



Seasonal evaluation of heavy metals and zooplankton distribution and their co-relationship in the Rosetta branch area of the Nile Delta in Egypt

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Abstract

The River Nile is the artery of Egypt, as it presents more than 96% of the municipal, industrial, and irrigation necessities of Egypt. This study was dedicated to providing data about the effect of pollution at six stations on the River Nile at the Rosetta branch during the period from August 2019 to April 2020, using heavy metals analysis and zooplankton as biological indicators. It was found that the average of heavy metals concentration follows the descending order $Al > Fe > Mn > Zn > Ni > Co$. Most of the heavy metals recorded its highest values at El-Rahawy station. Zooplankton community was represented by 32 species in addition to 4 Meroplanktons. Five groups of zooplankton were recorded, viz. Rotifera (1717 org./L), Protozoa (552 org./L), Cladocera (54 org./L), Nematoda (46 org./L), and other Meroplankton (44 org./L), dominated by Rotifera followed by Protozoa, Cladocera, Nematoda, and other Meroplanktons contributing 71%, 23%, 2%, 2%, and 2%, respectively. The highest average density of total zooplankton was recorded during spring while the lowest was recorded during summer. The highest similarity of (79.12%) was observed between (Site 1) and (Site 5). Almost all diversity indices were conducted and showed its highest values in site 6. The principal component analysis conducted between heavy metals, and zooplankton showed a significant negative correlation was shown for the dominant zooplankton with the heavy metals except with cobalt. Nematoda and the rotifer *Brachionus angularis* recorded a positive correlation with heavy metals except cobalt.

Keywords Nile Delta · Heavy metals · Zooplanktons · Seasonal distribution

Introduction

Nile River is the longest river on Earth; its physical setup together with the high population density of the surrounding nations makes the Nile River system one of the highly vulnerable water sources in Africa (Dumont 2009). Particularly, Egypt is considered the most populous country in the Nile River riparian, observing the highest water budget-deficit in Africa. The Nile River represents the main source of

freshwater (i.e., more than 97%) for more than 100 million Egyptians (Nikiel and Eltahir 2021).

The Nile Delta, approximately 22,000 km² in area, is among the largest deltas in the world (Dumont 2009), hosting more than 50% of Egypt's population, although it represents only 2% of total Egypt's area, showing one of the top densely populated areas on Earth (Hegazy et al. 2020).

Irrigation in Egypt mainly depends on River Nile water through a system of main canals and rayahs, secondary canals, third order, and meskas (Radwan and El-Sadek 2008). These irrigation canals are widespread over the Nile Delta area and run toward the Mediterranean coastal plain and discharge their water into the northern lakes or the sea (Elewa 2010). The main use of irrigations canals and rayahs is drinking, irrigation, navigation, and fishing (Khalifa and Bendary 2016).

At the north of Cairo, the Nile divides into two branches, the Rosetta branch to the west and the Damietta to the east (Helal 1981). Rosetta branch represents the area of investigation, and its length is about 225 km. The width of the branch

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varies from 150 to 200 m, and its average depth varies from 2 to 3.5 m. Recently, the Rosetta branch has been suffering from several environmental problems. It receives pollutants from three main sources: The first source is El-Rahawy drain which receives domestic and agriculture wastes from Giza city and pours more than 1,900,000 m³ per day of its effluents into Rosetta branch, the second source results from Kafr El-Zayat industrial area, and the third source of pollution is several small agricultural drains that discharge their wastes into the branch in addition to sewage discharged from several cities (El Bouraie et al. 2011). Consequently, several ecological effects have been detected in the Nile River environment (Goher et al. 2019). The serious health consequences, environmental degradation, and global life quality issues are results of water pollution that have an expensive cost to all humanity as well as increasing the severity of water deficiency problems (El-Amier et al. 2015). Hence, increasing water pollution not only causes the impairment of water quality, biodiversity, and the balance of aquatic ecosystems but also threatens human health, economic development, and social prosperity (Hassan et al. 2017).

Although several adverse health effects of metals have been known for a long time, exposure to heavy metals is continuous and even increasing in some parts of the world, particularly in developing countries (Badr et al. 2011). Heavy metals contamination is a very serious threat due to their toxicity, bioaccumulation, and biomagnifications in the food web (Eisler 1988). Also, metals are regarded as dangerous pollutants in the aquatic ecosystem due to their toxicity impact on living organisms (Ali et al. 2013; Khalil et al. 2007). Many countries with arid or semiarid climates that are sensitive to climate change (Mohammed et al. 2022; Abdel-Fatteh et al. 2020) face a delicate and crucial issue with surface water quality. Water pollution has a direct effect on human health, economy, and water resources availability (Brkic et al. 2019). About 80% of infections in low-income and developing countries are directly linked to contaminated drinking water and unhygienic settings (Das and Nag 2015). In order to decrease pollution and enhance water quality, researchers must examine and control the fluctuations in the amounts of heavy metals and other substances in both surface and groundwater (Gabr and Sousaa 2023).

Zooplanktons are a diverse group of heterotrophic organisms that consume phytoplankton, regenerate nutrients via their metabolism, and transfer energy to higher trophic levels (Steinberg and Condon 2009). They are major components in the trophic dynamics of freshwater ecosystems as they occupy an intermediate position in the food chain and indicate the environmental status. They respond to a wide variety of disturbances including nutrient loading, (Dodson 1992), acidification (Armored and Kormann 1993), fish densities (Carpenter and Kitchell 1993), contamination, (Yan et al. 1996), and sediment inputs, (Cuker 1997). So, they

could be used as a bioindicator for the physical, chemical, and biological processes in freshwater ecosystems, and safety of water. Moreover, zooplankton populations are considered bioindicators of eutrophication, as they are related to environmental conditions, responding more rapidly to changes than fish, and are easier to identify than phytoplanktons (Murugan et al. 1998; Sladeczek 1983). Changes in zooplankton community composition can affect the degree of up and down regulations of phytoplankton communities, influence the amount of nutrient availability, processing, and determine the capacity of aquatic ecosystems to uptake carbon dioxide (Bruet et al. 2010).

This work aimed to study heavy metals levels and zooplanktons' distribution seasonally in waters of different locations on Rossetta branch of the River Nile in Egypt, and their co-relation to assess to conclude and evaluate the extent of water quality in these areas.

Materials and methods

Samples collection

Seasonal samples were collected from six main stations from August 2019 to April 2020 in the Nile River. (Table 1, Fig. 1) summarize the monitoring sites.

