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Population Dynamics of Rotifers as Influenced by Physicochemical Parameters and Heavy Metals at Khanki Headworks, Gujranwala, Pakistan

Muhammad Ahsan Raza[1,2]*, Nabila Roohi[2], Abdul Qayyum Khan Sulehria[3] and Muhammad Khalil Ahmad Khan[4]

[1]*Department of Zoology, Govt. Graduate College (Boys), Satellite Town, Gujranwala, Pakistan*
[2]*Institute of Zoology, University of the Punjab, Lahore, Pakistan*
[3]*E-154/A9, Yasrab Colony, Walton Road, Lahore Cantt, Lahore, Pakistan*
[4]*Department of Zoology, University of Okara, Okara, Pakistan*

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Authors' Contribution
MAR collected samples, analyzed the data and wrote the paper. NR supervised the work. AQKS helped in data analysis. MKAK helped in sampling.

Key words
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ABSTRACT

Present investigation was conducted to evaluate the seasonal impact of physicochemical parameters of water and heavy metals on the population dynamics of rotifers at specific sites of Khanki Headworks, District Gujranwala, Punjab, Pakistan from February 2021 to January 2022. During this time period, 34 species of rotifers belonging to 10 families and 12 different genera were identified. Brachionidae was proved to be the most abundant family followed by Asplanchnidae. Population density was maximum in June and lowest in January. Rotifers displayed positive relationship with electrical conductivity, pH, temperature and total hardness while negative correlation was recorded with dissolved oxygen. Atomic absorption spectrophotometer was used for the determination of heavy metals in water. Three heavy metals Nickel, Zinc and Arsenic were found in the following order of their concentrations Zn > Ni > As. Analysis of variance also revealed significant difference in population density of rotifers in various months. Shannon-Weaver diversity index reflected greater diversity among rotifer species in June. Environmental variables and heavy metals generally drive the population dynamics of rotifers during the whole year cycle. Findings of this study suggested that rotifer fauna could be a robust bioindicator of water quality in aquatic ecosystem.

INTRODUCTION

Zooplanktons are tiny organisms dependent on water currents for their locomotion. Rotifers, protozoans, cladocerans and copepods are major groups of zooplanktons (Ejaz *et al*., 2016). Rotifers are multicellular microscopic organisms with 2000 species (Raza and Toama, 2021). Rotifers are present in diverse habitats i.e ponds, lake bottoms, rivers and oceans. Few zooplankton are parasitic as well (Lan *et al*., 2021; Coelho *et al*., 2021).

These are natural source of food for most types of fish larvae as well as adult fish (Clarke *et al*., 2013; Lynam *et al*., 2017). Productivity of an ecosystem cannot be maximized without them (Kaymak *et al*., 2018; Ayub *et al*., 2018; Hayee *et al*., 2019). Physicochemical properties of water such as temperature, pH, dissolved oxygen, transparency and electrical conductivity have pronounced effect on the abundance and species composition of rotifers (Sulehria and Malik, 2013; Hussain *et al*., 2016; Ejaz *et al*., 2016; Dastgeer *et al*., 2020). Anthropogenic activities such as excessive use of pesticides, herbicides and insecticides greatly alter the normal range of these water quality variables and reduce the life span of many aquatic organisms (Naseem *et al*., 2022). Suitable temperature range favors the abundance and diversity of rotifers (Havens *et al*., 2015). During summer season, their abundance is on higher side as compared to winter (Golmarvi *et al*., 2018). Rotifers serve as bioindicators of metallic pollution.

Extensive reliance on heavy metals i.e., Hg, Cd,

* Corresponding author: ahsanraza1810@gmail.com
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Cr, Co, Pb, As and Zn is befouling aquatic environment badly (Sharaf *et al*., 2020). Deposition of heavy metals in water bodies is disturbing the rotifer communities (Dural *et al*., 2007; Bat *et al*., 2016; Rauf *et al*., 2019). Khanki Headworks is one of the oldest headworks in Pakistan and it has a great significance for aquaculture projects. Present study is the first attempt to explore the effects of abiotic parameters on the population density and diversity of rotifers at Khanki Headworks. Hence, the main objectives of this work are; (1) to evaluate the population dynamics of rotifers during one year time period (2) to examine the affects water quality parameters and heavy metals on rotifer abundance and diversity (3) to investigate the role of rotifers as bioindicator of pollution in freshwater ecosystem.

