

# Ecology and Diversity of Freshwater Zooplankton in Laghouat Province (Algeria) and their Relationship with Environmental Factors

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## ABSTRACT

In order to investigate the distribution and richness of zooplankton on the Wadi M'zi River from November 2018 to October 2019, samples were collected on a monthly basis from four stations. Thirty-three zooplankton species belonging to four taxa have been described from the Wadi M'zi River; Copepoda, Rotifera, Cladocera and Nematoda. The following diversity indexes were used to measure the diversity of zooplankton species in the lake; Shannon Index 3.14 to 3.8, Pielou Evenness Index 0.72 to 0.84 and Margalef Index 1.08 to 1.45. The zooplankton communities were observed to be abundant in spring and summer compared to autumn and winter. Rotifers were found to be dominant followed by copepods, cladocerans and nematods for the entire research duration. CCA analysis indicates that WT, pH, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>2-</sup> and conductivity were responsible for most differences in zooplankton organisms. Finally, we concluded that all sampling area were oligosabrobic indicating the good environmental quality of Wadi M'zi River.

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## Authors' Contribution

HB designed the study, carried out the surveys, collected samples, performed laboratory work and the statistical analysis, wrote the manuscript. RC supervised surveys and laboratory work. FB and LB supervised the statistical analysis and manuscript writing. AN corrected the language.

## Key words

Zooplankton, Diversity, Environmental factors, Canonical correspondence analysis, Wadi M'zi River.

## INTRODUCTION

Freshwater ecosystems contain a wide range of microorganisms this variety is indispensable for maintaining the balance of such ecosystems. Regrettably, water and biodiversity are exposed to many environmental stressors, particularly those resulting from intensive human activities (Xiong *et al.*, 2019). Environmental and human activities are thought to be a significant cause of environmental degradation (Thietal., 2018). Desertification, agricultural expansion and industrial development,

urbanization and growing pollution, land-use change and climate change (rain and temperature) have exerted tremendous stress on the ecological conditions and sustainable development of many aquatic habitats. Protists, copepods, rotifers, nematods and cladocerans are rapid responders to many environmental factors (Pawlowski *et al.*, 2016) because of their rapid reaction to changes in the aquatic ecosystem considered to be an excellent biological sensor of water quality. Many researches types confirm that zooplankton communities have been significantly affected by excessive nutrient loading (Antony *et al.*, 2020). Several distinctive elements may be used as system health indicators, including essential chemical elements such as (nitrate, nitrite, phosphate, ammonium and dissolved oxygen) or biological characteristics such as zooplankton abundance. For aquatic organisms' lives, nutrients are essential, but excessive loading of nutrients into water sources may affect water's designated uses. In this research, a 1-year survey was conducted in Wadi M'zi River Laghouat Province, located in the south of Algeria,

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about 400 miles from the capital of Algeria. Wadi M'zi is one of the continental aquatic environments that are complex and fragile. It is the most important valleys in Algeria; it flows through the states of Djelfa, Biskra, Wadi Souf, and Laghouat on which there is the Seklafa Dam in Tadjmout province. Despite the importance of this river for the both region and population and according to our knowledge no previous study did reported the influence of water degradation on the Wadi M'zi biological populations. In fact, the use of biological indicators as zooplankton is recommended for water quality evaluation especially in freshwater ecosystems (Benoit, 2014; Li and Chen, 2020). Hence, our objectives were: i) to assess the environmental quality status using distribution patterns of the zooplankton communities in relation to the hydrological condition in this ecosystem, ii) to document the taxonomic composition of zooplankton populations, iii) to classify their distribution patterns. It can be mentioned that zooplankton diversity can be used as a methods for evaluating the state of water quality in small and shallow water bodies.

