N, 31º 11′ E) as follow: 1- Source site (S): a canal where treated water is settled from Arab El-Madabegh WWTP effluent. 2- Junction site (J): Where treated water mixed with Nil River water. 3- Reference site (R): River Nile upstream before the WWTP discharge. 4- After site (A): River Nile downstream after the WWTPs discharge.

Analysis of physicochemical parameters

During sampling, some physicochemical parameters includeing air and water temperature (AT and WT, °C), electrical conductivity (Cond, µSCm^{-1}), water pH, total dissolved solids (TDS, ppm), Transparency (Turb, cm), and dissolved oxygen (DO, mgL^{-1}) were measured by portable water quality instruments. In laboratory sediment total organic matter (OM, %) was measured [14]. According to APHA-AWWA-WPCF [15],

water phosphate (Po4, mgL^{-1}), nitrate (No3, mgL^{-1}) and ammonia (NH4, mgL^{-1}) were measured spectrophotometerically.

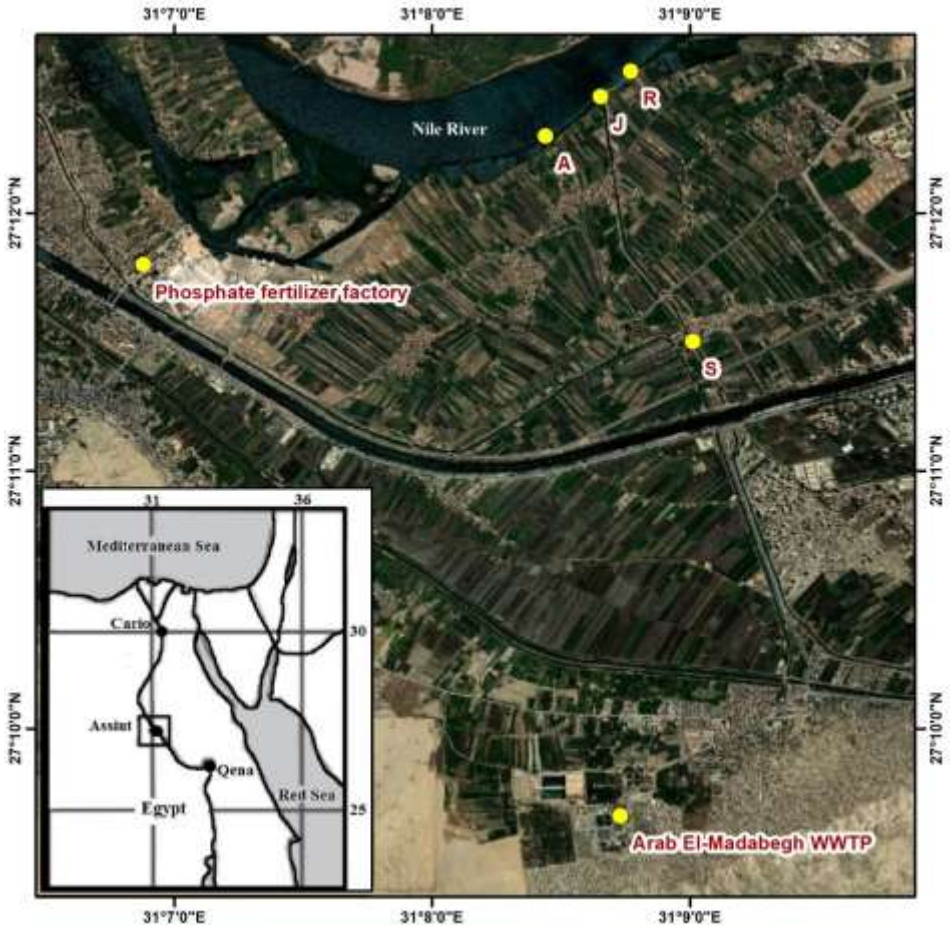

Figure 1. Maps show the studied sites at Assiut city, Egypt. (S): source site, (J): mixed Site, (R): Refrence site (River Nile upstream before the WWTP discharge) and (A): After site (River Nile downstream after the WWTPs discharge).

Determination of zinc and caffeine concentrations

According to Jackson [16], Zn concentrations in water and sediment samples were assessed. Caffeine concentrations in sediment and water samples were measured by a single flow-through UV multiparameter sensor responding with solid phase UV spectrophotometric recognition according to Vidal *et al*. [17]

Collection of Zooplankton

For quantitative analysis of zooplankton, 30 liters of water was filtered through the standard plankton net of 55 μm. The filtrate samples were transferred to labeled polyethylene vials, fixed immediately with 5% formalin, and stained with rose Bengal. Three replicates were collected from each site during the investigated season.

In the laboratory, each sample was reduced to a concentrated volume of 40 ml. For identification and counting of zooplankton species, 3 subsamples (one ml for each). The subsample (1 ml) was taken with a wide-mouthed pipette and poured into the counting slide. After allowing for some time they were examined, identified, and counted under an Optic Research Microscope. The zooplankton identification was made referring to the standard manuals, textbooks, and monographs [18, 19, 20, 21]. The number of zooplankton existing in 1 liter of water was calculated accordin to [22] using the following equation:

$$\mathbf{N} = \frac{n * v}{V}$$

Where, N = The number of zooplankton in liter of water; n = mean number of zooplankton in 1 ml of subsample; v = Volume (ml) of zooplankton concentrated and V = Volume (liter) of total water filtered. The zooplankton abundance was calculated and expressed in number per cubic meter. The dominance classification of the collected zooplankton was determined according to Engelmann's classification [23], subrecedent (bellow 1.3%), recedent (1.3-3.9%), subdominant (4-12.4%), dominant (12.5-39.9%), eudominant (40-100%).

Data analysis

Excel Office 2013, IBM SPSS Statistics (Version 20) and PAST4 program performed data summary and analysis for the collected data. Primer5 program was used for biodiversity parameters calculations. The collected zooplankton data was used to calculate the Wetland Zooplankton Index (WZI) according to Lougheed and Chow-Fraser [24]. The index was calculated using weighted means in the following fromula:

$$\mathbf{WZI} = \frac{\sum_{i=1}^{n} YiTiUi}{\sum_{i=1}^{n} YiTi}$$

Where Yi is the presence of plankter i, Ti is the tolerance (1–3), and Ui is the optimum (1–5). The index range from one (revealing of low quality) to five (revealing of high-quality wetland).

RESULTS

1. Physicochemical parameters

At the four study sites, the air temperature changes ranged from 15.57°C to 38.29°C in winter and summer, respectively. While, temperature of water ranged from 19.43°C in winter to 30.43°C in summer. The water pH ranged from 6.28 (summer at S site) to 8.23 (winter at R site). The mean electrical conductivity of the analyzed water samples fluctuated between 40 to 46 μSCm^{-1} while, the TDS was within the range between 131.33 to 561.00 ppm. Dissolved oxygen (DO) ranged from 1.08 to 6.87 mgL^{-1} at J and R sites in summer, respectively while DO ranged from 1.33 mgL^{-1} (winter at site S) to 6.70 mgL^{-1} (winter at site R).