MATERIALS AND METHODS

Study area

Khanki Headworks is situated at latitude of 32°24'11" and longitude of 73°58'19" on the River Chenab in Gujranwala district of the Punjab province, Pakistan. It was established in 1889 to control floods and irrigation purposes. A vast area of agricultural lands (3 million acres) is irrigated by Khanki Barrage. It is an attractive site for fishing because of its commercially important fish fauna. After its reconstruction in 2017, It was formally handed over to the Punjab Irrigation Department in June 2019.

Rotifer sampling and preservation

Rotifers were collected from four sampling sites with each site further categorized into three sub-sites. The duration of rotifer sampling was one year from February 2021 to January 2022. Standard plankton net of 37 µm mesh size was used. The net was placed in horizontal position in flowing water for few minutes so that fifty liters of water could pass through it. Plastic bottles (capacity: 50 ml) containing 4-5% formalin solution were used for rotifer preservation (Koste, 1978). Supplementary samples of rotifers were also extracted for live study of organisms.

Counting and identification

Rotifers were identified up to species level with the help of standard keys by considering their morphological features, behavior, size and shape (Ward and Whipple, 1959; Pennak, 1978; Shiel, 2014). Naming of rotifer species was also confirmed by consulting checklist (Segers, 2007). Relevant literature was also reviewed for that purpose (Malik and Sulehria, 2004; Ejaz *et al*., 2016; Dastgeer *et al*., 2020). Counting of rotifers was carried out by utilizing Sedgewick-Rafter chamber (APHA, 2005). Vital stain (1% neutral red) was used prior to live study of rotifers. Rotifer imaging was performed via LEICA HC 50/50 type 020-525.024 an inverted microscope equipped with 5 mega pixel camera.

Water sampling

Water samples (1 liter) were obtained on monthly basis from February 2021 to January 2022 to examine various parameters of water such as dissolved oxygen (DO), electrical conductivity (EC), temperature and heavy metals. Water and air temperature was measured by thermometer (HANNA HI-8053). DO meter (YSI-Eco Sense DO 200A) and pH meter (YSI-Eco Sense pH 100A) were utilized to measure DO and pH respectively, however, conductivity meter (YSI-Eco Sense EC 300) was used to determine the values of EC and total dissolved solids (TDS). Other parameters were examined in laboratory following APHA (2005). Water sampling was mostly conducted between 9 am and 12pm. Bottles were firstly immersed in 2 to 5% HCl solution and then washed with distilled water before using them for sampling. For the analysis of heavy metals, water samples (acidified with 1% HNO_3) were also collected. Estimation of different heavy metals performed by using Atomic Absorption Spectrophotometer (APHA, 2005).

Diversity indices

Two diversity indices namely Shannon-Weaver and Simpson were employed for the calculation of rotifer diversity and density. Species richness was calculated by using Margalef Formula (1968) and species evenness was counted by Shannon and Weaver (1949), Pielou (1966) and Margalef (1968).

Statistical analysis

Pearson's correlation was applied to find out any relationship between rotifer species and physicochemical characteristics (Schober *et al*., 2018). Rotifer data on monthly basis was subjected to one way ANOVA to observe statistically significant difference among rotifer density. ANOVA and Pearson's correlation utilized R programming language software, whereas MS Excel 2019 was employed in graphical representation.

PCA (principal component analysis) was executed to examine the interrelationship of rotifer species with various months (Nunes *et al*., 2021). Canonical correspondence analysis (CCA) was carried out to explore the correlation between rotifers and physicochemical parameters (Bouazzara *et al*., 2021; El-Tohamy *et al*., 2018). Software XL-Stat 2022 was utilized for both analyses.

