



Effect of sewage disposal in some torrents streams on zooplankton community structure

Montaser Hassan^{*1,2}, Hamada Mahmoud³, Madlen Habashy⁴ and Khaleid Abd El-Wakeil⁵

1- Zoology Department, Faculty of Science, Ain Shams University, Egypt.

2- Department of Biology, Faculty of Science, Taif University, KSA.

3- Faculty of Science, Beni-suef University, Egypt

4- National Institute of Oceanography and Fisheries, El-Qanater El-Khayria, Egypt.

5- Zoology and Entomology Department, Faculty of Science, Assiut University, Egypt

*Corresponding Author: shmontaser2@gmail.com , montasermohamed@sci.asu.edu.eg

ARTICLE INFO

Article History:

Received: April 16, 2022

Accepted: May 29, 2022

Online: Aug. 2, 2022

Keywords:

Zooplankton,
Community structure,
Sewage disposal,
Taif,
Saudi Arabia

ABSTRACT

Zooplankton is an important group in aquatic ecosystems. They act as biological indicators as they are influenced by both biotic and abiotic factors reflecting the change in water quality, eutrophication and pollution over time because they respond quickly to changes in the water body. Zooplankton of some torrent streams in Taif governorate, KSA were studied. Thirty-nine zooplankton species were recorded, in the studied water bodies during the present work. They were belonging to five zooplankton groups, Rotifera (20 species), Cladocera (5 species), Copepoda (3 species), Protozoa (6 species) and meroplankton (5 species). Rotifera was the dominant group and highly represented in site I, while Cladocera was the lowest according to their population densities. On the other side, site IV showed the lowest Rotifer density and the highest cladoceran density. This is attributed to the pollution caused by sewage discharge from the nearby treatment plant.

INTRODUCTION

Zooplankton as heterogeneous assemblage of microscopic organisms, help in the trophic dynamics in freshwater ecosystems (Aloufi and Obuid Allah, 2014), they not only regulate the aquatic productivity, by occupying the middle position in the food chain linking the lower and higher trophic levels but also indicate the environmental status in a given time due to the ability of certain species to indicate the changes caused by pollution and eutrophication in the water quality (Peroš-Pucar and Ternjej, 2014; Ismail and Zaidin, 2015; Sarkar and Saha, 2016; Hassan *et al.*, 2017). Some zooplankton significantly recycles of nutrients and builds up food materials for predators (Khalil *et al.*, 2008; Jia *et al.*, 2017).

Spatial and temporal distribution of zooplankton was not studied before in Taif, Saudi Arabia. Zooplankton distribution significantly varies according to the status of the water body, environmental variables and/or species interactions such as predation and

competition that shapes the species composition of this community (**Larson *et al.*, 2007; Hassan *et al.*, 2017; Eskinazi-Sant'Anna *et al.*, 2020**), therefore, species composition of zooplankton assemblage changed through time and place. Consequently, it is important to monitor the influence of environmental variables on the community structure of zooplankton continuously (**Bielan'ska-Grajner *et al.*, 2014**).

There is a few work has been done on zooplankton at different places in Saudi Arabia but not in Taif region. **Al-Asgah *et al.*, (1989)** carried out a survey study on freshwater zooplankton from Al-Hassa drainage canal, Al-Mahalla valley, Al-Dariyah, Abbha, and Gizan reservoirs. They found 8 species belonging to Copepods, Cladocera and Rotifera. **Al-Aidaros and Ghazali (1998)** investigated zooplankton assemblage at the highly eutrophic and sewage polluted Costal Lagoons of Jeddah. **Abdul Azis *et al.*, (2003)** studied the relationship between phytoplankton as producer and zooplankton as primary consumer at the Saudi Arabian Gulf coastal waters near the desalination plants of Al-Jubail. **Fathi and Al-Kahtani (2009)** examined the effect of water quality on zooplankton at Al-Khadoud spring in Al-Hassa Governorate, Saudi Arabia. **El-Bassat *et al.*, (2011)**; examined the effect of four drugs belonging to different therapeutic classes were chosen to examine their toxicity on some planktonic organisms of different trophic levels. **Al-Ghanim (2012)** studied the spatio-temporal distribution and composition of zooplankton in Wadi Hanifa Stream, Riyadh, Saudi Arabia.

Therefore this study was designed to contribute preliminary information to fill the gap of knowledge about zooplankton communities in some torrent streams in Taif region.

MATERIALS AND METHODS

Taif City lies at 21°16'00"N and 40°25'00"E at an elevation of 1,879 m (6,165 ft) above the sea level on the top of Al-Sarawat Mountains, Kingdom of Saudi Arabia. The elevation increases to the west and south till reach 2500 m. Most of the freshwater bodies in Taif come from the fallen rains on Al-Sarawat Mountains. There are five pathways for the torrent water, Wadi Wajj, Wadi Leyah, Wadi Al-Aqiq, Wadi Masarrah and Wadi Wakdan. Five main sites were selected in Wadi Wajj, Wadi Leyah for the present study (Fig.1).

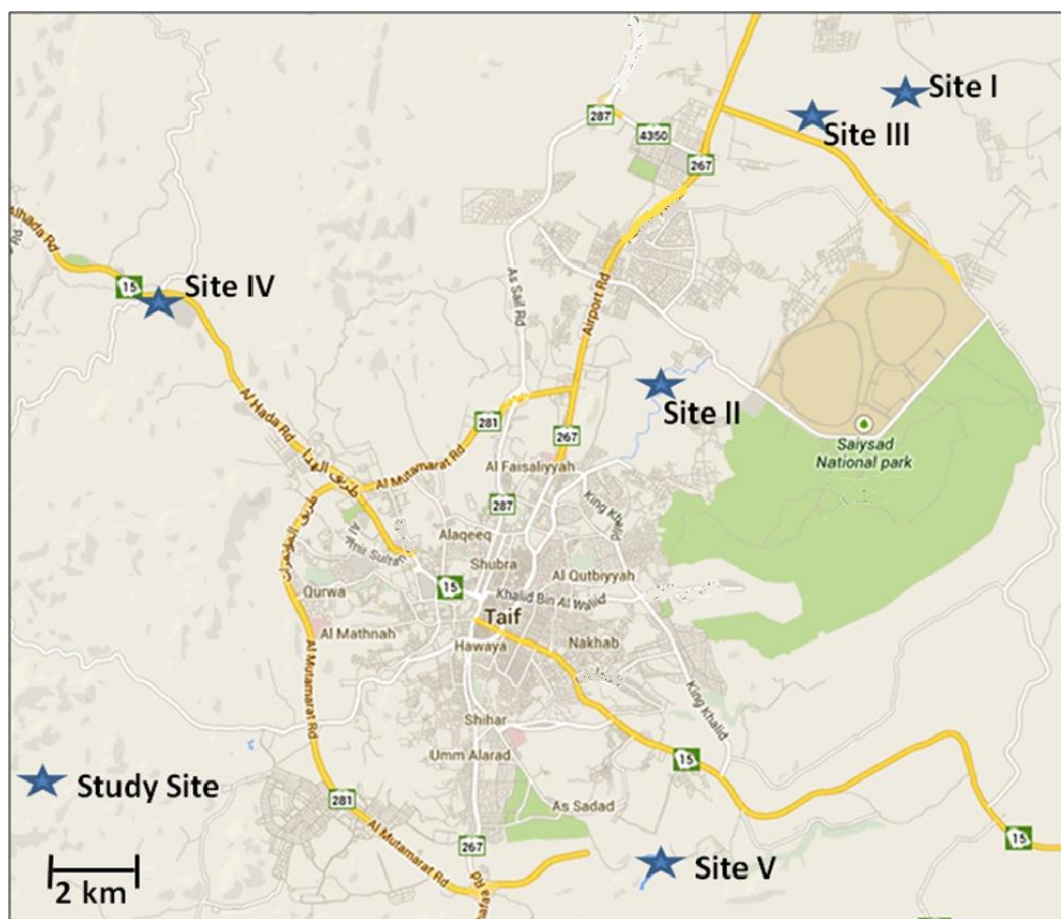


Fig. (1): Google map showing the five study sites in Taif Governorate, Saudi Arabia (Hassan *et al.*, 2014).