Heavy metals analysis

Six elements including aluminum (Al), cobalt (Co), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) were measured in water samples collected seasonally from the investigated area. The metal concentrations were determined after the digestion by nitric acid as follows in (APHA 2005). Atomic absorption spectrophotometer (ICP-MS QCAP Thermo USA) was used for measuring portions of digested solutions.

$$\text{Metal concentration mg L}^{-1} = A \times B/C$$

where *A* is the Conc. of metal in digested solution mg L⁻¹, *B* the final volume of digested solution, ml, *C* the Sample size, ml.

Table 1 Locations of sampling sites on Rosetta branch of River Nile

z	Location	Latitude	Longitude
1	El-Qanater	30° 12' 48.79" N	31° 2' 39.26" E
2	El-Rahawy	30° 12' 26.53" N	31° 2' 39.26" E
3	Kata	30° 13' 12.93" N	30° 58' 33.77" E
4	Tamalay	30° 30' 32.32" N	30° 49' 57.29" E
5	Kom Hamada	30° 42' 52.91" N	30° 45' 44.28" E
6	Kafer El-Zayat	30° 49' 22.64" N	30° 48' 38.93" E

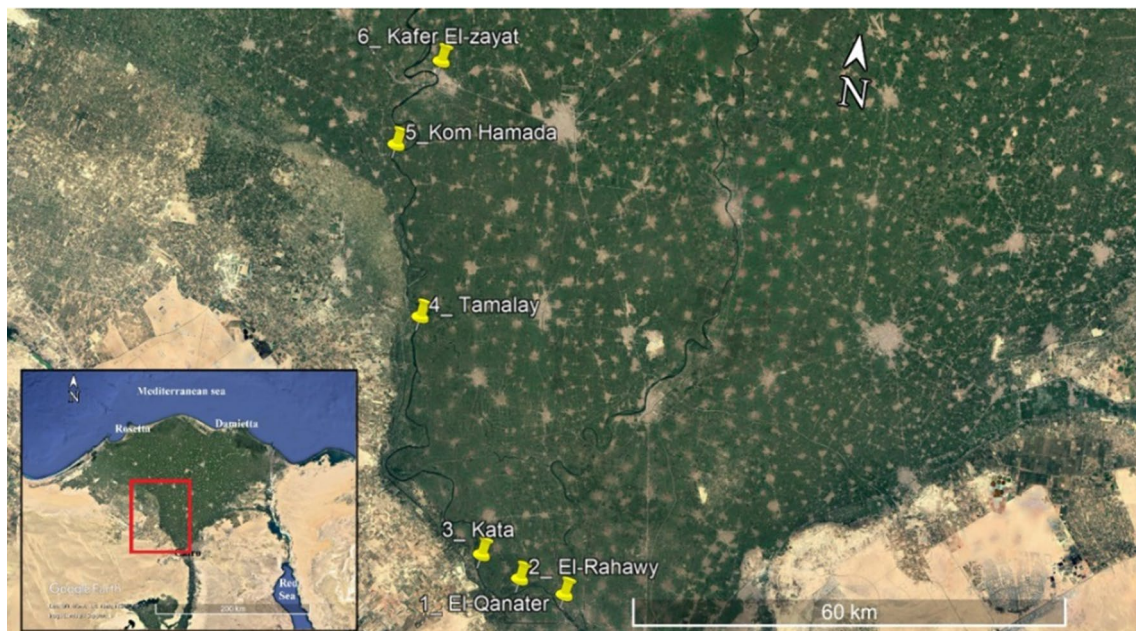


Fig. 1 Map of River Nile demonstrating the location of the collected stations

Collection and analysis of zooplankton

Zooplankton samples were collected from 30 L of water using a 55-µm-mesh-size plankton net. Samples were preserved in 5% neutral 33 formaldehyde solution. Each sample was shaken well, and then, the content was poured into a standard 150-ml total volume cup after washing the bottle with pure distilled water. One milliliter was dropped in a plastic counting grade with 2 mm sides' height and then completed with pure distilled water for counting, using Carl Zeiss's binocular stereomicroscope. This process was repeated three times. Zooplankton species were identified according to (Dang et al. 2015; Foissner and Berger 1996; Shiel and Koste 1992; Pennak 1978; Koste 1978; Ruttner-Kolisko 1974).

In the laboratory, three subsamples (one ml each) of the homogenized plankton samples were transferred into a counting cell, and zooplankton species were identified. The subsamples were examined under a binocular research microscope with magnification varied from 100 to 400X.

Zooplankton population density was then calculated as the number of individuals per liter from the equation conducted by (APHA 2005):

$$\text{No.} \times L = (c \times v') / (v'' \times v''')$$

where *c* is the number of organisms counted, *v'* the volume of concentrated sample/ml, *v''* the volume counted/ml, *v'''* the volume of the grab sample/L.

Statistical analysis

Similarity index

Similarity between different stations was performed by using primer 5.

Diversity indices

Diversity indices, e.g., Shannon–Wiener, species richness, evenness, and Simpson, were carried out on data at selected sites by using Premier Program version 5.

a. Shannon–Wiener index (*H'*):

The Shannon–Wiener index of species diversity was applied according to (Weber 1973) according to the following equation:

$$H' \sum (ni/N) \log_2 (ni/N)$$

where *ni* represents the number of individuals of *I* species. *N* represents the total number of individuals.

Diversity index between 0 and 3 means a medium pollution, and a diversity index > 3 means clean water (Wilhm 1972).

b. Species richness index or Margalef's diversity index (d):

It is expressed by simple ratio between total number of species (n) and total number of individuals (N). (Margalef 1958).

$$d = n - 1 / \log N$$

c. Evenness index (J):

It was calculated according the Shannon index (Shannon and Wiener 1963), using the formula:

$$J = H'_{\max} / S$$

where S is the total number of species of each sample and H'_{\max} is the number of maximal theatric diversity.

d. Simpson index:

The index of dominance (Simpson 1949) is the sum total of squares of the proportion of the species in the community and is expressed as follows:

$$C = \sum(n_i/N)^2$$

where C is the index of dominance, n_i the importance value for each species, N the total importance value.

Correlation coefficient

Correlation coefficient analysis was carried out using office Excel 2007 to show the relations between environmental parameters and the dominant group and species.

In addition, principal component analysis (PCA) between environmental and biological parameters was conducted by XL stat program 2019.