## MATERIALS AND METHODS

### *Study sites, sample collection and analysis*

Wadi M'zi originates in Djbel Chebka at an altitude of 1536 m, is considered a natural permanent freshwater wadi with a salinity of less than 1.2 g/L. There is an arid climate characterized by rainy spring and fall, dry summer and winter and significant seasonal changes. The estimated annual temperature and precipitation are 16.2°C and 184 mm, respectively. Field sampling was performed every month at 04 locations (St.1–St.4) along the longitudinal gradient of the M'zi Wadi River during the morning hours of November 2018 to October 2019 (7:00 am - 8:00 am). A complete explanation of the 04 sampling sites is given in Table I. Physical parameters (water temperature and pH) were analyzed in situ using multi-parameter (HANNA),

electrical conductivity (CE) measured with a conductivity meter (HANNA), while chlorophyll-*a* concentrations were measured using the Lorenzen (1967) monochromatic method and dissolved oxygen (DO) was analyzed by Winkler's method (Strickland and Parsons, 1979). Besides, nitrates ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), sulfate ( $\text{SO}_4^{2-}$ ) and orthophosphates ( $\text{PO}_4^{2-}$ ) have been studied using standard methods (APHA, 2005). Zooplankton samples were obtained at each station by vertical transport (from bottom to surface) from 0.5 m depth below the surface at each site using a typical plankton net of 20µm mesh size. Samples obtained were stored in a 4% neutral formalin solution and their volumes were concentrated at 100 mL. The water sample was collected from each site and subsequently stored with 1.5 % Lugol's iodine solution in the field and then concentrated to 50 mL after sedimentation for 48 h in the laboratory. After the entire mixture, 1 mL of the sample counting chamber was used to identify the zooplankton. It has been replicated three or four times (Zhao *et al.*, 2018). Zooplankton count and identification were made by a light microscope (Zeiss Axiostar) using the 40X objective phase contrast optics. Identification was made based on morphological characteristics using published guides and keys (Shiel, 2014; Glime, 2017a, b, c, d). The enumeration of the organisms was carried outnumbering them on the entire collection. The species' composition was expressed as the proportion of the species concerning the total number of individuals (%) whereas the abundance was expressed as the number of individuals per liter (ind/L).

### *Data analysis*

The species diversity (Shannon–Weiner,  $H'$ ), species evenness (Pielou's,  $J'$ ) and species richness (Margalef,  $D$ ) indices were used to summarize the biodiversity of the zooplankton communities (Shannon and Weaver, 1949; Pielou, 1966; Margalef, 1968). These measures were

**Table I.- Description of the studied sampling sites in the Wadi M'zi River.**

Sites	Location	GPS	Altitude	Depth
St.1	-1 Km from the River source; Low anthropogenic activities; Workshop (Seklafa Dam); Low population density (Bedouin communities); High concentration of fish in the lake	N: 33°59'55.90." E: 2°22'1.75."	976m	0.6m
St.2	- 2.7 Km from the river source; Agricultural activities; anthropogenic activities (medium); Low population density (Bedouin communities); High concentration of fish in the lake	N: 33°59'3.40." E: 2°22'23.58."	978m	0.5m
St.3	-7.2 Km from the river source; Low population density (Bedouin communities); High vegetation; Agricultural activities; High concentration of fish in the lake	N: 33°57'4.87." E: 2°24'0.86"	965m	0.25m
St.4	-15.6 Km from the river source, located after receiving water from the Tadjmout dam; High population density; High anthropogenic activities; Low concentration of fish in the lake; Contaminated with city sewage; Exposed to road traffic	N: 33°54'10.13" E: 2°28'19.59"	907m	0.6m

computed using the following formulae:

$$H' = -\sum p_i (\log_2 p_i)$$

$$J' = H' / \ln S$$

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

Where,  $P_i$  is proportion of the total counted arising from the  $i$ th species,  $S$  is total number of species, and  $N$  is total number of individuals.

The relationship between zooplankton organisms and environmental variables was drawn between 10 physicochemical parameters and was calculated by Canonical Correspondence Analysis using Palaeontological Statistics (PAST) Software Version 3.26. Indicator species have been described as the species with  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Environmental conditions

The mean temporal variation of the water parameters analyzed over the period is recorded in Figure 1. Similar seasonal variations of water temperature distinguished the water river under analysis; the highest WT value was recorded in summer (28.1°C) and the lowest in winter (15.52°C). Chl-*a*, CE and DO were higher in summer and were correlated positively with temperature ( $r = 0.6$ ;  $0.7$

and  $0.6$ ). pH was slightly alkaline during all season and it fluctuates between 8.1 and 8.25.  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were higher in winter and lowered in summer. However, ammonium was higher in autumn and it attenuated a value of 15.93 mg/L. Phosphate and sulfate have shown small variation among season and they were higher in autumn, spring and lower in summer. Phosphate was correlated negatively with conductivity, while sulfate was correlated positively with conductivity. These differences in the environmental factor are according to the sampling area, which is characterized by a dry and desert climate. The highest levels of the nutritive element are explicated to the heavy use of nitrogen fertilizers and the ultimate mineralization of other nitrogen forms ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ) (Rodier, 2009). Moreover, the occurrence of sulfate is primarily related to the geological structure of the parent rock and the land of the region.