1. Description of study sites:

1.1. Site I (Wadi Al-Arj):

Wadi Al-Arj is the downstream of Wadi Wajj that flows southwest to northeast through Taif City, western Saudi Arabia (20°30'49"N and 40°47'12"E). There are several agricultural activities and domestic sewage discharge along the stream. Additionally, it is a foraging area for camels and sheep. The sewage treatment station pours the excess untreated sewage in the stream. There is dense vegetation cover in the waterway consists of *Typha domingensis*, *Mentha longifolia*, *Abutilon pannosum*, *Calotropis procera*, *Solanum incanum*, *Cassia italica*, *Datura innoxia*, *Lycium shawii*, *Acacia laeta*, *Xanthium strumarium*, *Acacia ehrenbergiana*, *Tamarix nilotica* and filamentous algae. Water is shallow and almost clear. Four stations were chosen for sample collection along the stream; station (1) at the discharge point, station (2) flood water close to the discharge point, station (3) flood water 300m before the discharge point and station (4) represent the mixing point 300 m after the sewage discharge.

1.2. Site II (Saiysad):

Saiysad is the upstream of Wadi Wajj and then joins Wadi Al-Arj. It lies 9 Km northeast of Taif City at 21°18'56"N and 40°29'01"E. It is almost running shallow water comes from Al-Sarawat Mountains. There is mild vegetation cover on the banks, and filamentous algae in the water. This waterway is subjected to human activities where it receives large number of people every day especially in holydays for picnic. Three stations were selected for sampling from this site.

1.3. Site III (Wetland):

Wetland is a wide area of closed stagnant water lies adjacent to Wadi Al- Arj (20°30'49" N and 40°47'12 "E). It has dense vegetation cover consists of *Acacia gerrardii*, *Acacia laeta*, *Tamarix nilotica* and *Typha domingensis* covering most of the water body area. The water colour is green indicating presence of algae. The surface area of the wetland is about 1000 m². It lies at higher level than the waterway of Wadi Al- Arj. Water depth ranges between 0.3-1.5 m. The bottom is covered with a great amount of humus between the trees very rich with an aerobic bacteria; giving dark coulour to the water. The water level is highly fluctuated all over the year, and may reach dryness during winter and spring. Two sampling stations were selected for samples collection from the water body. The first in the covered part that having trees and humus in the bottom. The second is exposed to the sun. Some species of Tilapia fish are recorded in this area.

1.4. Site IV (Jabajeb):

This site locates at the right side of Al-Hada Road in the way to Taif City at 21°20'25" N and 40°20'17"E. Its area is about 1000 m². It is stagnant water, slightly turbid and has green coulour. Its water comes from the nearby mountains. The depth may reach 3-5 meters high in the middle. The water lens, *lemna gibba*, covers large parts of the water surface. Water level sharply decreases and even dries during dry season. Some plants grow on the banks such as *Rumex vesicarius*, *Acacia gerrardii* and *Tamarix nilotica*. Two stations were selected to represent this site.

1.5. Site V (Ghadeer Al-Banat):

It is 9 km southeast of Taif city close to Wadi Leyah Dam, at 21°12'58" N and 40°26'48" E. It receives the rain water from Al-Sarawat and Shafa Sofian Montains through Wadi Leyah to Rokba region. It is from 10-15m wide. The water level may exceeds 5 meters high and sharply decreases or completely dries during wenter; Wadi Leyah Dam that was constructed during the 80s of the twentieth century to conserve water for agricultural purpose. The water is turbid and containing filamentous algae and dense *Lemna gibba* covering wide areas of the water surface. This stream is highly impacted with human activities where they come for camping and enjoying the natural views in the place. Three points were chosen for samples collection.

2- Physical and chemical variables:

Water temperature (°C) was measured using digital thermometer. Acidity of water was measured using pen pH meter. Total dissolved solids (ppm) were measured using Hanna pen TDS meter.

3- Nutrients:

The dissolved nutrient salts such as ammonia NH^{-3} , nitrate NO^{-3} , nitrite NO^{-2} and phosphate PO_4^{-3} also were seasonally measured by spectrophotometric method according to methods for examination of water and waste water (APHA, 2017) American Public Health Association, New York. The water samples taken for analysis of nutrient salts were filtered through Whatman glass microfiber filters “GF/C” using Büchner funnel and a suction bump. The filtrate was processed for spectrophotometry.

4- Zooplankton sampling and examination:

Zooplankton samples were collected during the raining season only (late spring and summer) because water level sharply decreases and may dry in some places. One meter vertical sample was filtered using 55 μm mesh size, 30 cm diameter plankton net. Three replicates were taken from each station. Samples were immediately preserved in 10% neutral formalin. Zooplankton was counted in one milliliter from each sample using Carl Zeiss binocular stereomicroscope. This process was repeated three times for each sample. Zooplankton was identified to species level as possible according to Koste (1992), Shiel (1995), Shiel and, Einsle (1996), Smironov (1996), Fuentes-Reines and Roa (2013), and Perbiche-Neves *et al.* (2015).

5- Samples treatment

The dominance structure was determined according to Engelmann’s classification (Engelmann, 1978), sub-resident (below 1.3%), resident (1.3-3.9%), subdominant (4-12.4%), dominant (12.5-39.9%), eudominant (40-100%).

6- Statistical analysis:

The collected data were statistically analyzed using SPSS software package (version 20) and Microsoft Excel Office (V. 10). Analysis of Variance (ANOVA) and Pearson correlation coefficients were applied to the obtained data. In case of significant differences, the Duncan test was selected from the Post Hoc window on the same statistical package to detect the distinct variances between means. PRIMER 5 software package (Clarke, 1993; Clarke and Gorley, 2001) was used to calculate the diversity indices. The program Canoco for windows 4.5 was used for canonical corresponded analysis (CCA) as a unimodal method to analyze the response of the zooplankton community composition to environmental variables.

RESULTS

1-Environmental variables:

The statistical analyses showed significant differences among means of the measured environmental variables for the studied sites (Table 1). Water pH was in the alkaline side; the lowest average values were recorded in sites (I) and (V), Duncan test showed that there is no difference between these two sites but there are significant differences between them and the other remaining sites where they have the lowest pH values.

Site (III), wetland was significantly different from all stations with its highest average TDS while site (V), showed the lowest TDS. According to the obtained results, the water of all study sites contains relatively high amounts of water calcium except site (V) contains the lowest amount; the maximum value is recorded in site (III) also its water has the highest calcium concentration. Sites (III) and (I) were significantly different from the other sites as they have highest total hardness respectively. The lowest total hardness value was in sites (V) and (IV) which also showed the lowest calcium concentration.

Table (1): Means of physico-chemical variables and standard deviation of means of the study sites. (The similar characters for each factor show no significant difference).