RESULTS

Population attributes of rotifers

In this study, we recorded 34 rotifer species related to

12 genera and 10 families (Table I). *Brachionus* was the most abundant genus (Fig. 5) and *Brachionus angularis* (49±14.52) was the most dominant species followed by *Brachionus calyciflorus* (43±11.91). *Collurella ovalis* had the lowest (0.5±0.33) population density and it was observed only in September (Fig. 3). Highest values (2.42) of Shannon weaver index were recorded in June and lowest (1.21) were observed in January (Fig. 4). Greater biodiversity of rotifers was recorded in June and minimum in January which was also reflected by values of Simpson index of diversity being maximum (0.90) in June and minimum (0.67) in January (Fig. 4). In contrast Simpson density index value was highest (0.33) in January and lowest (0.10) in June. Values of species richness were calculated in the range of 2.92 and 1.07. Values of species evenness varied between 0.93 and 0.41 (Fig. 4). Statistically significant disparity in rotifer density was demonstrated by ANOVA (Table II).

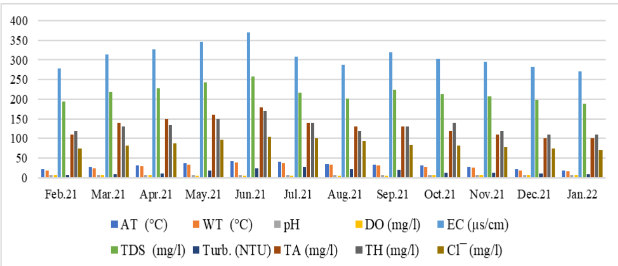

Fig. 1. Variations of different physicochemical parameters, temperature (°C), pH, dissolved oxygen (DO), electrical conductivity (EC), total hardness (TH), turbidity (NTU), total alkalinity (TA), total dissolved solids (TDS), chlorides (mg/L) at Khanki Headworks.

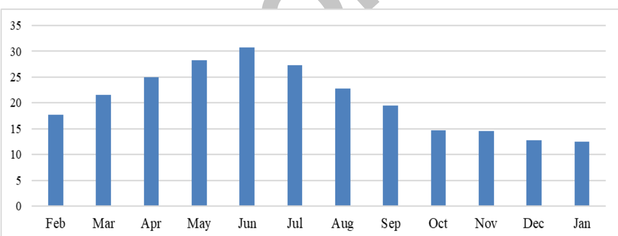

Fig. 2. Population density of rotifers on monthly basis from Khanki Headworks.

Physicochemical parameters and rotifers

We were able to explore the relationships between various physicochemical parameters and population dynamics of rotifer fauna. A direct correlation was recorded between temperature and population abundance of rotifers (Table III). Maximum temperature (39°C) was reported in June while the minimum (16°C) in January (Fig. 1).

Table I. List of Rotifer species identified from Khanki Headworks.

Family/ Genus	Species
Asplanchnidae	
Asplanchna	*1. Asplanchna brightwelli*
	2. A. girodi
	3. A. herricki
	4. A. priodonta
Brachionidae	
Brachionus	*5. Brachionus angularis*
	6. B. calyciflorus
	7. B. caudatus
	8. B. forficula
Collothecidae	
Collotheca	*9. Collotheca ambigua*
Keratella	*10. Keratella tropica*
	11. K. valga
Filinidae	
Filinia	*12. Filinia terminalis*
Gastropodidae	
Ascomorpha	*13. Ascomorpha saltans*
Lecanidae	
Lecane	*14. Lecane bulla*
	15. L. closterocerca
	16. L. hamata
	17. L. inopinata
	18. L. lunaris
	19. L. syngenes
Lepadellidae	
Lepadella	*20. Lepadella acuminata*
	21. L. cornuta
	22. L. lindaui
	23. L. patella
	24. L. vitrea
Collurella	*25. Collurella adriatica*
	26. C. obtusa
	27. C. ovalis
Notommatidae	
Cephalodella	*28. Cephalodella sterea*
Philodinidae	
Rotaria	*29. Rotaria rotatoria*
Testudinellidae	
Testudinella	*30. Testudinella ahlstromi*
	31. T. emarginata
	32. T. mucronata
	33. T. patina
	34. T. striata

Table II. Analysis of variance of rotifers (p<0.05).