### Zooplankton diversity patterns and dominant species

The composition and dominance of the zooplankton species in a particular water body are regulated by certain ecological factors, such as nutrient loading and pollution status. Thirty-three (33) species of zooplankton have been reported in Wadi M'zi River (Table II). Four taxa were

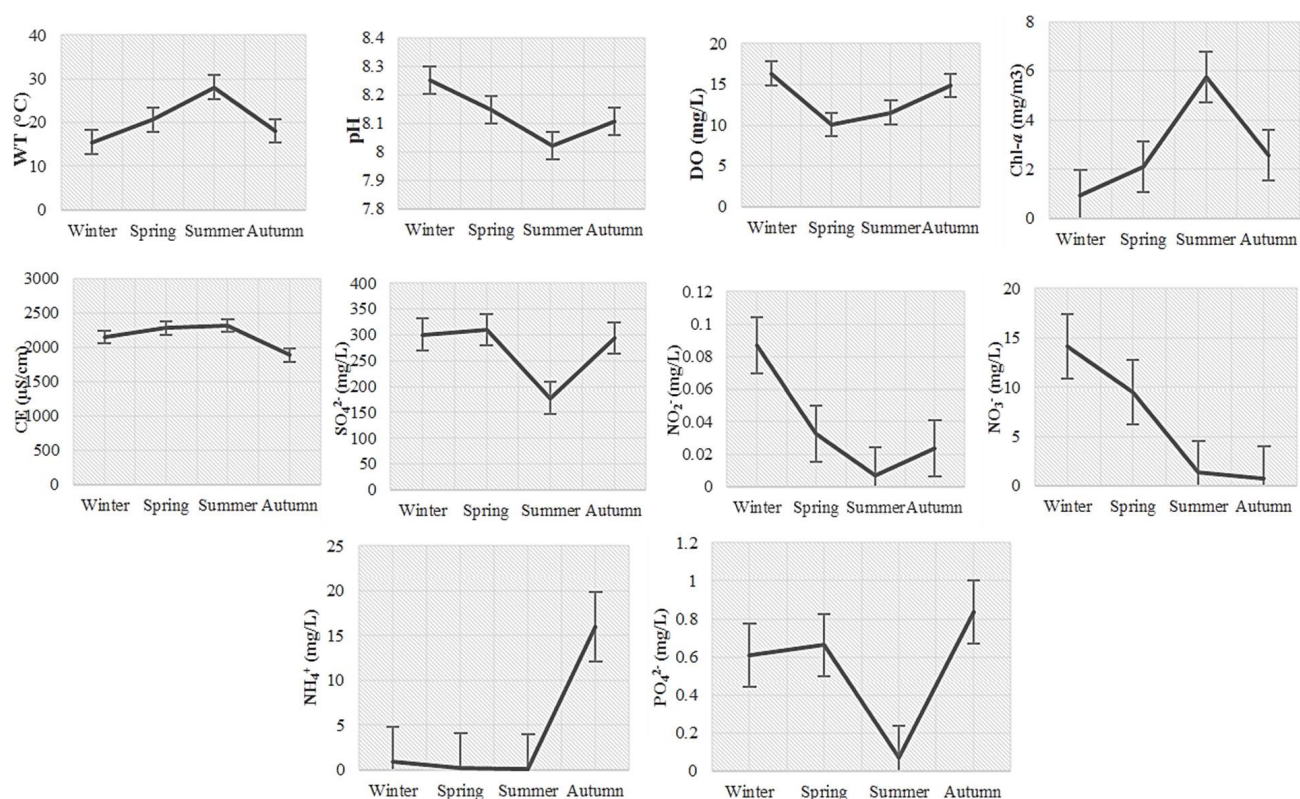


Fig. 1. Temporal variation in hydrological variables in Wadi M'zi River during the study period (Bar Standard Deviation).