The sediment OM values were ranged between 23.52% at S site in winter, and 1.94% at R site in summer. The concentrations of Po_4 in water from the four study sites

ranged from 0.10 mgL^{-1} to 7.43 mgL^{-1}, while the concentrations of No_3 ranged between 13.86 to 63.00 mgL^{-1}. NH_4 values ranged from 9.15 to 32.84 mgL^{-1} (in summer at R and S sites, respectively). On the other hand, in winter season NH_4 ranged from 21.90 to 54.36 mgL^{-1} at A and J sites, respectively. The differences in water caffeine (WCaf) flactuated between 5.73µg L^{-1} to 53.85 μgL^{-1} (at S site in winter and S site in summer, respectively), while ehe sediment caffeine concentrations (SCaf) ranged from 0.14 $mgKg^{-1}$ to 1.54 $mgKg^{-1}$ (at R site during winter to at S site during summer, respectively). The zinc concentration in water (WZn) flactuated from 0.08 mgL^{-1} to 0.22 mgL^{-1} (at S and J sites in winter and A site in winter, respectively), whereas sediment zinc ranged between 28.59 $mgKg^{-1}$ to 155.02 $mgKg^{-1}$ (at J site in winter and at J in summer, respectively).

2. Abundance of zooplankton:

A total of 30 taxa have been recorded, mainly for Protozoa (16.67 %), Rotifera (43.33 %), Cladocera (13.33 %), Copepoda (6.67 %) and Meroplankton (20 %). The recorded zooplankton were divided into dominancy classes; eudominant (12 species)، dominant (14 species), and subdominant (4 species) (Table 1). The protozooplankton were represented by *Arcella discoides*, *Centropyxis* sp., *Ceratium* sp., *Carchesium* sp. and *Euglena oxyuris*. All the genera seemed accompanying with the all sites samples, except for *Centropyxis* sp., *Ceratium* sp. and *Euglena oxyuris* in S site; *Centropyxis* sp. in J site and *Arcella discoides* in A site (Table 2).

The rotifer community dominated by seven genera: *Asplanchna*, *Brachionus*, *Keratella*, *Lecane*, *Lepadella*, *Polyarthra* and *Rotaria*. The *Brachionus* genus was represented by two, *Lecane* by five and *Lepadella* by two species. All the genera seemed accompanying with the all sites samples, except for *Lecane lunaris,* in R site; *Keratella cochlearis*, *Lecane bulla*, *Lecane cornuta*, *Lecane inermis*, *Lepadella akrobeles*, *Lepadella ovalis* and *Polyarthra vulgaris*, in S site; *Lecane cornuta*, *Lepadella akrobeles* and *Lepadella ovalis* in J site; and *Lecane inermis*, *Lepadella akrobeles* and *Lepadella ovalis* in A site (Table 2).

Copepoda were represented by Copepodite stage and Nauplius larva. All the taxa seemed accompanying with the all investigated sampling sites. The community of Cladocera was represented by four genera (*Bosmina longirostris*, *Chydorus sphaericus*, *Daphnia sp. and Moina micrura*). All the genera seemed accompanying with the all sampling sites, except for *Bosmina longirostris* and *Daphnia sp.* in J site. Meroplankton were represented by sex taxa: Chironomus larva, free living nematode, *Hydra* sp., *Cypris* sp., Water mite and Water spider. All the taxa appeared associated with the all investigated sampling sites, except for *Cypris* sp. and Water spider in R site; *Hydra* sp., *Cypris* sp. and Water spider in S site; *Hydra* sp. in J site and Chironomus larva and *Hydra* sp. in A site (Table 2).

Figure (2) shows the percentage composition of zooplankton groups at different investigated communities. Among the all zooplankton Rotifera forming the dominant group followed by Meroplankton, Protozoa, Cladocera, and Copepoda. Generally, the total zooplankton density was higher in winter than in summer at all studied sites. The density of zooplankton groups: Rotifera, Meroplankton, Protozoa, and Copepoda, varied significantly [($F= 13.038$, $p< 0.001$), ($F= 8.133$, $p< 0.001$), ($F= 7.006$, $p= 0.001$) and ($F= 3.920$, $p= 0.011$)] between investigated sites. However, no significant differences were

found in the total density of Cladocera between sampling sites (F= 1.958, *p*= 0.126). Figure (3) represent the average densities of zooplankton groups in each study communities and their significant differences.

Table 1. Taxonomic composition of the collected zooplankton during the presented study with their percentages of frequency and dominance (F: frequency, D: dominance, S.Dom: Subdominant, Dom: Dominant, E.Dom: Eudominant).

Zooplankton taxa		F	F%	D	Zooplankton taxa		F	F%	D
Protozoa	*Arcella discoides*	6	25.0	Dom	**Rotifera**	*Lepadella ovalis*	1	4.2	S.Dom
	Centropyxis sp.	3	12.5	Dom		*Polyarthra vulgaris*	7	29.2	Dom
	Ceratium sp.	12	50.0	E.Dom		*Rotaria rotatoria*	24	100.0	E.Dom
	Carchesium sp. (Part of colony)	15	62.5	E.Dom	**Cladocera**	*Bosmina longirostris*	10	41.7	E.Dom
	Euglena oxyuris	7	29.2	Dom		*Chydorus sphaericus*	6	25.0	Dom
Rotifera	*Asplanchna priodonta*	19	79.2	E.Dom		*Daphnia* sp.	3	12.5	Dom
	Brachionus calyciflorous	15	62.5	E.Dom		*Moina micrura*	6	25.0	Dom
	Brachionus plicatilis	19	79.2	E.Dom	**Copepoda**	Copepodite stage	10	41.7	E.Dom
	Keratella cochlearis	7	29.2	Dom		Nauplius larva	14	58.3	E.Dom
	Lecane bulla	6	25.0	Dom	**Meroplankton**	Chironomus larva	8	33.3	Dom
	Lecane cornuta	4	16.7	Dom		Free living nematode	24	100.0	E.Dom
	Lecane inermis	3	12.5	Dom		*Hydra* sp.	1	4.2	S.Dom
	Lecane luna	10	41.7	E.Dom		*Cypris* sp. (Ostracoda)	2	8.3	S.Dom
	Lecane lunaris	7	29.2	Dom		Water mite	14	58.3	E.Dom
	Lepadella akrobeles	1	4.2	S.Dom		Water spider	4	16.7	Dom

Two-way PERMANOVA for zooplankton taxa at the examined sites in summer and winter seasons showed significant differences (Table 3) between sites, seasons and interaction, respectively. PERMANOVA Pair-wise tests for changes between the examined sites according to abundance of zooplankton taxa during the period of study has showed significant differences (*p*= 0.0025, *p*= 0.0016, *p*= 0.0027 and *p*= 0.0033) between R and S, R and J, S and A, and J and A, respectively. However, no significant differences (*p*= 0.339 and *p*= 0.483) were found between sampling sites, R and A, and S and J, respectively (Table 4).

Table 2. Mean values of zooplankton density (D: 10^3Ind/m^3) and relative abundance (%) for different samples.