	Site1			Site2			Site3			Site4			Site5			F	P value
	Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D		
Temp. (°C)	21.83	±	4.33 b	24.11	±	0.80 ab	26.84	±	1.60 a	25.67	±	0.52 a	26.08	±	1.41 a	5.763	0.001
pH	7.62	±	0.21 b	8.13	±	0.30 a	8.13	±	0.51 a	8.45	±	0.37 a	7.37	±	0.26 b	14.860	<0.001
TDS (mg/l)	526.31	±	34.52 b	630.28	±	105.33 b	1180.92	±	583.89 a	274.08	±	51.10 c	171.00	±	1.89 c	21.278	<0.001
Depth (cm)	35.42	±	10.60 c	48.94	±	25.50 bc	55.67	±	25.55 bc	80.00	±	18.23 a	69.17	±	25.71 ab	5.828	0.001
Turbidity (cm)	33.33	±	10.13 bc	48.94	±	25.50 ab	40.25	±	15.63 abc	55.42	±	14.18 a	29.17	±	4.84 c	3.952	0.009
Org. M. (%)	11.28	±	1.92 a	10.98	±	2.51 a	6.20	±	0.74 b	5.60	±	1.10 b	6.93	±	0.36 b	23.016	<0.001
Ca (mg/l)	159.34	±	25.71 b	180.62	±	29.24 b	250.50	±	58.50 a	123.33	±	17.64 c	91.67	±	4.31 d	28.865	<0.001
Mg (mg/l)	60.43	±	29.66 a	20.58	±	5.04 bc	33.18	±	16.99 b	8.21	±	1.53 c	5.72	±	0.59 c	16.376	<0.001
PO ₄ (mg/l)	0.31	±	0.02 bc	0.28	±	0.04 c	0.51	±	0.38 a	0.18	±	0.13 c	0.45	±	0.03 ab	5.615	0.001
NH ₃ (mg/l)	0.49	±	0.15 b	0.13	±	0.07 d	0.27	±	0.05 c	0.27	±	0.14 c	0.63	±	0.09 a	28.693	<0.001
NO ₃ (mg/l)	0.90	±	0.23 a	0.92	±	0.32 a	0.39	±	0.27 b	0.28	±	0.36 b	0.95	±	0.22 a	9.844	<0.001
NO ₂ (mg/l)	0.05	±	0.01	0.09	±	0.10	0.05	±	0.03	0.05	±	0.01	0.07	±	0.01	1.115	0.364

SD: standard deviation, Temp. Temperature, TDS: Total Dissolved Solids, Org.M. Organic matter.

Nutrient salts were measured in the water to indicate the trophic status of the studied water bodies. Phosphates and nitrogen are considered as important factors for growth of algae and aquatic plants. According to the present results phosphorous concentrations are higher than 100 $\mu\text{g/l}$ in all the studied sites. Sites (III) and (V) showed the highest average phosphate values respectively. The lowest phosphate concentration was recorded in site (IV) where it was significantly different. Ammonia is one of the most effective nutrients; its mean was high in sites (V) and (I) respectively where they were significantly different from other stations. The lowest mean was found in site (II). Sites (II), (V) and (I) recorded high average nitrate concentrations respectively; however, site IV showed the lowest concentration. Nitrite concentration does not show significant variations among different sites.

The sediments in sites (I) and (II) was significantly different among study sites, having the highest organic matter content while, the lowest average value was in site (IV).

2- Zooplankton community structure:

According to the present results in Fig.(2), , zooplankton density was highly varied among the study sites. The maximum zooplankton density, was 650123 ind./m³, recorded in site (I) followed by site (III) 211075 ind./m³, site (V) 152394 ind./m³ and site (IV) 124734 ind./m³ respectively; while the lowest value was 50837 ind./m³, recorded in site (II), which was characterized by the lowest densities among all zooplankton groups.

Five zooplankton groups were recorded in the present study (Table 2), Rotifera, Protozoa, Cladocera, Copepoda and Meroplankton. The maximum average rotifer and protozoan densities were 367834 and 192763 ind./m³, respectively in site (I), followed by site (III) with (118365 and 36093 ind./m³). However the minimum average densities were (1238 and 1415 ind./m³) recorded in site (IV).

Cladocera showed their highest average density (12208 ind./m³) in site (IV) and in front of 589 and 1415 ind./m³ in sites (II) and (III) respectively. The highest copepod average densities were 112408 ind./m³ and 109872 ind./m³ respectively recorded in sites (V and IV).

Site (II) displayed the highest average value of meroplankton (9554 ind./m³) among the study localities; the lowest values were in sites (IV) and (V), recorded 0 ind./m³ and 235 ind./m³ respectively (Fig. 2).

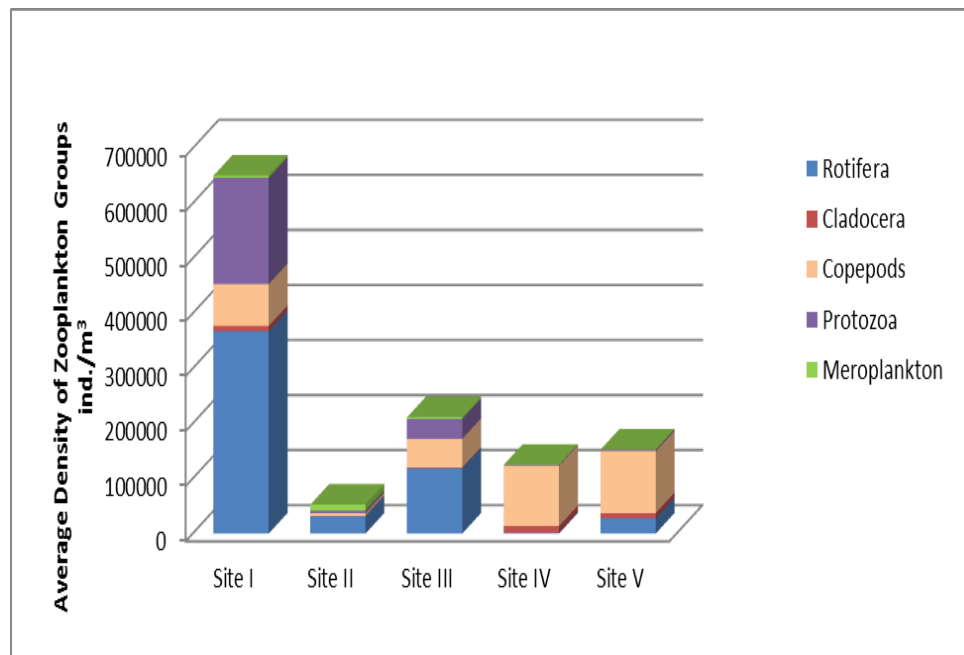


Fig. (2): Average densities zooplankton and different groups in each study site.

3-Zooplankton diversity:

Thirty nine zooplankton species were recorded in the present study (Table. 2); the first site contributed 33 total species number, site (III) 20 species, sites (II) and (V) 17 species for each and site (IV) attained 13 species. Rotifers represented the major component where 20 species were recorded in the five localities; 19 of them were found in sites (I) and 12 species in site (III); however, only three rotifer species were recorded in site (IV).

On the other side, six cladoceran species were recorded in the studied areas; five of them were found in site (IV); only one species, *Ceriodaphnia dubia* was recorded in site (I). In the present study, *C. dubia* was recorded in all sites except site (II); however, *Daphnia* was only restricted to site (IV). Three species of copepod were recorded in this study; all of them are found in sites (II) and (IV). Six protozoan species were recorded in the five sites especially site (I). *Arcella* was recorded in the five sites.

Five meroplankton taxa were found in the five sites. All the five species were recorded in site (I), however only one species was in site (V).

Table (2) shows the species frequency in the five sites and there are nine rotifer species have 40-71%, only one cladoceran species 45%, two copepod species 60-74%, two protozoan species 43-74% and one meroplankton species 50%.

Table. 2: Mean values of zooplankton density (Indi./m³) and relative abundance (%) for the collected plankton taxa at investigated sites and their percentage of frequency (%F).