Source of variation	SS	DF	MS	F	P-value	F crit
Between groups	1193.565	1	1193.565	45.15193	0.000	4.30095
Within groups	581.5573	22	26.43442			
Total	1775.122	23				

Table III. Pearson correlations between rotifers and physicochemical parameters.

	Rotifers	AT	WT	pH	DO	EC	TDS	Turb	TA	TH	Cl^-
Rotifers	1										
AT	0.845	1									
WT	0.839	0.995	1								
pH	0.112	0.406	0.409	1							
DO	-0.784	-0.956	-0.952	-0.486	1						
EC	0.818	0.780	0.755	-0.136	-0.645	1					
TDS	0.819	0.783	0.758	-0.133	-0.649	0.999	1				
Turb	0.710	0.899	0.897	0.527	-0.939	0.537	0.539	1			
TA	0.944	0.835	0.819	-0.022	-0.737	0.943	0.945	0.609	1		
TH	0.810	0.821	0.789	0.021	-0.699	0.931	0.931	0.616	0.912	1	
Cl^-	0.925	0.957	0.950	0.298	-0.935	0.766	0.768	0.897	0.866	0.813	1

Similarly, highest rotifer density (30.75) was documented in June and lowest (12.5) in January (Fig. 2). Rotifer diversity was also at its peak in June (13 species) and its number (4 species) was significantly reduced in January. Values of pH were calculated in the range of 7-7.9. Maximum pH was recorded in July and minimum in January (Fig. 1). Pearson correlation revealed that pH affect rotifer diversity and density in a positive manner (Table III). Both EC and TH exhibited positive relationship with population abundance of rotifers (Table III). Highest values of EC (369 µs/cm) and TH (170 mg/l) were noted in June and lowest values of EC (270 µs/cm) and TH (110 mg/l) were observed in January (Fig. 1). On the contrary DO presented a negative correlation with rotifer density and diversity (Table III). Highest mean value of DO (6.97) was recorded in January and its minimum value (5.34) was observed in June (Fig. 1). Alkalinity range was noted maximum (180mg/l) in June and minimum (100mg/l) in January (Fig. 1). Turbidity showed positive impact on population density of rotifers (Table III). Range of TDS varied from 258 mg/l in June to 189 mg/l in January (Fig. 1).

Seven principal components were selected for PCA, depicting 90.828% of total variance. Axis F1 (24.10%) and axis F2 (20.45%) indicated total 44.55% variance in rotifer community structure (Fig. 7). Symmetric map of CCA represented the effects of different physicochemical factors of water on the density and diversity of rotifers from February 2021 to January 2022 (Fig. 8). The first two CCA axes for rotifers abundance (34 species) recorded 47.60% of the trended information (26.97% and 20.62%).

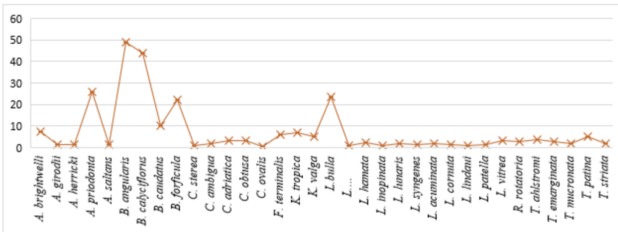

Fig. 3. Relative abundance of rotifer species isolated from Khanki Headworks.