**Table II.- Species of the zooplankton population in the Wadi M'zi River and their percentage of occurrences.**

Groups	Family	Taxa	Abbr.	St.1	St.2	St.3	St.4	Occurrence %
Rotifers	Brachionidae	<i>Anuraeopsis fissa</i>	Brd		+			25
		<i>Brachionus durgae</i>	Anf	+	+			50
		<i>Brachionus urceolaris</i>	Bru		+	+	+	75
	Dicranophoridae	<i>Aspelta beltista</i>	Asb			+	+	50
		<i>Dicranophorus biastis</i>	Dibi			+	+	50
		<i>Dicranophorus colastes</i>	Dico			+	+	50
		<i>Dicranophorus robustus</i>	Diro	+	+	+	+	100
		<i>Encentrum felis</i>	Enf			+	+	50
		<i>Lecane bulla bulla</i>	Lbb	+	+	+	+	100
	Lecanidae	<i>Lecane closterocerca</i>	Lec	+	+	+	+	100
		<i>Lecane cornuta</i>	Lco	+		+	+	75
		<i>Lecane curvicornis</i>	Lecr				+	25
		<i>Lecane depressa</i>	Ledp	+	+	+	+	100
		<i>Lecane flexilis</i>	Lefx			+		25
		<i>Lecane martensi</i>	Lmt				+	25
		<i>Lecane pyriformis</i>	Lep	+	+		+	75
		<i>Lecane unguolata</i>	Leung			+	+	50
	Lepadellidae	<i>Colurella adriatica</i>	Coad			+		25
		<i>Colurella geophila</i>	Colg	+	+	+	+	100
		<i>Lepadella discoidea Segers</i>	LediS	+	+		+	75
		<i>Lepadella naplius</i>	Lepna		+	+		50
		<i>Lepadella ovalis</i>	Lov		+			25
		<i>Lepadella patellaa</i>	Lepp			+		25
	Philodinidae	<i>Rotaria rotatoria</i>	Ror	+	+			50
	Trichocercidae	<i>Trichocerca brachyura</i>	Trb		+			25
		<i>Trichocerca obtusidens</i>	Tro	+	+	+	+	100
		<i>Trichocerca parvula</i>	Trp	+	+		+	75
Copepods	Cyclopidae	<i>Copepoda nauplius</i>	Cpn		+	+	+	75
		<i>Macrocyclus albidus</i>	Mal	+		+		50
Cladocerans	Bosminidae	<i>Bosmina meridionalis</i>	Bosm			+		25
	Daphniidae	<i>Daphnia lumholtzi</i>	Dapl	+	+			50
		<i>Daphnia pulex</i>	Dapu	+	+	+	+	100
Nematods	Plectidae	<i>Plectus murrayi</i>	Plm	+	+	+	+	100

presented in the lake; Rotifera is the dominant zooplankton group and present more than 88% of the total zooplankton population followed by Nematoda 4.6%, Cladocera 4.4% and Copepoda 2%. We identified ten (10) families during the study, where six (6) belonging to rotifers, one (1) to copepods, two (2) to cladocerans and one (1) to nematods. Dicranophoridae was dominant, with 30% of the total zooplankton composition (Fig. 2). This was followed by Lecanidae 25% and Lepadellidae 19%. *Dicranophorus robustus*; *Colurella geophila*; *Lecane bulla bulla*; *Lecane closterocerca*; *Lecane depressa*; *Trichocerca obtusidens*; *Trichocerca parvula*; *Lepadella naplius* and *Lepadella patellaa* were the most presented in the river during all season of the study period. The number of Bosminidae and

Cyclopidae species in Wadi M'zi during the study period are not high, *i.e.*, only one Bosminidae species (*Bosmina meridionalis*) and two Cyclopidae species (*Macrocyclus albidus* and *Copepoda nauplius*) have been found. This rotifers dominance is due to the proliferation of *Colurella geophila*, *Dicranophorus robustus*, *Lecane closterocerca* and *Trichocerca obtusidens* in all seasons, associated to nitrates, phosphates concentrations and dissolved oxygen rate. Such situation can be explained by the fact that, in a desert environment, the waters are relatively calm and have many aspects conducive to the growth of Rotifers, which is not the case in times of flooding where heavy winds and flows are undesirable to the installation of planktonic species (Appiah *et al.*, 2018).