Plankton Taxa	Site 1		Site 2		Site 3		Site 4		Site 5		%F
	Ind./m ³	%	Ind./m ³	%	Ind./m ³	%	Ind./m ³	%	Ind./m ³	%	
Rotifera											
<i>Brachionus calyciflorus</i> (Pallas, 1766)	13446.6	2.07	0	0.00	5661.7	2.68	0	0.00	0	0.00	43
<i>Brachionus angularis</i> (Gosse, 1851)	265569.4	40.84	0	0.00	1415.4	0.67	0	0.00	353.9	0.23	33
<i>Brachionus leydigii</i> (Cohn, 1862)	4600.1	0.71	0	0.00	4954.0	2.35	0	0.00	0	0.00	33
<i>Brachionus plicatilis</i> (Müller, 1786)	1061.6	0.16	0	0.00	0	0.00	0	0.00	0	0.00	14
<i>Brachionus urcularis</i> (Muller, 1773)	0	0.00	0	0.00	0	0.00	353.9	0.28	471.8	0.31	12
<i>Monostyla bulla</i> Gosse, 1851	176.9	0.03	2594.9	5.10	0	0.00	0	0.00	0	0.00	21
<i>Lepadella patella</i> (Müller, 1786)	1857.7	0.29	6251.5	12.30	0	0.00	0	0.00	118.0	0.08	40
<i>Lecane luna</i> (Müller, 1776)	3007.8	0.46	13328.6	26.22	1061.6	0.50	176.9	0.14	353.9	0.23	67
<i>Lecane mira</i> (Murray, 1913)	88.5	0.01	118.0	0.23	0	0.00	0	0.00	0	0.00	5
<i>Philodina citrina</i> (Ehrenberg, 1832)	3361.6	0.52	2005.2	3.94	4777.1	2.26	0	0.00	118.0	0.08	50
<i>Habrotrocha angusticollis</i> (Murray, 1905)	11765.7	1.81	7077.1	13.92	17692.8	8.38	707.7	0.57	471.8	0.31	71
<i>Anuraeopsis fissa</i> (Gosse, 1851)	6015.6	0.93	0	0.00	32731.7	15.51	0	0.00	0	0.00	40

<i>Polyarthra vulgaris</i> (Carlin, 1943)	30343.2	4.67	0	0.00	35385.7	16.76	0	0.00	2594.9	1.70	57
<i>Hexarthra mira</i> (Hudson, 1871)	15481.2	2.38	0	0.00	3892.4	1.84	0	0.00	22528.9	14.78	62
<i>Keratella vulga</i> (Ehrenberg, 1834)	884.6	0.14	0	0.00	0	0.00	0	0.00	0	0.00	14
<i>Keratella tropica</i> (Ehrenberg, 1834)	707.7	0.11	0	0.00	176.9	0.08	0	0.00	0	0.00	17
<i>Keratella quadrata</i> (Ehrenberg, 1834)	707.7	0.11	0	0.00	5838.6	2.77	0	0.00	353.9	0.23	29
<i>Asplanchna herricki</i> (Guerne, 1888)	4334.7	0.67	0	0.00	4777.1	2.26	0	0.00	0	0.00	40
<i>Asplanchna priodonta</i> Gosse, 1850	2919.3	0.45	0	0.00	0	0.00	0	0.00	0	0.00	17
<i>Filinia longiseta</i> (Ehrenberg, 1834)	1503.9	0.23	0	0.00	0	0.00	0	0.00	235.9	0.15	14
Cladocera											
<i>Daphnia longispina</i> (Müller, 1776)	0	0.00	0	0.00	0	0.00	3007.8	2.41	3892.4	2.55	19
<i>Ceriodaphnia dubia</i> Richard, 1894	9288.7	1.43	471.8	0.93	884.6	0.42	884.6	0.71	1651.3	1.08	45
<i>Simocephalus vetulus</i> (Müller, 1776)	0	0.00	0	0.00	0	0.00	6900.2	5.53	0	0.00	14
<i>Alona rustica</i> (Scott, 1895)	0	0.00	118.0	0.23	530.8	0.25	1238.5	0.99	3892.4	2.55	33
<i>Moina micrura</i> (Kurz, 1874)	0	0.00	0	0.00	0	0.00	176.9	0.14	0	0.00	2
Copepoda											
Nauplius larvae	55378.6	8.52	4482.2	8.82	44409.0	21.04	46886.0	37.59	97192.6	63.78	74
<i>Mesocyclops edax</i> (Forbes, 1891)	20789.1	3.20	118.0	0.23	7784.8	3.69	2653.9	2.13	15215.8	9.98	60

<i>Acanthodiaptomus denticornis</i> (Wierzejski, 1887)	0	0.00	707.7	1.39	0	0.00	60332.6	48.37	0	0.00	21
Protozoa											
<i>Stylonichia</i> Ehrenberg, 1830	442.3	0.07	0	0.00	0	0.00	1061.6	0.85	0	0.00	17
<i>Carchesium polypinum</i> Linnaeus, 1758	9908.0	1.52	0	0.00	2300.1	1.09	0	0.00	0	0.00	29
<i>Acineta tuberosa</i> Ehrenberg, 1833	1769.3	0.27	0	0.00	0	0.00	0	0.00	0	0.00	12
<i>Arcella vulgaris</i> Ehrenberg, 1832	1150.0	0.18	2948.8	5.80	1592.4	0.75	353.9	0.28	2712.9	1.78	74
<i>Centropyxis aculeata</i> (Ehrenberg, 1838)	265.4	0.04	1061.6	2.09	0	0.00	0	0.00	0	0.00	24
<i>Euglena oxyuris</i> (Schmarda 1846)	179228.4	27.56	0	0.00	32201.0	15.26	0	0.00	0	0.00	43
Meroplankton											
<i>Bradleystrandesia reticulata</i>	619.2	0.10	0	0.00	0	0.00	0	0.00	0	0.00	5
Chironomus larvae	707.7	0.11	3774.5	7.42	0	0.00	0	0.00	0	0.00	36
May fly nymph	176.9	0.03	2594.9	5.10	0	0.00	0	0.00	0	0.00	21
Mosquito larva	176.9	0.03	118.0	0.23	0	0.00	0	0.00	0	0.00	7
Free living nematodes	2477.0	0.38	3066.8	6.03	3007.8	1.42	0	0.00	235.9	0.15	50

Site (I) recorded the highest average species number 18, followed by 17 species at site (III) (Fig. 3a), 11 species in sites (II); while site (V) included average 9 species, however the lowest average species number, was 8 in site (IV). Although site (I) has the highest species number but sites (II) and (III) recorded the highest Shannon diversity index and both sites have high equitability of species Figs. (3b,d).

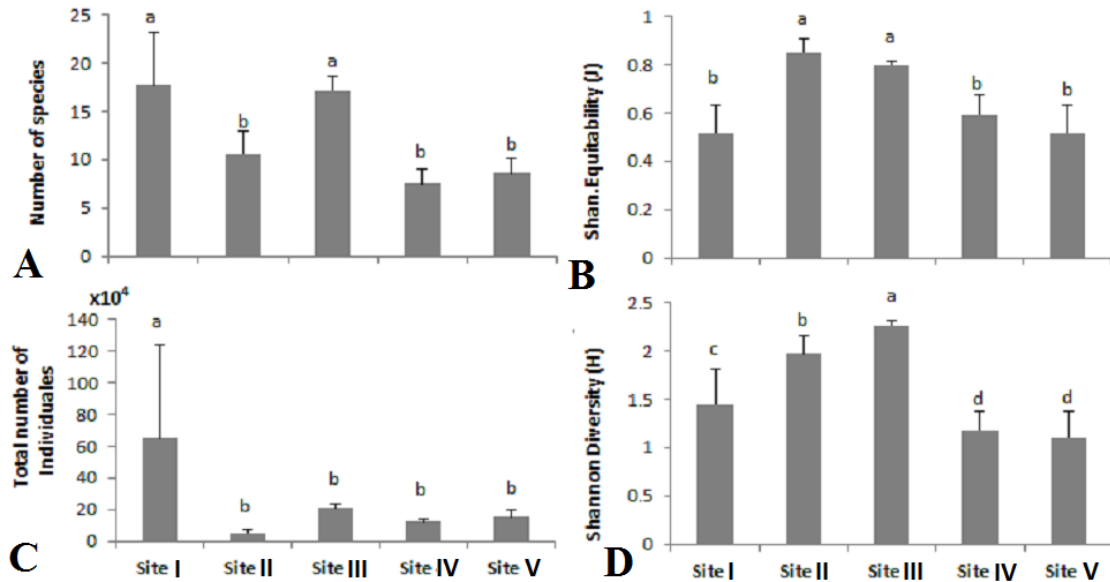


Fig. (3): Average species diversity indices of zooplankton in the five sites.