Zooplankton taxa	R				S				J				A			
	Sum		Win		Sum		Win		Sum		Win		Sum		Win	
	D	%	D	%	D	%	D	%	D	%	D	%	D	%	D	%
Arcella discoides	0.67	9.10	0.00	0.00	0.30	100.00	0.00	0.00	5.11	85.17	0.00	0.00	0.00	0.00	0.00	0.00
Centropyxis sp.	0.00	0.00	0.67	2.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	9.42	0.00	0.00
Ceratium sp.	5.56	75.79	29.33	89.78	0.00	0.00	0.00	0.00	0.00	0.00	2.22	25.61	5.33	74.96	14.44	78.31
Carchesium sp. (Part of colony)	0.00	0.00	2.67	8.17	0.00	0.00	11.78	100.00	0.00	0.00	6.44	74.28	0.67	9.42	4.00	21.69
Euglena oxyuris	1.11	15.16	0.00	0.00	0.00	0.00	0.00	0.00	0.89	14.83	0.00	0.00	0.44	6.19	0.00	0.00
Protozoa	**7.33**	**21.14**	**32.67**	**22.11**	**0.30**	**0.66**	**11.78**	**17.21**	**6.00**	**13.24**	**8.67**	**12.67**	**7.11**	**24.43**	**18.44**	**12.48**
Asplanchna priodonta	4.22	20.21	25.56	23.00	0.59	2.18	1.56	4.03	3.33	15.14	0.22	0.61	0.89	6.07	44.00	36.94
Brachionus calyciflorous	0.00	0.00	4.67	4.20	0.89	3.29	13.33	34.47	0.67	3.05	13.11	36.64	0.22	1.50	2.00	1.68
Brachionus plicatilis	9.56	45.74	37.11	33.40	0.00	0.00	6.44	16.65	1.33	6.05	10.22	28.56	2.89	19.70	3.33	2.80
Keratella cochlearis	0.00	0.00	24.89	22.40	0.00	0.00	0.00	0.00	0.00	0.00	1.33	3.72	0.00	0.00	25.11	21.08
Lecane bulla	0.44	2.13	0.00	0.00	0.00	0.00	0.00	0.00	0.89	4.05	0.00	0.00	0.44	3.00	0.00	0.00
Lecane cornuta	0.89	4.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.56	10.63	0.00	0.00
Lecane inermis	0.89	4.26	0.00	0.00	0.00	0.00	0.00	0.00	0.22	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Lecane luna	1.33	6.38	0.22	0.20	0.00	0.00	0.22	0.57	0.67	3.05	0.00	0.00	3.11	21.20	0.22	0.18
Lecane lunaris	0.00	0.00	0.00	0.00	7.93	29.33	0.00	0.00	3.33	15.14	0.00	0.00	0.44	3.00	0.00	0.00
Lepadella akrobeles	0.22	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lepadella ovalis	0.22	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polyarthra vulgaris	0.00	0.00	7.33	6.60	0.00	0.00	0.00	0.00	0.00	0.00	0.89	2.49	0.00	0.00	26.44	22.20
Rotaria rotatoria	3.11	14.89	11.33	10.20	17.63	65.20	17.11	44.25	11.56	52.55	10.00	27.95	5.11	34.83	18.00	15.11
Rotifera	**20.89**	**60.25**	**111.11**	**75.18**	**27.04**	**59.82**	**38.67**	**56.50**	**22.00**	**48.53**	**35.78**	**52.27**	**14.67**	**50.40**	**119.11**	**80.60**
Bosmina longirostris	0.89	66.84	1.11	62.36	0.15	50.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	41.57	1.33	74.72
Chydorus sphaericus	0.22	16.72	0.44	24.72	0.00	0.00	0.22	50.00	0.00	0.00	0.22	100.00	0.22	8.24	0.00	0.00
Daphnia sp.	0.00	0.00	0.22	12.36	0.15	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	12.36
Moina micrura	0.22	16.72	0.00	0.00	0.00	0.00	0.22	50.00	0.22	100.00	0.00	0.00	1.33	49.81	0.22	12.36
Cladocera	**1.33**	**3.84**	**1.78**	**1.20**	**0.30**	**0.66**	**0.44**	**0.64**	**0.22**	**0.49**	**0.22**	**0.32**	**2.67**	**9.17**	**1.78**	**1.20**
Copepodite stage	1.11	71.22	0.00	0.00	0.00	0.00	0.89	28.62	0.44	100.00	0.00	0.00	1.56	63.93	0.22	32.84
Nauplius larva	0.44	28.50	0.67	100.00	0.00	0.00	2.22	71.38	0.00	0.00	4.00	100.00	0.89	36.48	0.44	65.67
Copepoda	**1.56**	**4.50**	**0.67**	**0.45**	**0.00**	**0.00**	**3.11**	**4.54**	**0.44**	**0.97**	**4.00**	**5.84**	**2.44**	**8.38**	**0.67**	**0.45**
Chironomus larva	0.89	24.96	0.67	42.95	0.81	4.61	0.00	0.00	0.22	1.32	0.00	0.00	0.00	0.00	0.00	0.00
Free living nematode	2.00	56.17	0.89	57.05	14.22	80.98	14.00	96.95	13.33	79.96	16.89	85.39	2.00	90.09	7.11	91.39
Hydra sp.	0.22	6.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cypris sp. (Ostracoda)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	1.32	0.00	0.00	0.22	9.91	0.00	0.00
Water mite	0.44	12.49	0.00	0.00	2.52	14.35	0.44	3.05	2.89	17.34	0.89	4.50	0.00	0.00	0.44	5.66
Water spider	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	10.11	0.00	0.00	0.22	2.83
Meroplankton	**3.56**	**10.27**	**1.56**	**1.06**	**17.56**	**38.85**	**14.44**	**21.10**	**16.67**	**36.78**	**19.78**	**28.90**	**2.22**	**7.63**	**7.78**	**5.26**
Total	**34.67**		**147.79**		**45.20**		**68.44**		**45.33**		**68.45**		**29.11**		**147.78**	

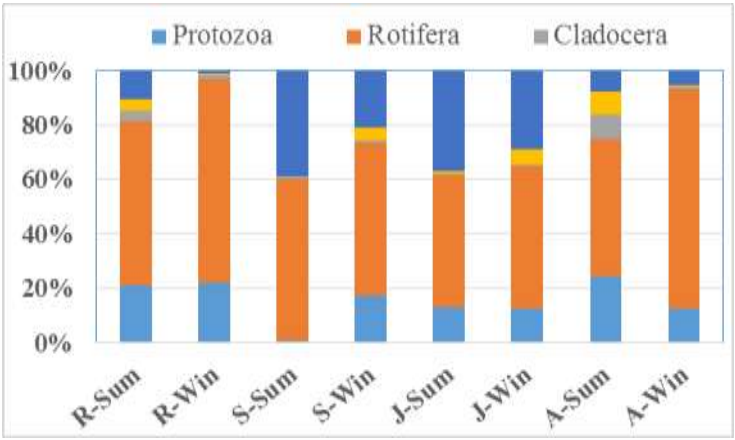

Figure 2. Percentage composition of zooplankton groups at different investigated communities.

Table 3. Two-way PERMANOVA for zooplankton taxa at the studied samples.

Source	Sum of sqrs	df	Mean square	F	p-value
Sites	1.966	3	0.65533	9.273	**0.0001**
Season	1.1157	1	1.1157	15.787	**0.0001**
Interaction	0.96107	3	0.32036	4.5331	**0.0001**
Residual	1.1307	16	0.070671		
Total	5.1735	23			

Table 4. PERMANOVA Pair-wise tests for differences between the studied sites based on abundance of zooplankton taxa.