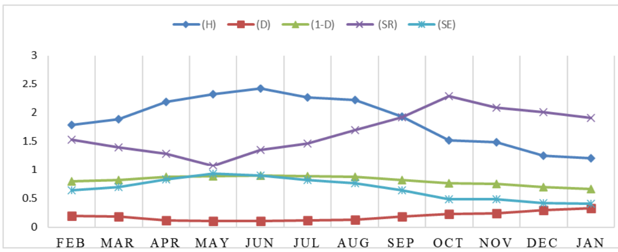

Fig. 4. Variations of diversity indices of rotifers isolated from Khanki Headworks. H, Shannon-weaver diversity index; D, Simpson index of dominance; 1-D, Simpson index of diversity; SR, species richness; SE, species evenness.

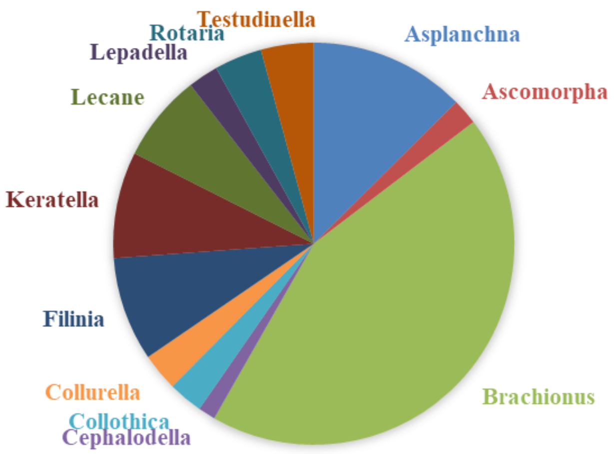

Fig. 5. Percentage representation of rotifer genera isolated from Khanki Headworks.

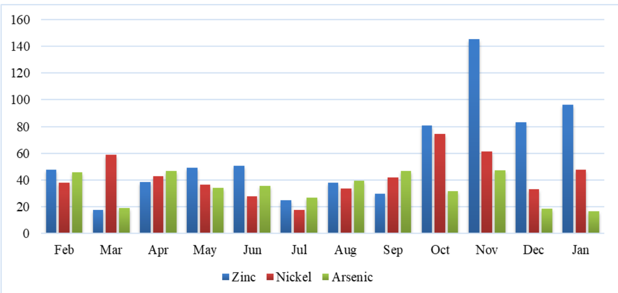

Fig. 6. Relative concentrations of heavy metals collected from Khanki Headworks.

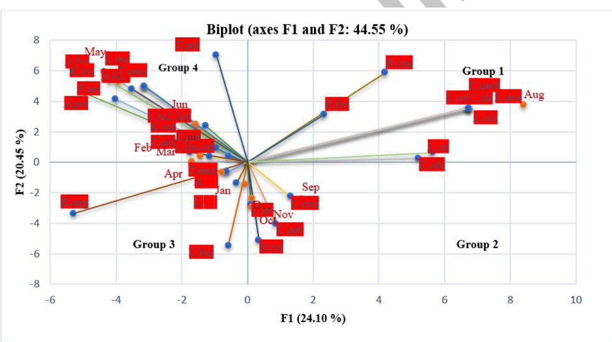

Fig. 7. PCA biplot of 34 rotifer species of Khanki Headworks from February 2021 to January 2022.

Heavy metals and rotifers

Three heavy metals Nickel, Zinc and Arsenic were reported at Khanki Headworks throughout the year. Concentrations of these heavy metals were recorded in following order Zn > Ni > As (Fig. 6). It was observed that high concentrations of heavy metals influence the rotifer density and diversity in negative manner.

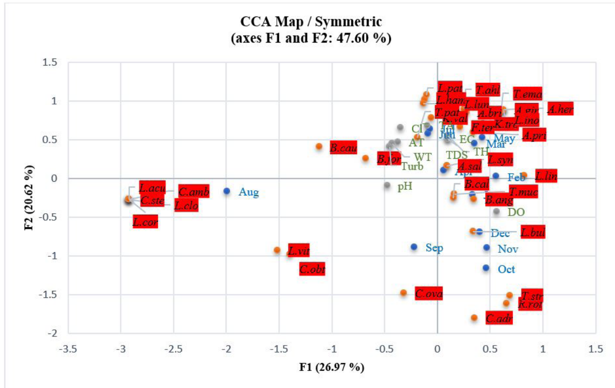

Fig. 8. CCA ordination triplot depicting the rotifer environment relationships at Khanki Headworks.