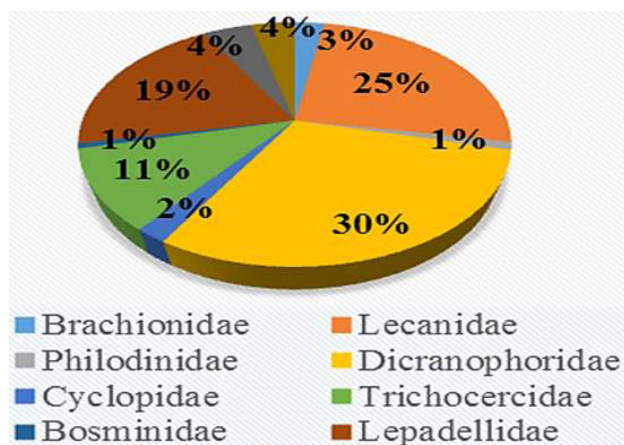


Fig. 2. Percentages of the species comprising different zooplankton groups relative to the total number of zooplanktons.

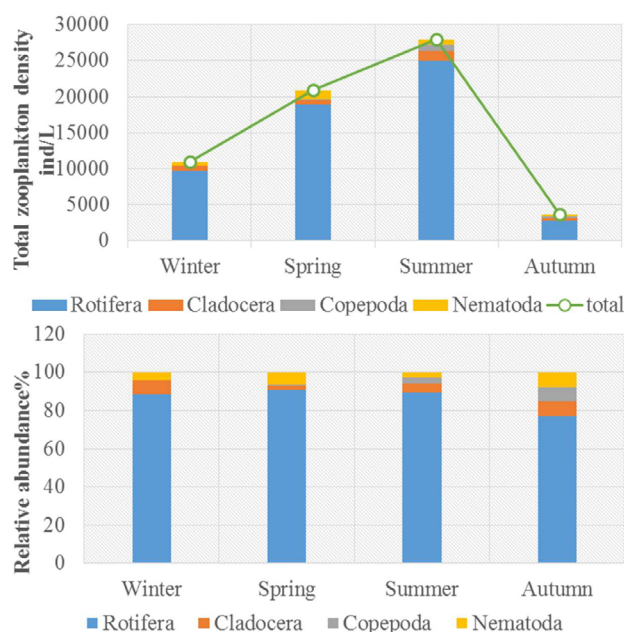


Fig. 3. Variation in the relative density of different zooplankton taxa and total zooplankton density during sampling periods.

#### Temporal and spatial variation in the zooplankton community

Rotifera always dominated the zooplankton community during the sampling phases. The density accounts for more than 75% at each season (Fig. 3). The relative density of Cladocera, Nematoda and Copepoda were highest in autumn, at 7.68%. The higher value of Cladocera density was observed in winter and summer with (800.7 ind/L and 1271.7 ind/L), then decreased gradually

with the lowest in autumn. Copepods had a similar tendency. The higher Copepoda density was 894.9 ind/L in summer and the lowest 282.6 ind/L in autumn. Then it proposed that water temperature was among the most significant factors influencing the seasonal occurrence of crustacean zooplankton population structure. For copepods in autumn and summer, the most common species were *Macrocyclus albidus* and *Copepoda nauplius*. For Rotifera, the dominant species included *Lecane bulla bulla*, *Colurella geophila* and *Dicranophorus robustus* in spring and summer at the four studied lakes. Furthermore, the abundance of zooplankton can vary depending on the species and the abundance of the fish in different lakes. This would be one of the reasons for the lower or higher zooplankton density in the lake. Nematoda also showed some variations at each season the highest value was recorded with 1318.8 ind/L in spring and the lowest in autumn 282.6 ind/L. The total Rotifera density was significantly higher than that of the other zooplankton within the range of 2827.1 and 25009.3 ind/L. There were different spatial changes in zooplankton density in the four water samples, as seen in Figure 4. The highest zooplankton density (10644.6 ind/L) was reported at St.3 in summer and St.2 in spring with (9608.4 ind/L).