Sites	R-S	R-J	R-A	S-J	S-A	J-A
F	8.355	5.211	1.088	0.855	5.616	3.802
p-value	**0.0025**	**0.0016**	0.339	0.483	**0.0027**	**0.0033**

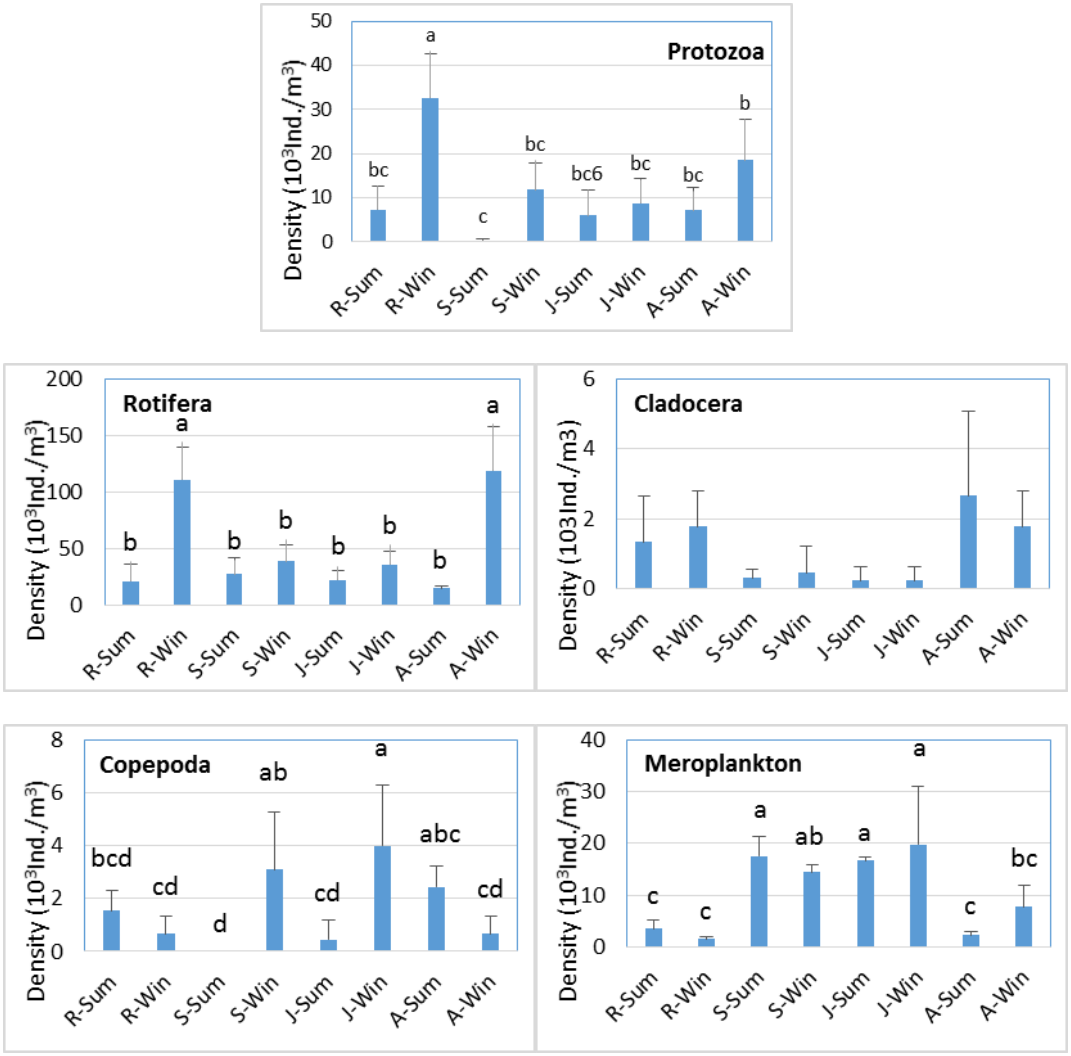

Figure 3. Average densities of zooplankton groups in each study communities. (The similar characters show no significant difference).

2. Biodiversity of zooplankton

The results of various zooplankton biodiversity indices are presented variations between collectd samples**.** During summer and winter seasons, values for biodiversity indices: Shan. diversity (H), Margalef species richness, and species numbers were higher in site R and site A than in dite S and site J (Figure 4). Also, total number of individuals was higher in R and A sites (during winter season) than other investigated sites. ANOVA results for biodiversity indices showed significant differences for zooplankton assemblages at different investigated samples; species numbers (F= 4.782, *p*= 0.005), total number of individuals (F= 11.968 *p*< 0.001), Margalef species richness (F= 5.545, *p*= 0.002), Shan. equitability (J) (F= 3.605, *p*= 0.016) and Shan. diversity (H) (F= 7.11, *p*= 0.001).