Results and discussion

Heavy metals

Pollution by heavy metals is a severe issue because of their toxicity, accumulation in biota, and inability to biodegrade in the environment. (Khadija et al. 2021) and industrial drainage threatens those sources. All heavy metals exhibit their toxic effects via metabolic interference and mutagenesis. They can enter into the water via drainage, atmosphere, soil erosion, and all human activities in different ways; these elements enter the biogeochemical cycle leading to toxicity in animals living in this water and then to humans consuming these animals (Pandey and Madhuri 2014). Industrial sector in Egypt consumes about 7% of the

available water resources, and the industrial sector dispose of their waste, which is loaded with pollutants belonging to each industry, had a negative impact on the quality of wastewater (ElMassah 2018).

In this study, it was found that the average of heavy metals concentration follows the descending order $Al > Fe > Mn > Zn > Ni > Co$. This arrangement agreed with the studies of Abotalib et al. (2023) and Khallaf et al. (2021). This is because Al and Fe are the most common metals in Earth's crust. Adding to that, the higher values of Al and Mn are possible because of the decay of organic materials, agricultural wastes, fertilizers, insecticides, sewage, and illegal wastewater discharges (Khalaf et al. 2021).

As shown in (Table 2), the highest average value of aluminum occurred during autumn (0.33 mg/l), but the lowest one (0.18 mg/l) was listed during spring, agreeing with Khalaf et al. (2021). At all sampled sites, the highest value of aluminum (0.94 mg/l) was recorded at site 2 during autumn, but the lowest one (0.078 mg/l) occurred at site 5 during winter. With regard to sites, the maximum average value (0.49 mg/l) was noticed at El-Rahawy drain (site 2) which may have been attributed to the increase in discharged wastewater rich with aluminum from El-Rahawy drain, as it is considered the most abundant cocking bowels in Egypt. Lowest average of Al was noticed in Tamalay (site 4) in Monoufia, contradicting with (Authman 2008; 2011; Authman et al. 2008) who found that higher concentrations of Al were detected in some drainage canals water in Menoufia Province, Egypt.

Alnenaï and Authman (2010) reported that Al is released into aquatic ecosystems through the recycled Al industries emissions and the stations of water purification discharge that contains an enormous amount of Al sulfate, which is used as suspended solid particles coagulant.

According to Egyptian law no. 485, (2007), the permissible limit of Al is 0.2 mg/l, which was exceeded in both sites 2 and 6.

The highest average value of cobalt occurred during summer (0.0004 mg/l), but the lowest one (0.00023 mg/l) was listed during autumn. With regard to sites, the maximum average value (0.0005 mg/l) was noticed at site 6, but the minimum one (0.0002 mg/l) was recorded at site 3 and site 5. At all sampled sites, the highest value of cobalt (0.0014 mg/l) was recorded at site 6 during summer.

Cobalt is one of the most important transition metals which play double dealing in both harmful and beneficial impacts on human beings. The increased use of Co in many industries, such as petrochemical and dye industries, generates large quantities of effluent and thus contaminates water (Mahmud et al. 2016). It was noticed that the exact same industries dominated Kafr El-Zayat (site 6), which witnessed the highest Co average value. It was proved that a lot of physical and mental problems, such as vomiting, nausea,

Table 2 Seasonal variation in heavy metals in the studied areas

Heavy metals	Seasons																	
	Summer				Autumn				Winter									
	Alu- minum	Cobalt	Iron	Manga- nese	Nickel	Zinc	Alu- minum	Cobalt	Iron	Manga- nese	Nickel	Zinc	Alu- minum	Cobalt	Iron	Manga- nese	Nickel	Zinc
1) El-Qanater	0.168	0.0002	0.11	0.007	0.0026	0.009	0.271	0.0002	0.15	0.019	0.003	0.022	0.151	0.0002	0.1	0.015	0.0028	0.016
2) El-Rahawy	0.196	0.0002	0.09	0.008	0.0022	0.011	0.943	0.0004	0.48	0.089	0.003	0.026	0.564	0.0004	0.36	0.064	0.0032	0.009
3) Kata	0.417	0.0002	0.5	0.016	0.0026	0.018	0.256	0.0002	0.18	0.052	0.002	0.013	0.112	0.0002	0.1	0.038	0.0022	0.006
4) Tamalay	0.399	0.0002	0.33	0.011	0.003	0.012	0.233	0.0002	0.16	0.018	0.002	0.014	0.284	0.0004	0.22	0.039	0.0032	0.008
5) Kom Hamada	0.118	0.0002	0.15	0.007	0.0022	0.015	0.119	0.0002	0.09	0.005	0.002	0.003	0.078	0.0002	0.21	0.037	0.0034	0.006
6) Kafer El-Zayat	0.566	0.0014	0.35	0.012	0.0034	0.018	0.143	0.0002	0.1	0.015	0.002	0.017	0.406	0.0002	0.28	0.018	0.0022	0.005
Average	0.311	0.0004	0.26	0.01	0.0027	0.014	0.327	0.0002	0.19	0.033	0.003	0.016	0.266	0.0003	0.21	0.035	0.0028	0.008
Heavy metals	Average																	
	Spring				Summer				Autumn				Winter					
	Aluminum	Cobalt	Iron	Manganese	Nickel	Zinc	Aluminum	Cobalt	Iron	Manganese	Nickel	Zinc	Aluminum	Cobalt	Iron	Manganese	Nickel	Zinc
1) El-Qanater	0.081	0.0004	0.2	0.032	0.0032	0.007	0.168	0.0003	0.14	0.019	0.003	0.0135	0.244	0.0004	0.07	0.486	0.0004	0.0129
2) El-Rahawy	0.216	0.0002	0.06	0.012	0.0024	0.007	0.25	0.0002	0.21	0.029	0.002	0.0111	0.216	0.0002	0.06	0.269	0.0003	0.0096
3) Kata	0.159	0.0002	0.08	0.006	0.0024	0.004	0.119	0.0002	0.13	0.014	0.004	0.0095	0.159	0.0002	0.07	0.119	0.0002	0.0095
4) Tamalay	0.159	0.0002	0.07	0.009	0.0058	0.014	0.119	0.0002	0.13	0.014	0.004	0.0095	0.159	0.0002	0.07	0.119	0.0002	0.0095
5) Kom Hamada	0.23	0.0002	0.02	0.016	0.0032	0.006	0.336	0.0002	0.02	0.016	0.0032	0.006	0.23	0.0002	0.02	0.336	0.0002	0.006
6) Kafer El-Zayat	0.182	0.0003	0.08	0.015	0.0033	0.007	0.271	0.0003	0.19	0.023	0.003	0.0113	0.182	0.0003	0.08	0.271	0.0003	0.0113
Average	0.182	0.0003	0.08	0.015	0.0033	0.007	0.271	0.0003	0.19	0.023	0.003	0.0113	0.182	0.0003	0.08	0.271	0.0003	0.0113

diarrhea, asthma, pneumonia, kidney congestion, skin degeneration, and weight loss, can occur due to excess Co in water (Rengaraj et al. 2002; Shibi and Anirudhan 2005; Naeem et al. 2009; Shahat et al. 2015). The permissible limits of cobalt allowed to be in drinking water is 0.01 mg/l, which was not exceeded in any of the studied areas.