4-Influence of environmental variables on zooplankton community structure and diversity

Zooplankton community structure is greatly influenced by the environmental variables. Pearson correlation Table (3) shows, Rotifers were negatively correlated to water temperature. Cladocera and copepods displayed significant negative correlation to calcium concentration. Copepods showed significant negative correlation with the sediment organic matter, Calcium and ammonia. Protozoa and meroplankton were negatively correlated to water depth and turbidity and correlated positively to magnesium and organic matter. Species richness in this study was positively correlated to TDS, Ca^{+2} , Mg^{+2} and organic matter and negatively influenced by depth. Total number of zooplankton in the study sites was negatively influenced by water temperature. Zooplankton equitability was positively correlated to water temperature and Ca^{+2} however; it was negatively influenced by ammonia. Species diversity was positively correlated to TDS, Organic matter, Ca^{+2} and Mg^{+2} but it was negatively influenced by depth and turbidity.

Table (3): Correlation coefficients between different zooplankton groups and environmental variables (* $p \leq 0.05$, ** $p \leq 0.01$)

	Rotifera	Cladocera	Copepods	Protozoa	Meroplankton	Species richness	Total number of Individuals	Equitability (J)	Shannon Diversity (H)
Temp. (°C)	-0.569**	-0.321*	-0.265	-0.045	-0.077	-0.279	-0.569**	0.363*	0.000
pH	-0.197	-0.099	-0.211	-0.243	-0.232	-0.178	-0.196	0.165	-0.121
TDS (mg/l)	0.064	-0.253	-0.271	0.090	0.102	0.438**	0.064	0.292	0.509**
Depth (cm)	-0.124	0.167	0.269	-0.508**	-0.520**	-0.503**	-0.123	-0.201	-0.533**
Turbidity (cm)	0.086	0.160	0.109	-0.354*	-0.345*	-0.262	0.087	-0.062	-0.285
Org. M. (%)	0.139	-0.213	-0.347*	0.551**	0.574**	0.423**	0.137	0.243	0.472**
Ca ⁺² (mg/l)	0.008	-0.333*	-0.391*	0.112	0.129	0.404**	0.008	0.425**	0.564**
Mg ⁺² (mg/l)	0.249	-0.133	-0.245	0.631**	0.655**	0.560**	0.248	0.259	0.581**
PO ₄ (mg/l)	-0.007	-0.059	0.040	-0.046	-0.052	0.167	-0.007	-0.045	0.179
NH ₃ (mg/l)	0.242	0.287	0.428**	0.262	0.242	0.120	0.242	-0.334*	-0.088
NO ₃ (mg/l)	0.131	0.017	0.005	0.220	0.226	0.182	0.130	-0.204	0.137
NO ₂ (mg/l)	-0.089	-0.082	-0.091	-0.117	-0.105	-0.086	-0.088	0.028	0.017

Canonical correspondence analysis (CCA) ordination was applied to the recorded data of zooplankton groups (Rotifera, Cladocera, Copepoda, Protozoa and Meroplankton) and the environmental variables (water temperature, pH, TDS, turbidity, depth, sediment organic matter, and Water Mg⁺², Ca⁺², PO₄⁻³, NH₃⁻³, NO₃⁻³, NO₂⁻²) for the collected samples from the investigated sites. As shown in canonical correspondence analyses diagram (Fig.4), the first two CCA axes together account for approximately 88.4% of the relations between zooplankton groups and environmental data. The results of CCA reveal that zooplankton composition mostly related to sediment organic matter, water temperature and depth followed by water Mg⁺², Ca⁺², TDS, Nitrate and water pH, while concentration of phosphorus (PO₄⁻³), nitrite (NO₂⁻²) and NH₃⁻³ have relatively small effects on zooplankton. Rotifera and meroplankton indicate negative correlation with water temperature, while Protozoa is negatively correlated with turbidity. Cladocera and Copepoda are positively correlated with water depth and negatively correlated with water TDS, Ca⁺², NO₃⁻³, Mg⁺² and sediment organic matter.

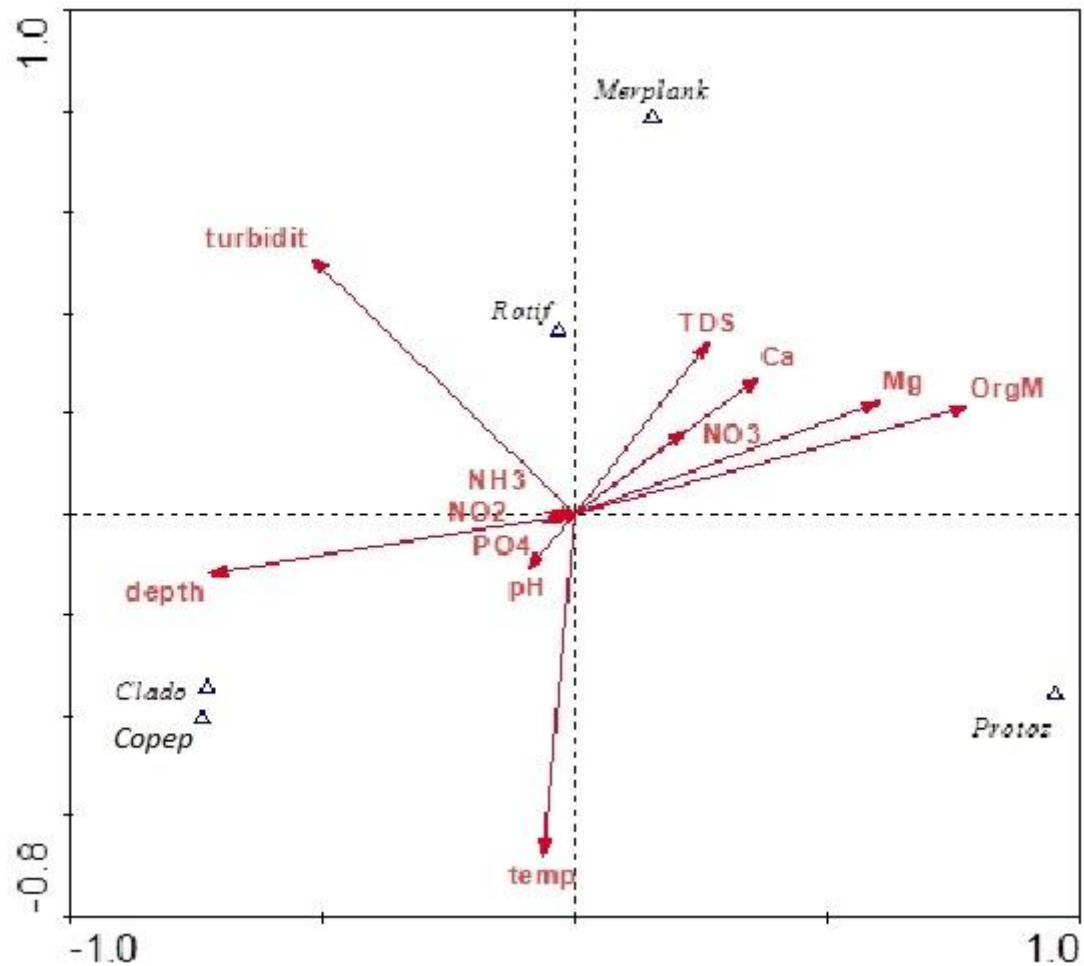


Fig. (4): Ordination bi-plot of the canonical correspondence analysis (CCA) with mean abundance of zooplankton groups and different environmental variables.

Zooplankton data used in this cladogram (Fig. 5) indicate that, sites (II) and (III) are similar to each other where they are the more diverse sites. Site (IV) and (V), are close to each other. However site I is completely different and separated from the other four sites.