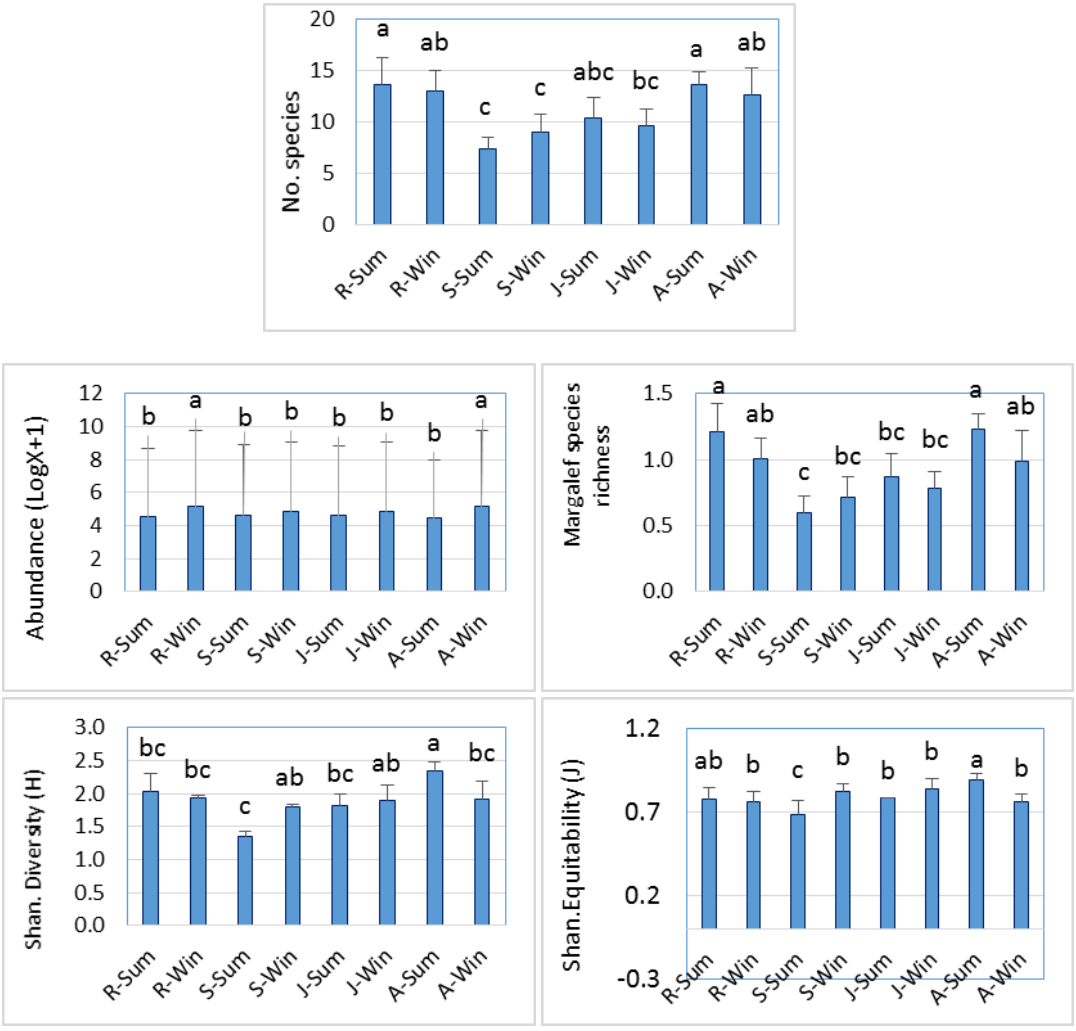

Figure 4. The mean ± standard deviation of diversty indices of the documented zooplankton at different samples collected from the investigated sites during the two different seasons. (The similar characters show no significant difference).

4. Contributions of zooplankton

The relative contributions of zooplankton taxa participating in about 90% of the average dissimilarity between the different sites are shown in Table (5)**.** *Brachionus plicatilis* (15.90%), *Ceratium* sp. (13.28%), free living nematode (12.71%), *Rotaria rotatoria* (12.26%) and *Asplanchna priodonta* (10.08%) are participated with higher dissimilarity (SIMPER) between R and S sites, *Brachionus plicatilis* (16.19%), free living nematode (14.31%), *Ceratium* sp. (13.35%), *Asplanchna priodonta* (10.83%), *Keratella cochlearis* (8.57%) and *Rotaria rotatoria* (7.11%) between R and J sites, *Asplanchna priodonta* (19.44%), *Brachionus plicatilis* (17.46%), *Ceratium* sp. (12.32%), *Keratella cochlearis* (11.97%) and *Polyarthra vulgaris* (9.78%) between R and A sites, *Brachionus calyciflorous* (16.54%), *Rotaria rotatoria* (13.31%), *Ceratium* sp. (11.43%), *Brachionus plicatilis* (10.75%), free living nematode (9.59%), and *Lecane lunaris* (9.35%) between S and J sites, *Asplanchna priodonta* (14.36%), free living nematode (12.08%), *Rotaria rotatoria* (11.94%), *Ceratium* sp. (8.96%), *Polyarthra vulgaris* (8.68%) and *Keratella cochlearis* (8.54%) between S and A sites and *Asplanchna priodonta* (15.16%), free living nematode (13.38%), *Polyarthra vulgaris* (9.02%), *Keratella cochlearis* (8.97%), *Ceratium* sp. (8.52%) and *Rotaria rotatoria* (7.55%) J and A sites.

5. The similarity between the studied zooplankton communities

The similarity between the studied zooplankton communities based on the investigated biodiversity indices, a dendrogram allowed to partition them into four clusters. The first cluster grouped S summer, the second cluster grouped R summer and A summer, third cluster grouped R winter and A winter and fourth cluster grouped J summer, S winter and S summer (Figue 5). Also, Bary-Curtis similarity distance among the investigated zooplankton communities related to the abundance of the effected zooplankton taxa, a dendrogram of hierarchical cluster analysis divided them into five groups (Figure 6**Error! Reference source not found.**): Group 1 comprising R summer; Group 2 comprising A summer; Group 3 comprising S summer and J summer; Group 4 comprising J winter and S winter and group five comprising R winter and A winter.

6. Wetland Zooplankton Index (WZI):

Values of Wetland Zooplankton Index (WZI) for different samples collected from the investigated sites during the two different seasons are shown in Figure (7). In summer season, the WZI was recorded the highest value at A site (3.64) followed by J (3.08), S (2.92) and R (2.88) sites. Whereas, during winter season WZI value was high at A site (2.45) followed by R (2.33), J (2.08) and S (2.05) sites. The average value of WZI was higher in summer than that in winter at the investigated sites. ANOVA for WZI at investigated sites during the two different seasons revealed significant difference ($F= 3.976$, $p= 0.011$) between studied groups (Figure 7).

Table 5. Results of similarity percentages analysis (SIMPER) procedures based on taxa composition between the study sites. The relative contributions of plankton taxa participating in about 90%of the average dissimilarity between the different sites.

Comparisons	Taxa	Relative contributions (%)	Comparisons	Taxa	Relative contributions (%)
R vs.S	*Brachionus plicatilis*	15.90	**S vs. J**	*Brachionus calyciflorous*	16.54
	Ceratium sp.	13.28		*Rotaria rotatoria*	13.31
	Free living nematode	12.71		*Ceratium* sp.	11.43
	Rotaria rotatoria	12.26		*Brachionus plicatilis*	10.75
	Asplanchna priodonta	10.08		Free living nematode	9.59
	Keratella cochlearis	7.84		*Lecane lunaris*	9.35
	Brachionus calyciflorous	6.18		*Arcella discoides*	5.25
	Carchesium sp.	5.41		Nauplius larva	4.14
	Lecane lunaris	3.84		*Asplanchna priodonta*	4.09
	Polyarthra vulgaris	2.26		Water mite	3.08
R vs. J	*Brachionus plicatilis*	16.19		*Ceratium* sp.	2.18
	Free living nematode	14.31	**S vs. A**	*Asplanchna priodonta*	14.36
	Ceratium sp.	13.35		Free living nematode	12.08
	Asplanchna priodonta	10.83		*Rotaria rotatoria*	11.94
	Keratella cochlearis	8.57		*Ceratium* sp.	8.96
	Rotaria rotatoria	7.11		*Polyarthra vulgaris*	8.68
	Brachionus calyciflorous	6.37		*Keratella cochlearis*	8.54
	Carchesium sp.	3.21		*Brachionus calyciflorous*	7.05
	Arcella discoides	2.79		*Carchesium* sp.	6.27
	Polyarthra vulgaris	2.61		*Lecane lunaris*	4.44
	Nauplius larva	2.09		*Brachionus plicatilis*	3.94
	Lecane lunaris	2.08		*Lecane luna*	2.65
R vs. A	*Asplanchna priodonta*	19.44	**J vs. A**	*Asplanchna priodonta*	15.16
	Brachionus plicatilis	17.46		Free living nematode	13.38
	Ceratium sp.	12.32		*Polyarthra vulgaris*	9.02
	Keratella cochlearis	11.97		*Keratella cochlearis*	8.97
	Polyarthra vulgaris	9.78		*Ceratium* sp.	8.52
	Rotaria rotatoria	7.60		*Rotaria rotatoria*	7.55
	Free living nematode	3.05		*Brachionus calyciflorous*	6.70
	Lecane luna	2.24		*Brachionus plicatilis*	5.67
	Carchesium sp.	2.11		*Carchesium* sp.	3.65
	Brachionus calyciflorous	1.97		*Arcella discoides*	3.05
	Lecane cornuta	1.49		*Lecane luna*	2.50
				Nauplius larva	2.33
				Water mite	2.31

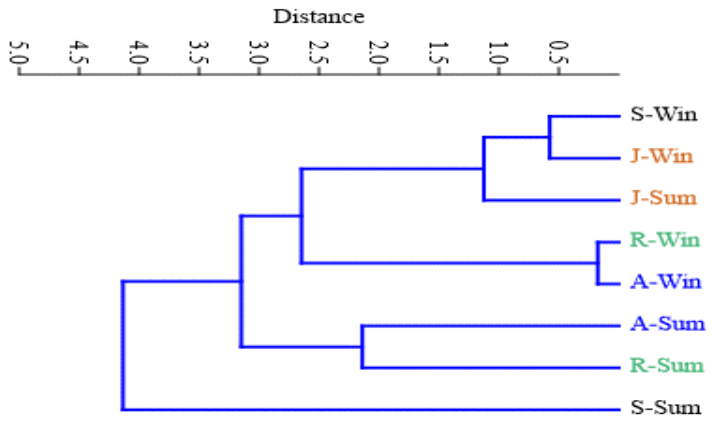

Figure 5. Dendrogram showing the similarity distance between the studied zooplankton communities based on the studied diversity indices (After standardizing the data).