The highest average of iron (0.26 mg/l) was recorded in summer, while the lowest average value (0.08 mg/l) was recorded in spring. With regard to sites, the highest average value (0.25 mg/l) was noticed at site 2, but the lowest one (0.13 mg/l) was recorded at site 5. Generally, the highest value of iron was recorded at site 3 (0.50 mg/l) in summer, but the lowest one was measured at site 6 during spring (0.02 mg/l).

It was rational that Fe would be in its highest level in summer, and at site 2, when and where the agriculture of rice thrives, as Fe is commonly reported from agricultural areas, especially those dominated by rice cultivation, where the pH of soil and water highly control the Fe uptake in rice fields (Gao et al. 2016; Muehe et al. 2013; Vatanpour et al. 2020). It is worth to be mentioned that the Nile Delta is dominated by rice cultivation with ~1.5 million acres representing 32.7% of the total cultivated area in the Nile Delta (Tolba et al. 2020), and that site 2 is considered the largest area for rice agriculture in the whole Nile Delta (182,550 acres). The high level of Fe was also explained by Lasheen et al. (2008), who reported iron release from different types of water pipes used in Egypt namely polyvinyl chloride (PVC), polypropylene (PP), and galvanized iron (GI).

Fe levels were at the permissible levels in all sites according to Egypt's Law No. 458 (2007) (0.3 mg/l). Only in El-Rahawy station (site 2) in winter, the limit was considered a borderline (0.36 mg/l).

Lowest level of Fe was recorded during spring. Similar results were found by (Mohamed et al. 2020). The low iron content in spring is possibly due to the consumption of iron by phytoplankton and oxidation of Fe^{2+} to Fe^{3+} and subsequent precipitation as hydroxide at high dissolved oxygen content (Ghallab 2000).

The highest average value of manganese occurred during winter (0.035 mg/l), but the lowest one (0.01 mg/l) was listed during the summer. With regard to sites, the maximum average value (0.04 mg/l) was noticed at site 2 due to the effect of El-Rahawy drain, but the minimum one (0.01 mg/l) was recorded at site 5. At all sampled sites, the highest value of manganese (0.089 mg/l) was recorded at site 2 during autumn, but the lowest was (0.005 mg/l) occurred at site 5 during autumn. The same seasonal results were found by (Khallaf et al. 2021), who stated that higher concentrations of Mn during winter than in summer may be attributed to the water's lower flow in winter, which could help in the heavy metals accumulation (Mohiuddin et al. 2011; Islam et al. 2014). Adding to that,

the Mn contents lower values during summer due to the dissolution of Mn hydroxides and oxides to the overlying water under low dissolved oxygen values and high water temperatures (Elewa 1993).

The lowest average value of nickel (0.0026 mg/l) was recorded in autumn, but the highest average (0.0033 mg/l) was recorded in spring. The highest average value (0.0035 mg/l) was noticed at site 5, but the lowest one (0.0024 mg/l) was recorded at site 3. Generally, the highest value of nickel was recorded at site 5 (0.0058 mg/l) in spring. Increased industrialization is the major source of increment in environmental pollutants including Ni and their increased health hazards. There are two sources (Anthropogenic and natural release) for increased Ni in the environment and its increased exposure to humans. Ni is considered to be an important element for various vital body functions, but its increased exposure may lead to toxic levels in the human body (Diagomanolin et al. 2004; Haber et al. 2000; Scott-Fordsmand 1997). It was concluded that workplaces such as those related to welding and battery manufacturing that Ni concentration as a result of occupational exposure may vary widely from micrograms to milligrams of Ni (Bencko 1982). Leaching or corrosion processes are also the main reason by which Ni present in pipes and containers gets dissolved in drinking water and beverages. These processes may lead to daily oral Ni intake (Grandjean 1984a, 1984b).

Our results didn't exceed the permissible levels of Ni by (WHO 2011) (0.07 mg/l) and by (Egypt's Law No. 458 2007) (0.02 mg/l).

The highest average value of zinc occurred during autumn (0.016 mg/l), but the lowest one (0.007 mg/l) was listed during spring. With regard to sites, the maximum average value (0.013 mg/l) was noticed at site 1, but the minimum one (0.010 mg/l) was recorded at site 5. At all sampled sites, the highest value of zinc (0.026 mg/l) was recorded at site 2 during autumn, but the lowest one (0.003 mg/l) occurred at site 5 during autumn. These results came along with results by (Abdelhamid et al. 2013) and contradicted with results by (Mohamed et al. 2020).

Trace amounts of some metal ions, such as zinc (Zn) and cobalt (Co), are required by organisms as cofactors for enzymatic processes (Zhang et al. 2014; Kozlowski et al. 2009). However, an excess of these metals would cause serious problems in living organisms due to their high toxicity, carcinogenic, and bioaccumulation. (Zhang et al. 2014; Kozlowski et al. 2009; Sebastian and Srinivas 2015; Omraei et al. 2011). Zn is one of the most common pollutants for water, and due to its nonbiodegradability and acute toxicity, Zn-containing liquid and solid wastes are considered hazardous wastes. Anyway, our results didn't exceed either WHO permissible limits (5 mg/l), or (Egypt's Law No. 458 2007) (3 mg/l).