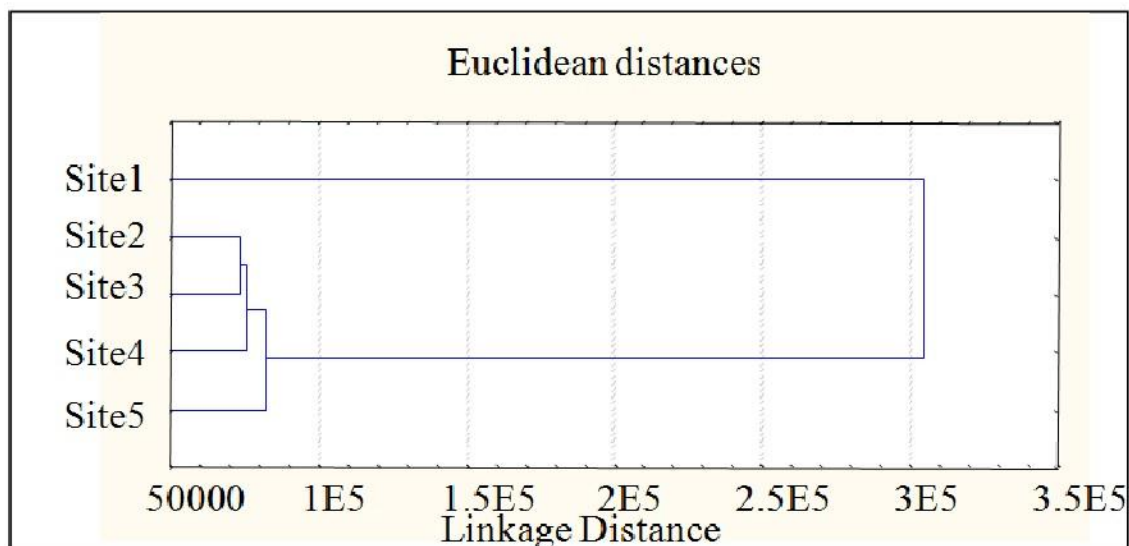


Fig. (5): Dendrogram for the similarity distance for the study sites.

DISCUSSION

Environmental variables play important roles in aquatic ecosystems; they reflect the impact of human activities on the ecosystem such as community structure, species distribution and diversity. Monitoring of habitat conditions and structure are important components of any ecological study (Hassan *et al.*, 2017; Omar and Hassan 2018). Water temperatures in all studied sites are within normal temperature range indicating there is no thermal pollution.

The increased concentrations of TDS, and Phosphates in site (III) (wetland) could be due to presence of a lot of vegetation, plant debris and it is close the main road and some agriculture lands. This may help at the beginning to increase zooplankton diversity as this site is considered as mesotrophic (Muller and Helsel, 1999), and has more rotifer species than site (II); after that, rotifer will dominate over Cladocera and Copepoda decreasing diversity and ecosystem functions (Coldsnow *et al.*, 2017).

As appeared from the present study, site (I) considered as a source of contamination due to the discharge point that increased pollutants; Raza (2004) declared that this stream contaminates the aquifer of the groundwater in this area, which is the main source for irrigating crops and animal grazing in this area additionally, Bahabry (2011) found similar results for water and soil pollution at Uranah in Makkah region. High ammonia concentration also in sites (V) may result from presence of grazing animals and baboon monkeys inhabiting that site (V). The fecal pellets are clearly observed in the stream of both sites. Nitrogen and phosphorous are limiting factors in the aquatic ecosystem controlling the algal blooms and water quality; this could be happened through

construction of the new road or salts driven to the stream through precipitation runoff from constructions and surrounding mountains (Jones *et al.*, 2017). Muller and Helsel (1999) categorized trophic status according to phosphorus concentration. Water bodies with phosphorus concentrations below 0.010 mg/L are classified as oligotrophic, phosphorus concentrations between 0.010 and 0.020 mg/L are indicative of mesotrophic status, and those having phosphorus concentrations exceeding 0.020 mg/L are eutrophic; therefore all the studied sites are eutrophic except site (III) is mesotrophic.

The positive correlation between calcium and certain zooplankton was explained by Merlini *et al.* (1968); they found that *Daphnia* and certain species of this genus display higher calcium concentration in their bodies than other Cladocera. Additionally, Barrow *et al.* (2014) stated that there are Ca-rich taxa, mainly *Daphnia pulex* and Ca-poor taxa such as *Bosmina* spp. and *Holopedium glaciale*. Additionally, Merlini *et al.* (1968) found the lowest calcium concentration in copepods and the highest in Cladocera from the same water body and attributed this difference to the physiological control not to water concentration of the element. Lewis *et al.* (2013) declared that copepods have chitinous exoskeleton therefore; they are not vulnerable to aqueous calcium concentration unlike daphniids which need calcium due to continuous shedding and have calcium is incorporated in their carapace formation. They found a shift from larger size Ca-rich daphniids to smaller Ca-poor taxa in soft water due to decrease of calcium as a result of increased acidity due to the industrial sewage; and this indicates rotifer dominance than cladocera in site (I) (Hassan *et al.*, 2017).

Site (I) less exposed to human activities and has slightly faster water current. The highly increased rotifer density in sites (I) and (III) indicated the impact of sewage discharge; the lowest rotifer density was in site (IV) and higher cladoceran density than all other sites indicates its good water quality. Harris and Vinobaba (2012) indicated that water quality can influence species composition. Bodiou *et al.* (1990) examined the relation between copepods and dissolved organic matter where they observed increase in copepod densities and decrease in organic carbon; and at certain threshold of the organic matter the population decreases. They attributed this change to the trophic richness and food type where copepods depend on specific bacterial populations or diatoms. This was explained by Urban-Rich *et al.* (2006) where copepod can change and recycle the composition of organic matter in the water body. Ward *et al.* (2011) and De Sousa *et al.* (2012) examined the sensitivity of the copepod *Nitokra* sp. and found that it is tolerant to ammonia; they added toxicity of ammonia depends on water pH.

In the present study, zooplankton was negatively correlated to water temperature; this could be attributed to the water current where those sites receive flood water during summer. Mainly zooplankton in closed or semi-closed water bodies is positively correlated to water temperature due to egg hatching (Khalil *et al.*, 2008) unlike rivers and streams (Sleem and Hassan, 2010) like sites (I) and (II). Eddy (1934) stated that water current greatly influences zooplankton density by decreasing feeding ability, production

or draining plankton from upstream to downstream. This also explains the lower zooplankton density in upstream at site (III) than downstream at site (I).

Zooplankton abundance and diversity depend mainly on the physico-chemical variables of the water body (**Khalifa and Abd El-Rahman, 2010**) and interactions among biotic factors (**Hassan *et al.*, 2017**) which affect the community structure in aquatic habitat (**Eskinazi-Sant'Anna *et al.*, 2020**). Site (II) and site (I) represent upstream and downstream for the same flood plain. The results showed that, site (II) is more diverse than site (I) indicating the influence of sewage pollution where rotifers increase and dominate the community of site (I) more than site (II). Similarly, site (V) has higher ammonia and lower diversity index than site (I). Rotifers are important link between microbial loop and higher trophic levels; they are highly dispersed group and characteristic for eutrophication and anthropogenic impacted water bodies especially genus *Brachionus* (**Pedrozo and Rocha, 2005; Lijing *et al.*, 2012; Bielan'ska-Grajner *et al.*, 2014**). Rotifers and Protozoa were dominating sites (I) and (III) in the present work unlike Cladocera and Copepods. Several studies indicated that, rotifers and protozoa dominate organically polluted water bodies (**Sleem and Hassan, 2010; Hassan *et al.*, 2017; Fathibi *et al.*, 2020**). Zooplankton clearly reflects the status of the water body especially sensitive species due to their quick response to changes of the environmental factors; additionally, zooplankton community structure helps in monitoring environmental changes in the water bodies such as eutrophication, pollution, global warming and long-term changes (**Al-Ghanim, 2012**). **Miralto *et al.* (1999)** and **Pinel-Alloul *et al.* (1988)** clarified that, eutrophication greatly influenced species composition where it shifts from dominance of large species to small species. Similar results were obtained by **Hassan *et al.* (2017)** found that zooplankton community structure of impacted water bodies shifted from dominance of large cladocerans to dominance of rotifers after long-term changes to water eutrophication. This was confirmed by **McPherson *et al.* (2002)** and **Isyaku *et al.* (2019)** where habitat pollution leads to disappear of sensitive species and increase of tolerant species. In other words, changes in environmental factors greatly influence passively dispersing than actively dispersing organisms and showed weaker influence on the smaller body animals than those having larger bodies therefore, rotifers with their small sizes, dormant eggs, high reproduction rate (**Lijing *et al.*, 2012**), parthenogenesis (**Xu *et al.*, 2014 and Xiong *et al.*, 2016**) and high acclimatization (**Coldsnow *et al.*, 2017**) make them easily colonize and dominate the new condition of the ecosystem.