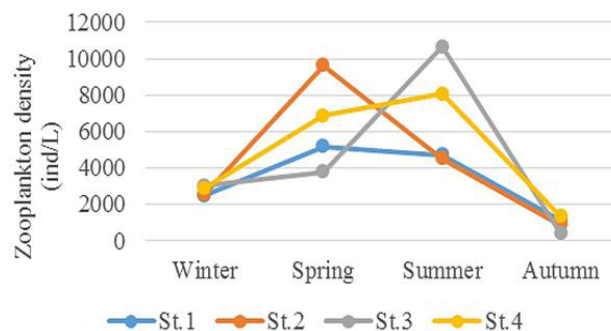


Fig. 4. Distribution of zooplankton density at four studied lakes.

In comparison, the lowest density (only 377.93 ind/L) was reported at St.3 in autumn these variations among the sites is explicated to their exposition to different factors such as industry, agriculture and anthropogenic activities. In our study, zooplankton diversity was the highest in spring and summer rather than during the other sampling periods. This may be due to favourable spring temperatures (10 to 20°C) with a significant variety of species (such as the Lucanidae, Trichocercidae, Lepadellidae and Dicranophoridae family) complementing the diversity of zooplankton in the water body. According to the findings of Bouazzara *et al.* (2020), cyanobacteria were habitually prevalent in the St.4 which is located after receiving water from the Tadjmout dam and specially in spring season, the

abundance of cyanobacteria in this lake may suggest the increased number of available resources for zooplankton. Another potential reason was that the lake ecosystems were more diverse due to the variety of aquatic vegetation in spring and summer, such as the emergent and submerged plants.

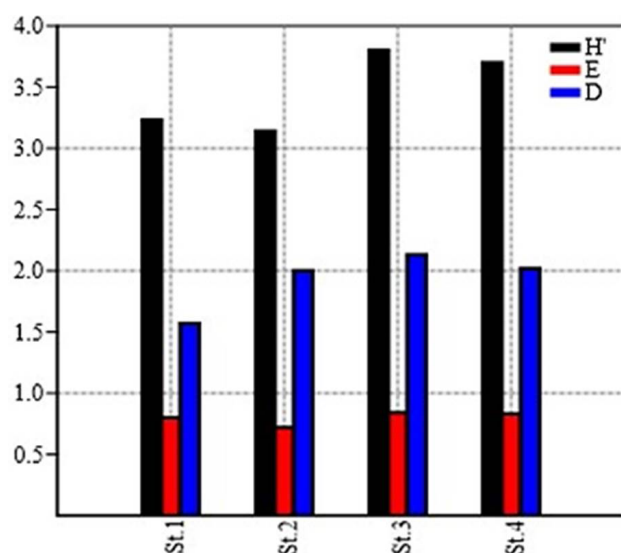


Fig. 5. The diversity index of zooplankton taxa in floodplain lakes collected in different stations.

#### Diversity indices

The result of diversity indices are shown in Figure 5. The highest Shannon-Weiner diversity was recorded at St.3 with 22 species: 17 *Rotifera*; 02 *Copepoda*, 2 *Cladocera* and just one species belongs to *Nematoda*. In contrast, the lowest Shannon-Weiner diversity was recorded at St.2 with 20 species: 16 *Rotifera*; 2 *Cladocera* and 01 for *Nematoda* and *Copepoda*. In addition, the Pielou evenness diversity shown the same result comparing to the Shannon-Weiner diversity index. Where the highest value also recorded at St.3 with (0.84) and the lowest in St.2 with (0.72). Similarly, there was also a significant trend of the highest Margalef richness diversity at St.3 than that the other stations sampling. The lowest Margalef richness diversity was 1.08 at St.1 and the highest was 1.45 at St.3. Their relation with the environmental factor can explain these diversities according to the result of correlation; there was a significant trend of the environmental factor's impact on diversity. The correlation analysis (Pearson, PAST) showed that the Shannon-Weiner diversity index was positively correlated with pH and  $\text{SO}_4^{2-}$  ( $r = 0.78$ ;  $r = 0.85$ ) and negatively correlated with  $\text{PO}_4^{2-}$  ( $r = -0.7$ ), which means that low quantity of  $\text{SO}_4^{2-}$  might limit the distribution of zooplankton species. As they prefer a slightly alkaline