Table 3 List of zooplankton species

Species/Taxa	Phylum	Summer	Autumn	Winter	Spring	Average
<i>Anuraeopsis fissa</i>	Rotifera	6	200	0	17	56
<i>Asplanchna priodonta</i>	Rotifera	122	0	0	0	31
<i>Brachionus calyciflorus</i>	Rotifera	133	0	28	827	247
<i>Brachionus patulus</i>	Rotifera	17	0	0	0	4
<i>Brachionus plicatilis</i>	Rotifera	67	78	0	0	36
<i>Brachionus angularis</i>	Rotifera	167	0	0	6	43
<i>Brachionus cadatus</i>	Rotifera	11	0	0	0	3
<i>Brachionus falcatus</i>	Rotifera	56	11	0	0	17
<i>Brachionus quadridentatus</i>	Rotifera	39	22	413	156	158
<i>Brachionus budapestinensis</i>	Rotifera	0	0	0	250	63
<i>Brachionus urceolaris</i>	Rotifera	0	28	0	0	7
<i>Keratella cochlearis</i>	Rotifera	6	51	666	228	238
<i>Keratella tropica</i>	Rotifera	17	44	11	17	22
<i>Keratella quadrata</i>	Rotifera	11	0	0	0	3
<i>Lecan luna</i>	Rotifera	0	0	22	28	13
<i>Lecan clortan</i>	Rotifera	11	0	0	0	3
<i>Cephalodaly</i>	Rotifera	11	0	0	0	3
<i>Epiphanes</i> sp.	Rotifera	0	0	0	17	4
<i>Monostyla clostercera</i>	Rotifera	0	6	0	0	2
<i>Philodina</i> sp.	Rotifera	144	437	394	455	358
<i>Polyarthra vulgaris</i>	Rotifera	0	500	344	126	243
<i>Trichocerca elongate</i>	Rotifera	0	523	22	83	157
<i>Platylas patulus</i>	Rotifera	6	0	0	0	2
<i>Synchata pectinata</i>	Rotifera	22	0	0	0	6
<i>Trichocerca tetratis</i>	Rotifera	0	6	0	0	2
<i>Bosmina longirostris</i>	Arthropoda, Cladocera	0	0	67	89	39
<i>Chydorus sphaericus</i>	Arthropoda, Cladocera	0	0	0	33	8
<i>Daphnia longispina</i>	Arthropoda, Cladocera	0	0	0	22	6
<i>Diaphanosoma excisum</i>	Arthropoda, Cladocera	0	0	0	6	2
<i>Sphenoderia</i>	Protozoa	67	0	0	0	17
<i>Vorticella</i> sp	Protozoa	239	627	705	611	546
Nauplius larvae	Arthropods larva	0	39	0	72	28
Copepodite stage	Arthropods larva	17	6	0	17	10
Harpacticoid stage	Arthropods larva	0	0	0	6	2
Insect larvae	Arthropods larva	11	0	11	0	6
Nematoda	Nematoda	0	13	144	28	46
Total species number	–	21	16	12	21	36

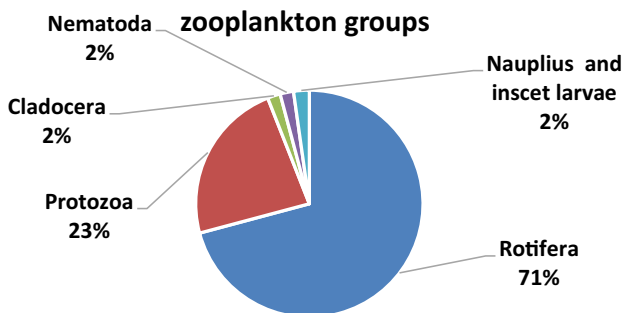


Fig. 2 Community composition (%) of zooplankton groups

Table 4 Seasonal variations in total zooplankton (Org. /l) at Rosetta branch, River Nile

Sites	Seasons				
	Summer	Autumn	Winter	Spring	Average
1) El-Qanater	599	3796	4463	1332	2548
2) El-Rahawy	1130	200	866	1398	899
3) Kata	598	344	1499	1864	1076
4) Tamalay	232	3431	4496	5284	3361
5) Kom Hamada	1066	4530	2864	2965	2856
6) Kafer El-Zayat	3364	3265	2399	5696	3681
Average	1165	2594	2765	3090	2403

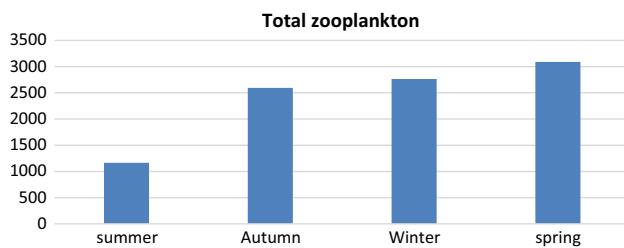


Fig. 3 Seasonal fluctuations of total zooplankton (Org./L)

Biological study of zooplankton

Zooplankton occupy an important position in pelagic food webs, as they transfer energy produced through photosynthesis from phytoplankton to higher trophic levels (fish) consumed by humans (Sommer et al. 2002). They are also considered as remarkable detectors for pollution.

Zooplankton community were represented by 32 species in addition to 4 Meroplankton (larval stages). The highest number of species (21 species) was recorded during summer and spring. On the other hand, the lowest number of species was observed during autumn (16 species) and winter (12 species). Rotifera showed the highest number of species (25 species). It is followed by cladocera (4 species) and protozoa (2 species) in addition to other Meroplanktons (4 Taxa) (Table 3). (Khalifa and Bendary 2016) showed also that the largest zooplankton population was recorded during spring.

Five groups of zooplankton recorded, viz. Rotifera (1717 Org./l), Protozoa (552Org./l) Cladocera (54 Org./l), Nematoda (46 Org./l), and other Meroplankton (44 Org./l). Zooplankton communities were dominated by Rotifera followed by Protozoa, Cladocera, Nematoda, and other Meroplanktons contributing 71%, 23%, 2%, 2%, and 2%, respectively (Table 5, Fig. 2). Khalifa and Bendary (2016) recorded the dominance of Rotifers, Protozoa, and then Cladocera in their studied area. Sheir et al. (2020) partially agreed with our results as they showed a dominance of Protozoa, then Rotifers in their studied areas. Moreover, Mola et al. (2018) found that zooplankton communities were dominated by rotifers, followed by copepods, Cladocera, Meroplankton, and Protozoa, contributing, respectively. It is worth mentioning that these studies were conducted also in the Nile Delta of Egypt.

Highest average density of total zooplankton was recorded during spring (3090 Org./l), while the lowest annual average was recorded during summer to 1165 Org./l (Table 4, Fig. 3). Sheir et al. (2020) revealed that the highest density of zooplanktons was recorded in winter, which was our second highest season of zooplanktons, and the highest for Protozoa and Nematodes. This came along with Gulati (1978) who regarded temperature as one of the main factors controlling the growth and composition of zooplankton. In

addition, winter was the favorable season for zooplankton to reproduce and increase in density in the lentic ecosystem (Sheir 2018).