CONCLUSION

Freshwater bodies are greatly influenced by sewage disposal, that impaires water quality and consequently changes the community structure and diversity of the aquatic animals especially planktonic component of the ecosystem.

REFERENCES

- Abdul Azis, P.K.; Tisan, I.A.A.; Daili, M.A.; Green, T.N.; Dalvi, A.G.I. and Javeed, M.A.** (2003). Chlorophyll and plankton of the gulf coastal waters of Saudi Arabia bordering a desalination plant1. *Desalination*, 154: 291-302.
- Al-Aidarroos, A.M. and Ghazali, F.M.** (1998). Zooplankton of Highly Eutrophic, Sewage Polluted Coastal Lagoons of Jeddah, Central Red Sea. *J. King Saud Univ. Sci., Mar. Sci.* 9: 131-148.
- Al-Asgah, N.A.; Adam, A.B. and Bedawi, R.M.** (1989). A check list of zooplankton species in Saudi Arabia. *J. King Saud Univ. Sci.* 1: 35-41.
- Al-Ghanim, K.A.** (2012). Spatio-Temporal Distribution and Composition of Zooplankton in Wadi Hanifah Stream Riyadh (Saudi Arabia) and Abu Zabaal Lakes (Egypt). *Pak. J. Zool.* 44: 727-736.
- Aloufi, A.A. and Obuid-Allah, A.H.** (2014). Zooplankton communities inhabiting inland waters of Al-madinah almonawwarah region; Saudi Arabia. *J. Adv. Res.* 2 (7): 706-713
- American Public Health Association (APHA)** (2017). Standard Methods for Examination of Water and Waste Water, 23rd. ed. American public Health Association (APHA), New York.
- Bahabry, A.A.** (2011). Environmental assessment of polluted water and soil due to sewage water at Uranah, South East Makkah. MSc Thesis, King Abdulaziz University, Mekka.
- Barrow, J.L.; Jeziorski, A.; Ru'hland, K.M.; Hadley, K.R. and Smol, J.P.** (2014). Diatoms indicate that calcium decline, not acidification, explains recent cladoceran assemblage changes in south-central Ontario soft water lakes. *J. Paleolimnol.* 52: 61-75. 10.1007/s10933-014-9778-2.
- Bielan'ska-Grajner, I.; Cudak, A.; Biala, A.; Szyman'czak, R. and Sell, J.** (2014). Role of spatial and environmental factors in shaping the rotifer metacommunity in anthropogenic water bodies. *Limnology.* 15(2): 173–183. 10.1007/s10201-014-0428-1
- Bodiou, J.Y.; Delille, D. and de Morais, L.T.** (1990). Influence of organic matter and climatic factors on the harpacticoid copepod (Crustacea) population from the well sorted fine sand of Banyuls Bay. *Helgolander Meeresun.*, 44: 265-274.
- Chen, L.; Liu, Q. and Peng, Z.** (2012). Rotifer community structure and assessment of water quality in Yangcheng Lake. China. *J. Oceanol. Limnol.* 30: 47-58. 10.1007/s00343-00012-00150-y.
- Coldsnow, K.D.; Mattes, B.M.; Hintz, W.D. and Relyea, R.A.** (2017). Rapid evolution of tolerance to road salt in zooplankton. *Environ. Pollut.*, 222: 367-373.

- De Sousa, E.C.P.M.; Zaroni, L.P.; Bergmann Filho T.U.; Marconato, L.A.; Kirschbaum, A.A. and Gasparro, M.R.** (2012). Acute sensitivity to *Nitokra* sp. benthic copepod to potassium dichromate and ammonia chloride. J. Braz. Soc. Ecotoxicol. 7 (1): 75-81. 10.5132/jbse.2012.01.011.
- Eddy, S.** (1934). A study of fresh-water plankton communities. Published by the University of Illinois under the auspices of the Graduate School Urbana, Illinois.
- Einsle, U.** (1996). Copepoda: Cyclopoida. Guides to the identification of the microinvertebrates of the continental waters of the world. Genera *Cyclops*, *Megacyclops*, *Acanthocyclops*. Belgium, State University of Gent, 82 pp.
- El-Bassat, R.A.; Touliabah, H.E.; Harisa, G.I. and Sayegh, F.A.Q.** (2011). Aquatic toxicity of various pharmaceuticals on some isolated plankton species. Int. J. Med. Med. Sci. 3: 170-180.
- Engelmann, H.D.** (1978). Dominanzklassifizierung von bodenarthropoden. Pedobiologia, 18: 378-380.
- Eskinazi-Sant'Anna, E. M.; Santos, G. de S.; Alves, N. J. da S.; Brito, L. A. F. and Leite, M. G. P.** (2020). The relative importance of regional and local factors in shaping zooplankton diversity in high-altitude tropical shallow lakes. J. Freshw. Ecol. 35(1):203–221.
<https://doi.org.sdl.idm.oclc.org/10.1080/02705060.2020.1770874>
- Fathi, A.A. and Al-Kahtani, M.A.** (2009). Water Quality and Planktonic Communities in Al-Khadoud Spring, Al-Hassa, Saudi Arabia. Am. J. Environ. Sci., 5: 434-443.
- Fathibi, K.; Sudhikumar, A. V. and Aneesh, E. M.** (2020). Species composition and abundance of rotifers (Rotifera: Eurotatoria) in Thrissur Kole wetland, Kerala, India. Egypt. J. Aquat. Biol. Fish. 24(6):439–451. <https://doi.org.sdl.idm.oclc.org/10.21608/ejabf.2020.117375>.
- Fuentes-Reines, J.M. and Roa, E.Z.d.** (2013). New additions to the cladoceran fauna of Ciénaga Grande de Santa Marta and Colombia. Check List. 9: 9-24.
- Harris, J.M. and Vinobaba, P.** (2012). Impact of Water Quality on Species Composition and Seasonal Fluctuation of Planktons of Batticaloa Lagoon, Sri Lanka. J. Ecosyst. Ecogr. 2(4):117. 10.4172/2157-7625.1000117.
- Hassan, M.M.; Mahmoud, H.M. and Abd El-Wakeil, K.F.** (2014). Community Structure of Zoobenthos in Some Freshwater Bodies in Taif, Saudi Arabia. J. Adv. Res. 2(4): 114-127.
- Hassan, M.M.; Khalil, M.T.; Shakir, S.H.; Saad, A.A. and El Shabrawy, G.M.** (2017). Zooplankton Community Structure of Lake Edku, Egypt. Egypt. J. Aquat.