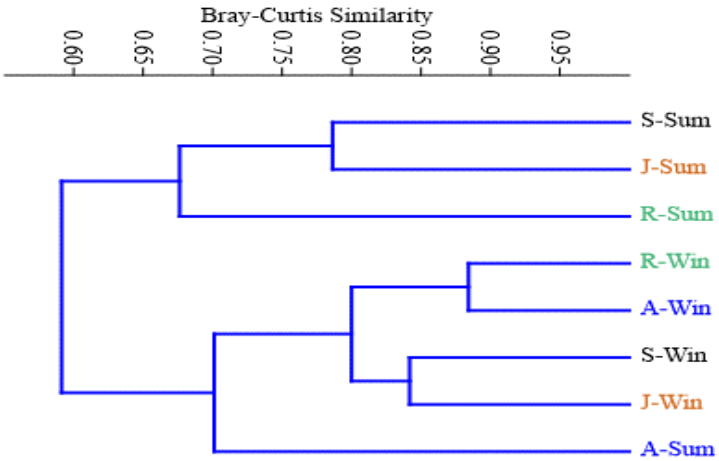

Figure 6. Dendrogram showing the Bary-Curtis similarity between the studied zooplankton communities based on the abundance of the effected zooplankton taxa (Data was transformed to Loge X+1).

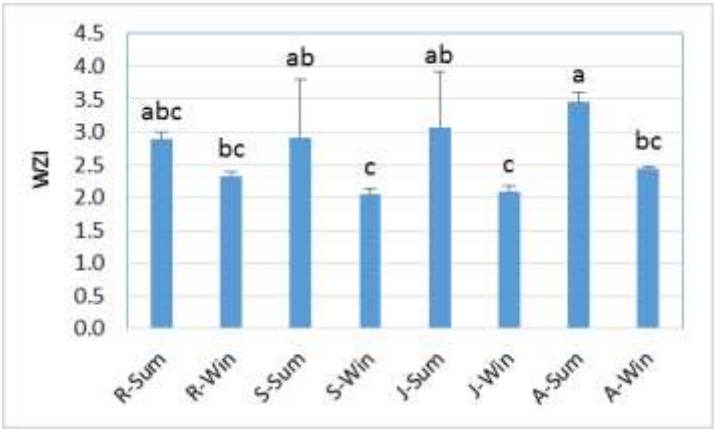

Figure 7. Mean ± standard deviation of Wetland Zooplankton Index (WZI) at investigated sites during the two different seasons (The similar characters for each variable show no significant difference).

7. Response of zooplankton to environmental characteristics

Biplot of CCA on environmental characteristics and zooplankton groups showed that Protozoa and Copepoda are associated with DO, pH, Turb and MO. Moreover, Ptotozoa are more associated with pH, Do and Turb. Cladocera, Rotifera and Meroplankton are associated with AT, WT, TDS, Po_4, No_3, NH_4, Wcaf, Scaf, WZn and SZn, and presented a negative association with pH, DO, and Turb. Rotifera are more associated with air/water temprtures (AT, WT), Cond, Scaf, Wcaf, SZn and WZn, while Meroplankton are more associated with Po_4, TDS, No_3 and NH_4. Most of the investigated environmental characteristics showed strong influences on zooplankton groups (Figure 8**Error! Reference source not found.**).

Also, CCA results of environmental characteristics and biodiversity indices of zooplankton, revealed that Margalef species richness, Shan. Equitability (J) and Shan. Diversity (H) and abundance are associated with MO, TDS, Po_4, No_3, NH_4, Wcaf, Scaf, and SZn. Species numbers and total number of individuals are associated with AT, WT, Cond, DO, pH, Turb and WZn. Total number of individuals are more associated with DO, Turb and pH. DO, pH, Turb, MO, TDS, Po_4, No_3, NH_4 and WZn values have shown strong influences on biodiversity indices of zooplankton (Figure 9).

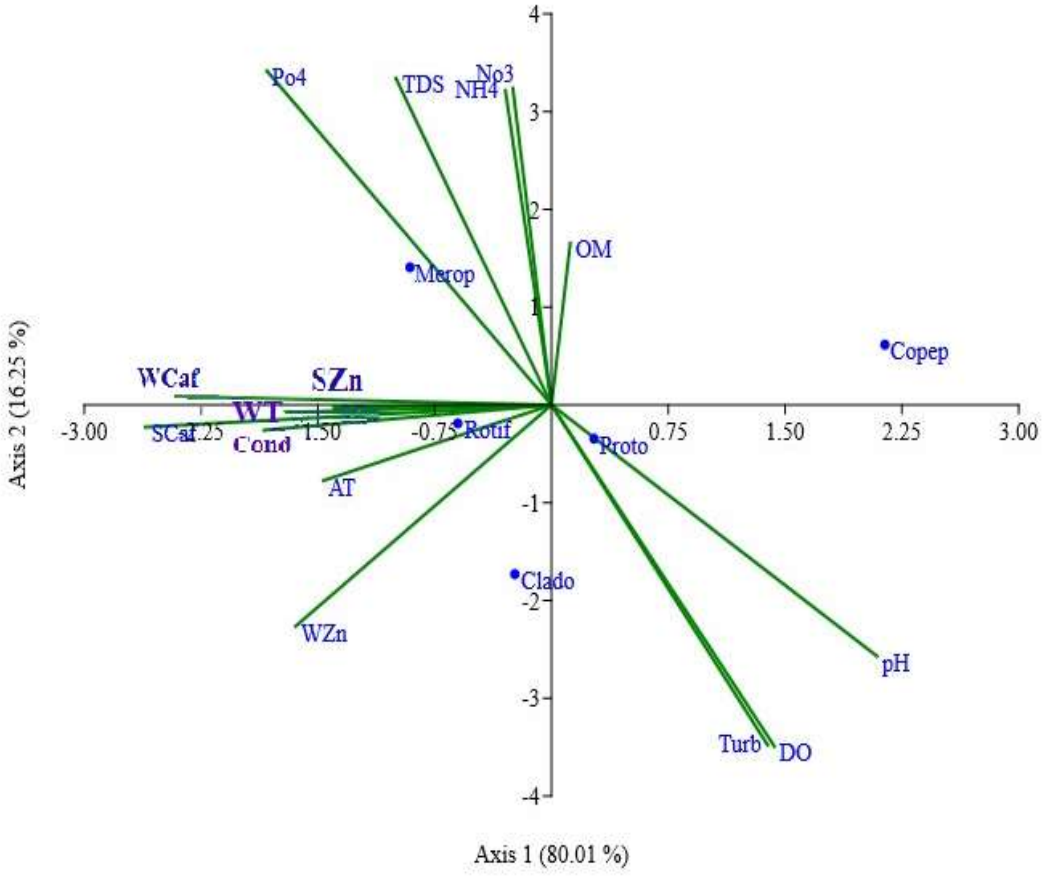

Figure 8. Biplot of the canonical correspondence analysis (CCA) results on environmental characteristics and groups of zooplankton at study sites both studied seasons. (Variables symbolization as mentioned in matrial and methods).