environment according to their strong correlation with pH, a high level of  $\text{PO}_4^{2-}$  might influence their distribution. The Pielou evenness index was positively correlated with  $\text{SO}_4^{2-}$  ( $r = 0.98$ ) and negatively with  $\text{PO}_4^{2-}$  ( $r = -0.86$ ). The Margalef richness index value were positively correlated with pH ( $r = 0.88$ ) and negatively with dissolved oxygen and  $\text{NH}_4^+$  ( $r = -0.95$ ;  $r = -0.86$ ). The Shannon-Weiner diversity index averaged between 3.14 to 3.8, a value higher than those reported in previous studies that used finer mesh size (Wang *et al.*, 2019a). Shannon-Weiner's value was higher in the area influenced by organic matter where it indicates that all sampling sites were oligosaprobic and there are in accordance with the assessment standard of biodiversity indices. Pielou-evenness index value had a similar distribution pattern as that of the Shannon-Weiner diversity, with an average of 0.79, the value was high compared to other studies (Wang *et al.*, 2019b; Appiah *et al.*, 2018). The evenness value reflects the species' more or less equally homogenous distribution in the pond environment at the time of sampling. So according to our result, this high value indicates an equal distribution of species. The Margalef diversity index averaged from 1.08 to 1.45, a value lower than which found by Wang *et al.* (2019b) and Ayub *et al.* (2018) this value might be explained to the nature of the study region and the influence of nutrients elements on their distribution. It is shown that sulfate ammonium and phosphate had a strong correlation with the diversity index. The high level of sulfate in the study area might be due to the parent rock's geological nature and the land of the region. The highest level of ammonium was reported in winter and autumn in the stations most vulnerable to leaching and anthropogenic activities partly due to the heavy use of nitrogen fertilizers and ultimate mineralization (Rodier, 2009). Thus, phosphate has a crucial role in the aquatic ecosystem's primary productivity by promoting the growth of organisms and limiting the production of aquatic species.

#### Relationship between zooplankton species and environmental factor

CCA has been used to discover the association between environmental variables and zooplankton composition and will further recognize the driving factors influencing the zooplankton community structure. Figure 6 shows the CCA sample triplots concerning the environmental gradients potentially influencing the zooplankton community structure. Based on the Dominance Value calculation ( $Y > 0.02$ ), 26 dominant species in the four sites during this study were chosen for analysis. The CCA ordination diagram for zooplankton species and environmental variables of water is shown in Figure 6. The length of the arrow is related to the significance of

the component. Thus, it demonstrates positive or negative associations with the axis. We noticed that percentages of variance and Eigenvalues of each site in axis 1 were higher than those found in axis 2. CCA has been drawn between 10 physicochemical parameters and species of zooplankton. Eigenvalues were equal to 0.062514 for axis 1 and 0.0398 for axis two at sampling stations. Thus, that result explained a percentage of correlation between physico-chemical parameters and zooplankton species equal to 45.6% for axis 1 and 29.07 % for axis two (2) at all sampling stations. Among the variables included in the CCA,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{2-}$ , WT, pH and conductivity significantly affected zooplankton community, the strong correlation between environmental variable and zooplankton species are different in each station, the first axis is negatively associated with  $\text{NH}_4^+$ ,  $\text{PO}_4^{2-}$ , WT and pH (-0.24; -0.013; -0.06 and -0.03), respectively, and positively correlated with  $\text{NO}_3^-$ . Nitrate was representing in the second axis and was negatively correlated with *Dicranophorus robustus*. These findings indicate that  $\text{NO}_3^-$  was the dominant factor that affected zooplankton populations more than any other environmental variables in terms of composition and distribution at all sampling sites and influenced the zooplankton population structure. *Lepadella nauplius*, *Lecane flexilis*, *Lepadella patellaa*, *Lecane closterocerca* were closer to each other on CCA ordination axis, indicating that they have similar ecological adaptation; they were positively correlated with  $\text{NO}_3^-$ . *Lecane pyriformis* and *Trichocerca parvula* were positively correlated with dissolved oxygen. This result is compared to which they found in a previous study (Banerjee *et al.*, 2018). Whereas, Mishra and Saksena (1998) reported that rotifer density was inversely proportional to the dissolved oxygen in the Morur (Kalpi) River, polluted with urban sewage comprising multiple abiotic and biotic factors that affected their abundance. From our CCA and Pearson correlation, analysis the dominant species density was found to be strongly influenced by WT, DO and  $\text{NO}_3^-$ . *Rotaria rotatoria*, *Trichocerca parvula* and *Daphnia lumholtzi* were positively correlated with temperature, suggesting that they prefer to live in a high temperature and high dissolved oxygen. *Lecane unguolata*, *Lecane curvicornis* and *Lecane depressa* were positively correlated with pH and conductivity, they inhabited a wide salinity and alkaline environment and negatively with  $\text{NO}_2^-$  did not support elevate the concentration of nitrite in the environment. *Dicranophorus biastis*, *Encentrum felis*, *Copepoda nauplius* were positively correlated with sulfate, which is suggested to be included in their life cycle and essential for their development. Suggesting these zooplankton species may have a higher tolerance to sulfate than other co-occurring species. On the other