- Biol. Fish. 21(3): 55-77. <https://doi.org.sdl.idm.oclc.org/10.3897/zookeys.497.8091>.
- Ismail, A. H. and Zaidin, S. A.** (2015). A comparative study of zooplankton diversity and abundance from three different types of water body, in 2nd International Conference on Agriculture, Environment and Biological Sciences (ICAEB'S'15), Bali (Indonesia). <http://dx.doi.org/10.17758/IAAST.A0715053>.
- Isyaku, I. H. and Ibrahim, S.** (2019). Spatio-Temporal Distribution of Zooplankton and Physico-Chemical Conditions of Wasai Reservoir, Kano State, Nigeria. Bayero J. Pure Appl. Sci., 12:186–192. <https://doi-org.sdl.idm.oclc.org/10.4314/bajopas.v12i1.30S>.
- Jia, J.; Shi, W.; Chen, Q. and Lauridsen, T.L.** (2017). Spatial and temporal variations reveal the response of zooplankton to cyanobacteria. Harmful Algae, 64: 63-73.
- Jones, D.K.; Mattes, B.M.; Hintz, W.D.; Schuler, M.S.; Stoler, A.B.; Lind, L.A.; Cooper, R.O. and Relyea, R.A.** (2017). Investigation of road salts and biotic stressors on freshwater wetland communities. Environ. Pollut., 221:159-167.
- Khalifa, N. and El-Rahman, H.H.A.** (2010). Some Investigations on Zooplankton and Biochemical Contents of Phytoplankton in Wady El-Rayan Lakes, Egypt. World Appl. Sci. J., 11:1035-1046.
- Khalil, M.T.; Shakir, S.H.; Saad, A.A.; El Shabrawy, G.M. and Hassan, M.M.** (2008). Physico -chemical environment of Lake Edku, Egypt. Egypt. J. Aquat. Biol. Fish., 12(2): 119 -132.
- Larson, G.L.; McIntire, C.D.; Buktenica, M.W.; Girdner, S.F. and Truitt, R.E.** (2007). Distribution and abundance of zooplankton populations in Crater Lake, Oregon. Hydrobiologia, 574: 217-233.
- Lewis, C.N.; Brownb, K.A.; Edwardsc, L.A.; Cooperd, G. and Findlay, H.S.** (2013). Sensitivity to ocean acidification parallels natural pCO₂ gradients experienced by Arctic copepods under winter sea ice. Proceedings of the National Academy of Science of the United States of America, PNAS 110 E4960–E4967 www.pnas.org/cgi/doi/4910.1073/pnas.1315162110.
- Lijing, C.; Qiao, L.; Ziran, P.; Zhongjun, H.; Junzeng, X. and Wu, W.** (2012). Rotifer community structure and assessment of water quality in Yangcheng Lake. Chinese J. Oceanol. Limnol., 30: 47-58. DOI: <http://dx.doi.org/10.1007/s00343-00012-00150-y>.
- McPherson, A.K.; Abrahamsen, T.A. and Journey, C.A.** (2002). Investigation of Water Quality and Aquatic Community Structure in Village and Valley Creeks, City of Birmingham, Jefferson County, Alabama, 2000–01. U.S. GEOLOGICAL SURVEY Water-Resources Investigations Report 02-4182. Montgomery, Alabama

- Merlini, M.; Ravera, O. and Bigliocca, C.** (1968). Modern trends in activation analysis. In: J.R. DeVoe, International conference U.S. Department of Commerce National Bureau of Standards Special Publication 312. National Bureau of Standards Gaithersburg, Maryland, U.S.A., pp. 475-481.
- Miralto, A.; Barone, G.; Romano, G.; Poulet, S.A.; Ianora, A.; Russo, G.L.; Buttino, I.; Mazzarella, G.; Laabir, M.; Cabrini, M. and Giacobbe, M.G.** (1999). The insidious effect of diatoms on copepod reproduction. *Nature*, 402: 173 – 176.
- Mueller, D. K. and Helsel, D. R.** (1999). "Nutrients in the Nation's Waters-Too Much of a Good Thing?" U.S. Geological Survey Circular 1136. National Water-Quality Assessment Program. <http://water.usgs.gov/nawqa/circ-1136.html>.
- Omar, W. A. and Hassan, M. M. S. M.** (2018). Environmental assessment of a floodplain exposed to pollution at Taif Governorate, Kingdom of Saudi Arabia. *Biosci. Res.*, 15(4): 3606-3618.
- Pedrozo, C.D.S. and Rocha, O.** (2005). Zooplankton and water quality of lakes of the Northern Coast of Rio Grande do Sul State, Brazil. *Acta Limnol. Bras.*, 17: 445-464.
- Perbiche-Neves, G.; Boxshall, G. A.; Previattelli, D.; Nogueira, M. G. and da Rocha, C. E. F.** (2015). Identification guide to some Diaptomid species (Crustacea, Copepoda, Calanoida, Diaptomidae) of “de la Plata” River Basin (South America). *ZooKeys*, 497:1–111.
- Peroš-Pucar, D. and Ternjej, I.** (2014). The relative importance of physical-chemical factors in the brackish shallow lake Vrana (Croatia) as determinant of crustacean zooplankton community. *Period. Biol.*, 116 (3):293-301.
- Pinel-Alloul, B.; Downing, J.A.; Perusse, M. and Codin-Blumer, G.** (1988). Spatial heterogeneity in freshwater zooplankton: Variation with body size, depth, and scale. *Ecology.*, 69(5): 1393-1400.
- Raza, M. J.** (2004). Groundwater quality evaluation and vulnerability assessment of Wadi Al-Arj alluvium aquifer, Taif, Saudi Arabia. King Fahd University of Petroleum and Minerals. M. Sc. thesis.
- Sarkar, C. and Saha, N.C.** (2016). A study on acute toxicity of an insecticide triazophos on Zooplankton *Cyclops viridis* (Jurine, 1820) along with the changes in their behaviour. *Glob. J. Res. Anal.*, 5: 279-280.
- Shiel, R. J.** (1995). A guide to identification of rotifers, cladocerans and copepods from Australian Inland waters. CRCFE Identification guide no. 3, Co-operative Research Center for Freshwater Ecology. Murray-Darling Freshwater Research Center, Albury: 144pp.
- Shiel, R. and Koste, W.** (1992). Rotifera from Australian inland waters. VIII. Trichocercidae (Rotifera: *Monogononta*). *Trans. R. Soc. S. Aust.*, 116: 1-27.

- Sleem, S.H. and Hassan, M.M.** (2010). Impact of Pollution on Invertebrates Biodiversity in the River Nile Associated With Dahab and El-Warrak Islands, Egypt. *Int. J. Environ. Eng.*, 1:15-25.
- Smironov, N.N.** (1996). Cladocera: the *Chydorinae anssayciinae* (Chydoridae) of the world. SPB Academic Publishing, 119 pp.
- Urban-Rich, J.; McCarty, J.T.; Ferna'ndez, D. and Acun, J.L.** (2006). Larvaceans and copepods excrete fluorescent dissolved organic matter (FDOM). *J. Exp. Mar. Bio. Ecol.*, 332:96- 05.
- Ward, D.J.; Perez-Landa, V.; Spadaro, D.A.; Simpson, S.L. and Jolley, D.F.** (2011). An assessment of three harpacticoid Copepod species for use in ecotoxicological testing. *Arch. Environ. Contam. Toxicol.*, 61:414-425.
- Xiong, W.; Li, J.; Chen, Y.; Shan, B.; Wang, W. and Zhan, A.** (2016). Determinants of community structure of zooplankton in heavily polluted river ecosystems. *Sci. Rep.* 6: 22043. DOI: <http://dx.doi.org/10.1038/srep22043>.
- Xu, X. P.; Xi, Y. L.; Huang, L. and Xiang, X. L.** (2014). The Life-Table Demographic Response of Freshwater Rotifer *Brachionus calyciflorus* to Multi-metal (Cu, Zn, Cd, Cr, and Mn) Mixture Interaction. *B. Environ. Contam. Tox.*, 93:165–170. DOI: <http://dx.doi.org/10.1007/s00128-014-1281-y>.