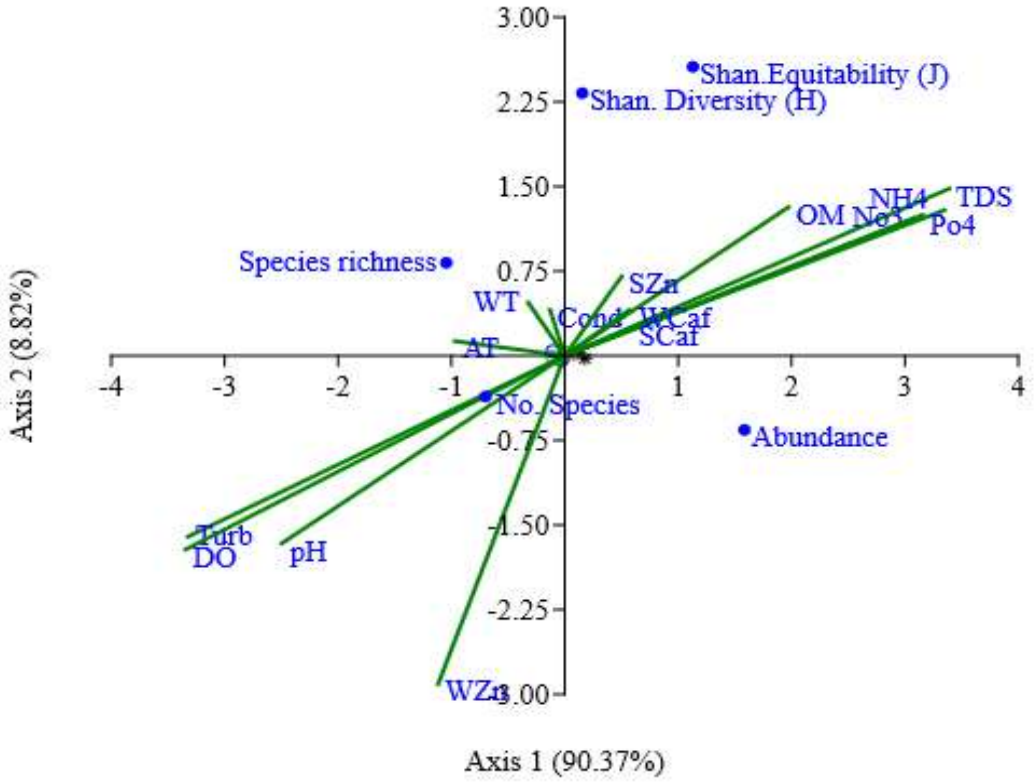

Figure 9. Biplot of the canonical correspondence analysis (CCA) results on environmental characteristics and biodiversity indices of zooplankton a at study sites both studied seasons. (Variables symbolization as mentioned in matrial and methods).

DISCUSSION

Results of the present study showed that zooplankton population density and species composition greatly vary with the physiochemical parameters of the water body at investigated sites. Zooplankton is one amongst the most important biotic organisms of any aquatic ecosystem. Their biodiversity and ecological linkages are reflected important for the health and homeostasis of any ecosystem. They are frequently utilized as environmental condition and trophic status indicators due to their distinctive characteristics [25, 26]. Furthermore, zooplankton communities react swiftly to variations in physical-chemical parameters, which can impact their species richness, increase or decrease their abundance, and induce changes in their diversity [27, 28].

In current study, a total of 30 taxa have been recorded with the maximum share in zooplankton structure was revealed by Rotifers (43.33%) followed by Meroplankton (20%), Protozoa (16.67%), Cladocera (13.33%), and least by Copepoda (6.67%). A relatively high abundance and dominance of Rotifera is reported in several freshwater bodies [29]. This mainly was caused by their short life cycle, the ability to speedily colonize new environments and to drift passively long spaces because of their small specific size and weight [30, 31].

In the present study, some zooplankton species disappear or become rare in the plankton strcture composition in S (WWTPs drain) and J (where WWTPs mixed with River water) sites compered to R (upstream before the WWTPs discharge) and A (downstream after the WWTPs discharge) sites. Previous studies showed that most recorded species of aquatic planktons, including Rotifera, Copepoda, Cladocera, and protozoa, withdraw or become rare in the plankton strcture in places of urban wastewater release [6].

In the Cuiaba River (Central Brazil), the species protozoa community increased in the areas of the river under pollution where water quality proved higher nutrient concentrations and lower oxygen levels [32]. Nutrient enrichment of the river, as a result of farming activities, industries, discharge of domestic wastes and effluents, has altered the structure of zooplankton community of the Anambra River, Nigeria [33]. However, even efficiently treated wastewater can have important impacts on the stream ecosystems [34].

According to Ogbeibu and Edutie [35], delicate species generally disappear as the water becomes stressed by polluation while tolerant ones survive the pollution stress and voluntarily improves downstream of the point of discharge. Similar outline was observed in the current study when pollution delicate plankter disappeared or decreased in number at polluted J site while their density recovered downstream of the site getting WWTPs wastes (A site).

Freshwater zooplankton, represented by Rotifera, Cladocera and Copepoda showed great seasonal variability in abundance [36, 37]. Zooplankton community in aquatic ecosystems depend mostly on the seasonal variations and physicochemical parameters of water [13] .Therefore, it is necessary to measure seasonal variations and abundance of zooplanktons to determine the status of fresh water body. Zooplankton yielded a relatively more pronounced seasonal change in the zooplankton community structure during the study period, where the total zooplankton density was higher in winter season and less in summer season at all studied sites. Results of the present work documented the earlier work done in the Nile River [38]. The current results are also well-matched with Kar and Kar [39] who stated that zooplankton of a freshwater enverionment in a Cachar district of Assam (India) have higher population density in winter and lower in summer season.

In the present study, the values of Margalef species richness and Shan. diversity (H) were higher in both sites R and A than in both sites S and J (during summer and winter seasons). Generally, significant differences were observed between groups, but not within groups. Also, the highest total number of individuals and species numbers were observed at sites R and A, while the lowest ones at sites S and J. diversity indices have been used as an important tool by ecologists to understand community structure in terms of richness, evenness, or the total number of existing individuals [40, 41]. Ismael and Dorgham [42] found that the diversity index (Shannon's) to be a suitable indicator for water quality assessment.