hand, *Lepadella ovalis* was negatively correlated with WT it is explicated that low or high temperature has a direct impact on their distribution. For the other hand, *Bosmina meridionalis* as a cladoceran and *Coleurella adriatica* as a rotifer are closer in each other in CCA ordination, they have shown a strong correlation with  $\text{NO}_3^-$  and water temperature ( $r=0.8$ ) it is explicated that they are both influenced by the presence of nitrate even though they have been included in different taxa. The previous study showed that these two species are positively correlated with temperature, such as Xiaoyu *et al.* (2014) reported a positive correlation between cladocerans and rotifers with nitrogen and temperature. The majority of species showed a negative relation with  $\text{PO}_4^{2-}$ , phosphate was generally the foremost factor altering the composition of the zooplankton group. Accordingly, Li and Chen (2020) reported that the zooplankton population dynamics are altering as phosphate increases confirming that phosphate was generally the most crucial  $\text{PO}_4^{2-}$  influenced zooplankton populations.

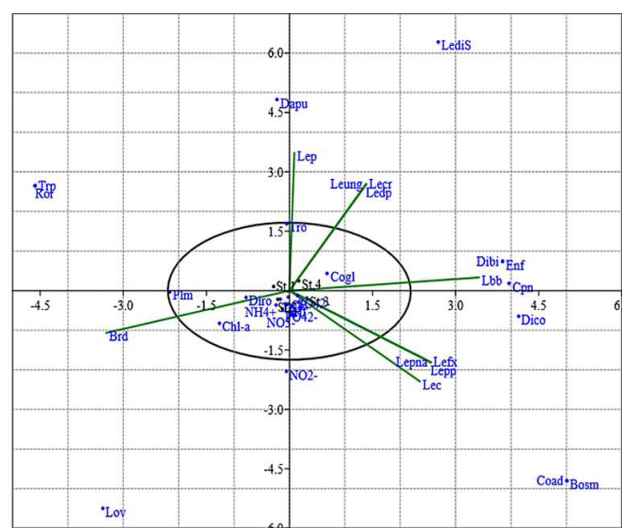


Fig. 6. CCA ordination triplot showing the zooplankton–environment relationships in the Wadi M’zi River and its adjacent areas.

## CONCLUSION

This research shows the richness of the species of the Wadi M’zi River zooplankton for the first time. It shows that population abundance and diversity differ through season and location which is dominated qualitatively and quantitatively by Rotifers. The species of Rotifers found in the stations are basically species markers of oligotrophic habitats that propagate extensively in this Wadi. The distribution of these species is primarily related to 10 parameters. In terms of zooplankton composition,



the Wadi M'zi has good environmental quality. It can be mentioned that zooplankton diversity can be used as a methods for evaluating the state of water quality in small and shallow water bodies.

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### Statement of conflict of interest

The authors have declared no conflict of interests.

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