DISCUSSION

During summer season population abundance was on the rise. In the month of June both rotifer density and diversity were recorded as maximum (Fig. 2). Such pattern was observed due to favorable conditions for rotifer proliferation. Suitable temperature, availability of food resources, lesser competition and normal range of pH all were facilitating the growth of rotifers. In contrast rotifer population was on decline during winter season and lowest in January. Similar observations are reported by other authors as well (Paturej *et al*., 2017; Joshua *et al*., 2018; Rauf *et al*., 2018; Hayee *et al*., 2019). Brachionidae was noted as most dominant family of rotifers and percentage composition of genus *Brachionus* (43%) was recorded as highest (Fig. 5). Review of previous literature also validate such findings (Sulehria and Malik, 2013; Vázquez-Sánchez *et al*., 2014; Ejaz *et al*., 2016; Dastgeer *et al*., 2020). Percentage composition of *Cephalodella* genus was found lowest throughout the year.

Water quality variables greatly affect the species composition and richness of rotifers. We were able to find out different kinds of relationships between physicochemical parameters of water and rotifers. Temperature was observed to impact the population density of rotifers in a positive manner. It was monitored that high values of temperature enhance the abundance of rotifers. An inverse correlation was recorded between the DO and rotifer density. Although DO level was minimum in June but growth rate of rotifers was increasing. It was also reported by other scientists (Sulehria *et al*., 2009; Ejaz *et al*., 2017; Golmarvi *et al*., 2018; Joshua *et al*., 2018; Rauf *et al*., 2018; Dastgeer *et al*., 2020). DO level drops in summer because of more decay of organic matter and high temperature. pH range between 6.5 to 8.5 favors the rotifers to increase in number (Neschuk *et al*., 2002; Ejaz *et al*., 2016). In our investigation pH range was calculated between 7 to 7.9 which paved the way for greater abundance of rotifers. We were able to explore a positive connection between EC and rotifers. Same positive link was found between the levels of total dissolved solids and rotifer density. High temperature and greater organic decay raise the level of EC during summer. TH was another parameter which was noted to have direct link with rotifer proliferation. These findings were also described by earlier researchers (Ejaz *et al*., 2017; Benedetti *et al*., 2019; Nwinyimagu *et al*., 2021). In Figure 7 PCA values depicted that there was high abundance of rotifer species during summer season as compared to winter months (Ejaz *et al*., 2016; Hussain *et al*., 2016). Group 1 was established on upper right corner of the biplot and manifested nine rotifer species related to the month of August while group 2 on the lower left side reflected only four species associated with the months of September, October, November and December. Group 3 on bottom left presented five species connected to the months of January and April and group 4 on the upper left side contained sixteen species linked to February, March, May, June and July. CCA was applied to explore the interrelationships between rotifer species and various water quality parameters (Fig. 8). According to its results higher population abundance was noted in summer months and a positive relationship was found between density and diversity of rotifers and different environmental factors (temperature, EC, TDS, TH and turbidity). Heavy metals were analyzed on monthly basis. Only three heavy metals namely Ni, Zn and As were detected in high concentrations. Concentration level of Zn was highest among them (Fig. 6). *Brachionus* was observed as most tolerant genus to heavy metals exposure followed by *Asplanchna*, *Keratella* and *Filinia* respectively. These observations were in accordance with results of previous researchers (Wilkozniak *et al*., 2011; Itigilova *et al*., 2016; Rauf *et al*., 2019; Lordache *et al*., 2022).

CONCLUSION

In conclusion, heavy metals and environmental variables showed strong influence on population attributes of rotifers. Summer season was found more suitable for rotifer abundance and diversity. No such study has been conducted earlier at this site. Our findings will provide insight on improvement of water quality for aquaculture projects at this site.

Statement of conflict of interest

The authors have declared no conflict of interest.

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