Biodiversity is constructed according to the principle that in natural clean water diversity is high, whereas low diversity in polluted water. During present investigation it was found to be true, the lowest biodiversity detected in bothr S and J sites compared to

R and A sites may be endorsed to the comparatively elevated anthropogenic activities, thus leading to poor water quality. Low biodiversity in polluted water may be due to the point that numerous pollution sensitive organisms were removed from the community and only a few pollution tolerant organisms succeeded in the absence of competitions.

Numerous studies have documented reduction in habitat and biodiversity as a result of pollutant impacts. The diversity of zooplankton species tends to be low in stressed and polluted ecosystem [43]. Ghosh and Biswas [44] reported low diversity indices of zooplankton in polluted sites compared to reference site at Ox-Bow Lake of West Bengal (India). Arab *et al*. [45] revealed that the increased values of biodiversity indices could mostly be considered a signal of community steadiness and improved trophic position. The fall in the value of Margalef index shows the rise in the level of pollution. Biodiversity indices (diversity, richness and evenness) were highest at minimum polluted sites while lowest at extremely polluted sites [46]. The higher value of species biodiversity showed the good health status of the waterbody [47].

Shann. Diversity index value greater than 3 specifies clean water, whereas, a value range of 1 to 3 specifies moderately polluted condition and value less than 1.0 specifies heavy polluted situation [48]. All values of Shannon index for R and A sites were below 3, which mean that these sites are moderately polluted, and there is a weak internal community structure in these sites.

For WZI, the values computed range from 2.05 to 3.46. The lowest WZI was observed in winter season at both S and J sites, while the highest was recorded at A site during winter season. According to Lougheed and Chow-Fraser [24], 1.0 WZI value is indicative of low water quality (high eutrophication), 5.0 indicates high water quality (low eutrophication), and a 3.0 value signifies mesotrophic conditions.

The cluster analysis was able to classify the sites as first site-type group (R site during summer), second site-type group (A summer), third site-type group (S and J sites during summer), fourth site-type group (S and J sites during winter) and fifth site-type group (R and A sites during winter). Overall, zooplankton structure of the river showed a diverse spatial distribution due to the influence of WWTPs discharge. According to Dorche *et al.* [49], zooplankton communities generally change in response to the quality of water.

Based on CCA for the current data, it was inferred that zooplankton groups and biodiversity indices are influenced by the cumulative effect of various physicochemical factors in addation to caffeine and Zn in water and sediment. The present results revealed that Cladocera, Rotifera and Meroplankton are associated with AT, WT, TDS, Po_4, No_3, NH_4, Wcaf, Scaf, WZn and SZn and showed a negative correlation with pH, DO, and Turb. While, Protozoa and Copepoda are associated with DO, pH, Turb and MO and showed a negative correlation with other studied physicochemical factors. Freshwater zooplankton, represented by Rotifera, Cladocera, Copepoda, and Protozoa showed spatially variable response relationships with changes in physicochemical characteristics of the aquatic environment. Mozumder *et al*. [50] observed positive relationship between protozoan and dissolved oxygen. The Protozoa have positive association with dissolved oxygen and negative correlation with temperature and nitrates [51].

Singh and Sharma [52] revealed that protozoans showed positive correlation with DO and negative correlation with water temperature turbidity, conductivity, nitrates and phosphates. Abundance of cladocerans showed negative correlation with pH [36] and positive correlation with TDS [53]. Cladocerans preferred high phosphorous level, showed inverse correlation with total alkalinity and pH [54]. Copepoda displayed a positive association with dissolved oxygen and negative with TSS and temperature [52].

Rotifers indicated a significant positive association with water temperature, conductivity, TDS and chlorides, whereas they showed an inverse correlation with DO and pH [55, 56]. Moreover, several studies had shown that Rotifer population density is positively related with total nitrogen and phosphorus content (Po_4-P, No_3-N and NO_2-N) Mohammed *et al*., [47]. Also, Minakshi and Madhuri [57] indicated that the rotifers show strong positive correlation with nitrate and phosphate. Yermolaeva *et al*. [58] showed that increasing phosphates and nitrates stimulate the growth of rotifera and cladocera in Ob River western of Siberia.

On the other side, heavy metal pollutants are affecting aquatic life in their enverionments [59]. Hoang *et al*. [60] reported that Cladocera were the most sensitive group to Zn and the least sensitive group was Rotifera. Cladocerans have been reported to be the most sensitive group to Zn [61]. Rotifers were the greatest tolerant to heavy metal pollution, followed by copepods and cladocerans [62].

The present study revealed that Margalef species richness, Shann. Equitability (J) and Shann. Diversity (H) and abundance are associated with MO, TDS, Po_4, No_3, NH_4, Wcaf, Scaf, and SZn, while species numbers and total number of individuals are associated with AT, WT, Cond, DO, pH, Turb and WZn. Srivastava and Reddy [63] revealed that surface water temperature, dissolved oxygen, and phosphate values have shown strong influences on diversity indices of zooplankton species. Negative relations among zooplankton richness and turbidity, conductivity, zinc, iron and vanadium were recorded Santos *et al*. by [64].

Shena *et al*. [65] reported that both heavy metals and nutrients were important factors that inhibited the richness of zooplankton species, and nutrients had a stronger inhibitory effect than heavy metals. Similarly, Santos *et al*. [66] revealed a decrease in richness sites stressed by Fe, Zn and Khan *et al*. [67] emphasizing that raises zooplankton richness under reductions of Zn, Cu, and Ni in water. Peng *et al*. [68] found that high concentrations of Zn, Cu Cd, Pb, and Hg in the sediment of river declined zooplankton abundance.

The Shannon index (H) values showed a positive correlation with temperature, DO and phosphate and exhibited a negative association with pH and nitrate [63]. Cr and Zn showed a negative influence on the diversity of zooplankton and only the tolerant opportunists raised in polluted waters [69]. Zooplankton density exhibited direct association with hardness, total alkalinity and chloride of water but converse association with pH, temperature and dissolved oxygen [70]. Also, Chen [71] recorded positive correlation between zooplankton abundance and total nitrogen, total phosphorus and suspended solids. No_3 showed direct influence on abundance of zooplankton [53]. The present findings are consistent with Kondowe *et al*. [72] and Mohammed *et al*. [47], who

found a negative association between zooplankton abundance and electrical conductivity. The effect of Zn on zooplankton abundance led to decreasing species richness [60, 64].

CONCLUSION

Zooplankton communities react swiftly to variations in physicochemical parameters, which can impact their species richness, increase or decrease their abundance, and induce changes in their diversity. So, Screening of zooplankton assemblage would offer a clear picture of water quality and the circumstances for ecosystem health. From the CCA, it was inferred that the zooplankton faunal dynamics were influenced by the cumulative effect of various physicochemical factors including WCaf, SCaf, WZn and SZn. This study gives valuable information to understand how caffeine, Zn and other physicochemical factores may interact with aquatic environment, using zooplankton commonly found in the aquatic habitat. Overall, the present results pointed out that contaminants from WWTPs discharge can origin variations in the quantitative and qualitative composition of zooplankton and influence their abundance. Hence, zooplankton can express to disorder of water body and can be used to assess over all river health, and they may serve as early warning signals that reflect the 'health' status of an aquatic system. On the other hand, despite the determinations to make improve wastewater treatment systems, still the technologies unable to provide the effective protection of water bodies from pollutant substances. Therefore, it is crucial that several WWTP treatment systems must be implemented or improved in order to reduce the release of contaminants in the Nile River from these point sources.

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