As shown in (Table 5, Fig. 4), average number of total Rotifera was (1717 Org./l). The maximum average density was recorded during spring (2208 Org./l) while the minimum average density (843 Org./l) was recorded in the summer. Total rotifer recorded the maximum average density (3090 Org./l) at site 6, while the minimum one (657 Org./l) was recorded at site 2. The maximum average density of Rotifers was recorded during spring (2208 org./l), while the lowest was during summer (843 org./l). These results came along with (Fishar et al. 2019; Khalifa and Bendary 2016; Khalifa 2014).

The apparent dominance of rotifers in rivers may be due to their relatively short generation time compared to the larger crustacean zooplankton (Van Dijk and Van Zanten 1995 and Mola 2011). In addition, its simple parthenogenetic reproduction (Herzig 1983) which in favorable conditions results in high production rates often manifested as very high population densities (Andrew and Fizesimons 1992) and they are less vulnerable to fish predation (Allan 1976; Brook and Dodson 1965). Rotifers are also able to reproduce in a wide temperature range (Galkovskaja 1987), and the eutrophication condition of water affects the composition of zooplankton, shifting the dominance from large species as in Copepods to smaller species as in Rotifers (Emam 2006; El-Shabrawy 2000). The dominance of rotifers over other zooplankton groups in many tropical waters could also be explained due to high predation pressure by fish larvae on microcrustaceans (Nandini et al. 2015).

Philodina was the main dominant and abundant species of Rotifera community; it attained to an annual average of 358 Org./l organisms counting. Spring recorded the highest average density (455 Org./l) while the lowest one (144 Org./l) was detected during summer. On the other hand, the highest average count (542 Org./l) of the species was harvested from site 4, it decreased to the lowest average of 223 Org./l at site 3. *Brachionus calyciflorus* was considered the second dominant species of total rotifers with averages of 247 Org./l. It recorded the highest average density (827 Org./l) during spring, while in winter it recorded the lowest average density (28 Org./l), and it completely disappeared during autumn. The highest average densities (733 Org./l) were recorded at site 6, while the lowest average density was recorded at site 1 (17 Org./l). *Polyathra vulgaris* was the third dominant species of total Rotifera with an annual average of 242 Org./l (Table 23). It recorded its highest average density (500 Org./l) during autumn, while in spring, it recorded the lowest average density (126 Org./l), and it completely disappeared in summer. The highest average density of *Polyathra vulgaris* (533 Org./l) was recorded at site 1, while the minimum ones were listed at sites 2 (8 Org./l).

Table 5 Seasonal variation in zooplanktons in the studied areas

Seasons/ Sites	Summer						Autumn						Winter							
	Rotifera		Cladocera		Protozoa		Nematoda		Other forms of zooplanktons		Rotifera		Cladocera		Protozoa		Nematoda		Other forms of zooplanktons	
1) El-Qanater	566	0	33	0	0	0	2531	0	1265	0	0	4097	133	200	0	33				
2) El-Rahawy	530	0	600	0	67	200	0	0	0	0	0	566	67	33	200	0				
3) Kata	365	0	0	0	0	336	0	0	0	8	0	1066	67	0	333	33				
4) Tamalay	232	0	0	0	0	1966	0	1299	0	166	1466	33	2997	0	0	0				
5) Kom Hamada	500	0	566	0	0	3331	0	1132	0	67	2398	100	366	0	0	0				
6) Kafer El-Zayat	2864	0	400	0	100	3098	0	67	67	33	1433	0	633	333	0	0				
Average	843	0	267	0	28	1910	0	627	13	44	1907	67	705	144	11					
Seasons/Sites	Spring						Average													
	Rotifera		Cladocera		Protozoa		Nematoda		Other forms of zooplanktons		Rotifera		Cladocera		Protozoa		Nematoda		Other forms of zooplanktons	
1) El-Qanater	932	300	67	0	33	0	67	0	33	2032	108	0	17							
2) El-Rahawy	1331	0	0	67	0	67	67	0	0	657	17	158	67	17	17	17				
3) Kata	1465	233	66	67	33	33	33	33	33	808	75	17	102	8	50	42				
4) Tamalay	2021	33	3164	33	100	0	2190	366	94	1421	17	1865	8	100	125	44				
5) Kom Hamada	2532	200	133	233	0	0	3090	33	54	2190	75	549	0	100	46					
6) Kafer El-Zayat	4964	133	611	28	28	28	1717	28	28	3090	33	333	100	46	44					
Average	2208	150	611	28	28	28	1717	28	28	1717	54	552	46	46	44					

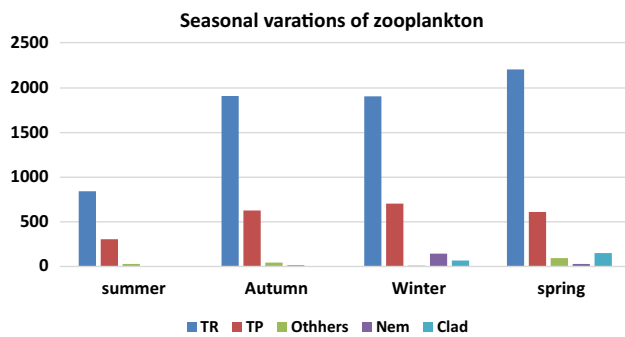


Fig. 4 Abundance and seasonal variations in zooplankton groups (Org./L). TR total rotifer; TP total Protozoa; others=nauplius and insect larvae; Nem nematoda; Clad cladocera

These results came along with (Fishar et al. 2019; and Khalifa 2014). *Keratella cochelaris* was the fourth dominant species of total Rotifera with an annual average of 238 Org./l. It recorded its highest average density during winter (666 Org./l) in winter and recorded the lowest average density (6 Org./l) in summer. The highest average density of *Keratella cochelaris* (658 Org./l) was recorded at site 1, while the minimum ones were listed at sites 2 (17 Org./l). (Fishar et al. 2019) almost found the same Rotifers as their dominant genera were *Proalides*, *Keratella*, *Brachionus*, *Trichocerca*, *Polyarthra*, and *Philodina*.

According to Perbiche-Neves (2013) and Abd El-Mageed (2008), the high richness of *Brachionidae* indicates eutrophic conditions. (Kumari et al. 2008) described *Keratella sp.* and *Brachionus sp.* as pollution indicator species. El-Bassat (1995) reported the high existence of these genus at Delta Barrage was attributed to their ability to tolerate pollution. In addition, the study of Kumari et al. (2008) recorded rotifer as an indicator of water pollution and described *Keratella sp.* and *Brachionus sp.* as pollution indicator species.

In our study, *Trachiounus elongata* was the 5th most abundant species in Rotifera. It flourished in autumn with an average of (523 org/l) and reached the lowest average in winter (22 org/l). These results came in accordance with (Fishar et al. 2019) and with (Bedair 2006) who believed that this species preferred low temperature.

Most of the 5 dominant species abundance of Rotifera were found in sites (1 and 6), which indicated that these sites are most likely to be eutrophic, while the least abundance of almost all of these species was recorded in site (2), which may indicate again to the high pollution in El-Rahawy drain area. Many other species of Rotifera were found as listed in (Table 6).

Protozoa was the second most abundant group in zooplankton. Composition and distribution of Protozoa recorded the highest density of total Protozoans during winter (705 Org./l) and showed the lowest density (267 Org./l) during summer. It recorded the highest average density (1865 Org./l) at site 4, while the lowest average density recorded

(17 Org./l) at sites 3. Protozoa was represented by two species (*Vorticella spp* and *Sphenoderia*). *Sphenoderia* has appeared only summer season with an average of (67 Org./l). It recorded an average of (100 Org./l) at site2. *Vorticella sp.* (Table 8) recorded the highest average density (536 Org./l) of total Protozoa. Its maximum density average recorded during winter (705 Org./l), while summer recorded the lowest average density of (200 Org./l). Regarding sites, the maximum density of *Vorticella spp* (1865 Org./l) appeared at site 4, while it recorded the minimum average density (17 Org./l) at site 3. Similar results were shown by Fishar et al. (2019) and Khalifa and Bendary (2016), who found that Protozoa was the second group of zooplankton during their study and that *Vorticella sp.* was the most abundant sp. among Protozoa. Emam, (2006) concluded that Protozoa are pollution tolerant group of zooplankton and attained its highest density in the polluted area, while Gideon et al. (2014) considered *Vorticella campanula* as one of the species that are indicators of high water pollution status. On the other hand, Fishar et al. (2019) didn't agree with our seasonal distribution as they stated that the highest Protozoa recordings were found in summer while it sharply decreased in winter.

Cladocera was the third abundant group in zooplanktons in our study. Its maximum average density was recorded during spring (150 Org./l). Total Cladocera recorded the maximum average density (108 Org./l) at site 1 while recorded the minimum average density (17 Org./l) at sites 2, 4 (Table 5). *Bosmina longirostris* was considered as the main dominant species of total Cladocera with an annual average of 39 Org./l (Table 7). It showed its peak density of 89 Org./l during spring. During winter, it recorded the minimum average density (67 Org./l), while it completely disappeared during summer and autumn. Site 5 recorded the highest average number (67 Org./l), while sites 2 and 4 showed the lowest average one (17 Org./l).

Nematoda recorded the highest average density (144 Org./l) during winter, while it completely disappeared in summer; the lowest average density (13 Org./l) is recorded in autumn. According to sites, it was found that the highest average density was found at site 2 (102 Org./l), followed El-Rahawy site and still affected by sewage water, while site 4 showed the lowest average (8 Org./l) and completely disappeared in sites 1 and 5 (Table 5). Bouwman et al. (1984) stated that an abundance of nematodes occurs in contaminated environment and they are more tolerant to low oxygen content than other taxa.

Other forms of zooplanktons are calculated by the summation of Nauplius larvae, copipodite stage, harpacticoid stage, and insect larvae. Its maximum average density was recorded during spring (94 Org./L), while it recorded the minimum average density (11 Org./L) during winter. The highest density (125 Org./L) was shown at site 6, while the

Table 6 Seasonal variation in Rotifers in the studied areas

Seasons/ Sites	Summer						Autumn						Winter																			
	Philo- dina sp	B. caly- ciflorus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. angu- laris	Lecan luna	Philo- dina sp	B. caly- ciflorus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. angu- laris	Lecan luna	Philo- dina sp	B. caly- ciflorus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. angu- laris	Lecan luna											
1) El- Qanater	133	0	0	0	200	167	33	0	433	0	700	100	899	33	0	0	333	1232	2231	67	167	0	67	0	233							
2) El- Rahawy	233	0	0	0	33	0	33	0	200	0	0	0	0	0	0	0	500	0	33	33	0	0	0	0	0	0						
3) Kata	133	0	0	0	0	33	0	0	291	0	0	4	8	0	0	0	233	0	233	333	0	167	0	0	67	0						
4) Tamalay	100	33	0	33	0	0	33	0	800	33	500	33	300	0	33	0	633	0	167	233	0	433	0	0	0	0						
5) Kom Hamada	0	0	0	0	0	300	0	0	500	0	1465	167	800	0	0	133	0	266	0	400	1166	67	466	0	0	33						
6) Kafer El-Zayat	266	766	0	0	0	500	0	0	400	766	333	0	1132	100	0	100	0	400	167	0	0	833	0	0	0	33						
Average	144	133	0	6	0	39	17	0	437	133	500	51	523	22	0	44	0	394	28	344	666	22	344	0	11	22						
Average																																
Seasons/ Sites	Spring						Summer						Autumn						Winter													
Heavy metals	Philo- dina sp	B. caly- florus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. angu- laris	Lecan luna	Philo- dina sp	B. quad- ridetatus	B. angu- laris	K. cochelar- is gate	T. elon- gate	B. angu- laris	Lecan luna	Philo- dina sp	B. caly- florus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. quad- ridetatus	B. angu- laris	Lecan luna	Philo- dina sp	B. caly- florus	P. vul- garis	K. cochelar- is gate	T. elon- gate	B. quad- ridetatus	B. angu- laris	Lecan luna		
1) El- Qanater	67	200	300	33	0	33	33	0	283	17	533	658	250	33	0	100	50	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2) El- Rahawy	300	932	0	33	33	0	0	0	0	308	233	8	17	8	8	8	8	0	8	0	8	0	0	0	0	0	0	0	0	0	0	0
3) Kata	233	633	133	167	0	0	266	0	0	223	158	92	126	2	108	8	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4) Tamalay	633	932	23	133	0	167	0	33	33	542	241	173	108	75	150	0	17	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5) Kom Hamada	799	400	233	400	133	300	500	0	67	391	100	525	433	250	242	75	33	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6) Kafer El-Zayat	533	1998	167	333	300	0	0	67	67	400	733	125	83	358	233	125	42	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average	455	827	126	228	83	155	6	17	28	358	247	242	238	157	140	43	22	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0

B. calyciflorus: *Brachionus calyciflorus*, *P. vulgaris*: *Polyartha vulgaris*, *K. cochelar*: *Keratella cochelar*, *T. elongate*: *Trichoerca elongate*, *B. quadridentatus*: *Brachionus quadridentatus*, *B. angularis*: *Brachionus angularis*, *K. tropica*: *Keratella tropica*.

Table 7 Seasonal variation in *Bosmina longirostris* (Org./l) (cladocera) at Rosetta branch, River Nile

Sites	Seasons				
	Summer	Autumn	Winter	Spring	Average
1) El-Qanater	0	0	133	67	50
2) El-Rahawy	0	0	67	0	17
3) Kata	0	0	67	133	50
4) Tamalay	0	0	33	33	17
5) Kom Hamada	0	0	100	167	67
6) Kafer El-Zayat	0	0	0	133	33
Average	0	0	67	89	39

Table 8 Seasonal variation in *Vorticella* sp (Org./l) (protozoa) at Rosetta branch, River Nile

Sites	Seasons				
	Summer	Autumn	Winter	Spring	Average
1) El-Qanater	33	1265	200	67	391
2) El-Rahawy	200	0	33	0	58
3) Kata	0	0	0	66	17
4) Tamalay	0	1299	2997	3164	1865
5) Kom Hamada	566	1132	366	133	549
6) Kafer El-Zayat	400	67	633	233	333
Average	200	627	705	611	536

minimum average density (17 Org./L) was recorded at sites 1, 2, 3 (Table 5). The Nauplius larvae were more abundant than the adult Copepoda. It represented more than 75% of the total copepoda.

These results agreed with (Fishar et al. 2019; Mola et al. 2018; Gaber 2013) who found that the maximum peak of copepod was recorded during spring, and explanation as this result may be due to the abundance of Naupilii larvae and the copepodite stages during this period. In addition, this may be attributed to the effect of high water temperature which accelerates the copepods' production in the presence of high nutrient concentrations. Moreover, (El-Bassat 2002) stated that the maximum abundance of this group was attributed to the high concentration of nutrients and high transparency. (Sheir et al. 2020) stated in their study that Arthropoda was the third population density (after Protozoa and Rotifera) of the collected zooplanktons during spring and summer seasons. (Manickam et al. 2015) outlined similar results and attributed that to temperature and availability of favorable food such as bacteria and suspended detritus where most planktonic arthropods are filter feeders, while (Wayaa et al. 2017) attributed the high abundance in rainfall seasons to that this is the entrance time of the nutrients through rainfall and runoff of water of agricultural lands.

Statistical analysis

Zooplankton may form an important component of the biological communities in large rivers due to their high abundance and their ability to cycle nutrients through the aquatic environment (Kobayashi et al. 1998; and Lehman 1980). The structure and function of the zooplankton community with regard to species composition and abundance are affected by several factors. These factors included the nature and availability of food resources, types of predatory interaction in the water environment, physical and chemical aspects of water, and anthropogenic changes (Sipaúba-Tavares et al. 2010).

Similarity index for zooplankton

A similarity index based on the two samples is calculated in the hope that it will indicate the degree of resemblance between the two ecological populations represented by the samples. If the resemblance is "high," the samples may be judged to come from the same population (Johnston 1976) (Table 8).

The similarity index between stations depending on zooplankton distribution in the studied sites is shown in cluster analysis (Table 9, Fig. 5). The highest similarity of (79.12 %) was observed between (Station 1) and (Station 5). Also, cluster analysis recorded high similarity between (Station 6) and (Station 5) being 69.27%, which may be attributed to its location at the northern part of the sites. On the other hand, the lowest similarity was observed between Stations 1 and 2 (40.44%).

In addition, site (6) showed the lowest similarity indices with all the other sites, which indicates that the environment of this location is different from the others.

Diversity indices for zooplankton

As shown in (Table 10), diversity indices were conducted depending on zooplankton species and zooplankton total number. The highest Richness Index values (2.75) were recorded at site 3 then site 2 (2.64), but the lowest one (2.17) occurred at site 1, at the same time, the Evenness Index showed the highest value (0.76) at site 5, but the lowest one (0.69) was recorded at site 2. Also, Shannon diversity index showed the maximum value (2.59) at site 6, but the lowest one showed at site 4 of 1.61. The highest Simpson index value (0.90) was recorded at site 6, but the lowest one (0.65) was recorded at site 4.

Ristau and Traunspurger (2011) mentioned the increase in species density, Shannon, richness, and evenness indices in the oligo and mesotrophic lakes. They explained these patterns as some species could tolerate different degrees of

Table 9 Similarity index (%) between sites sampled based on data of zooplankton

Stations	1) El-Qanater	2) El-Rahawy	3) Kata	4) Tamalay	5) Kom Hamada	6) Kafer El-Zayat
1) El-Qanater	100					
2) El-Rahawy	40.44	100				
3) Kata	51.42	60.01	100			
4) Tamalay	56.51	49.42	57.48	100		
5) Kom Hamada	79.12	44.50	54.91	65.63	100	
6) Kafer El-Zayat	59.47	46.19	53.04	63.17	69.27	100

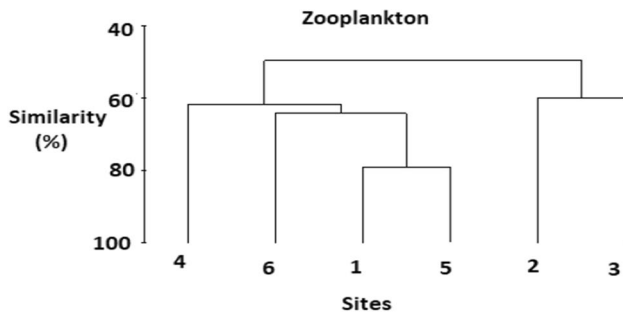


Fig. 5 Similarity index between sites sampled based on data of zooplankton

nutrient levels in the water. Also, they mentioned the distribution of some species were dependent on the water movement (lentic/lotic). Q

According to (Pyron 2010), species evenness is a description of the distribution of abundance across the species in a community. Species evenness is highest when all species in a sample have the same abundance. Evenness approaches zero as relative abundances vary. Site (6) recorded the highest evenness indices. And as Shannon indices are related to evenness indices, the highest Shannon indices were also recorded at site (6). That means that the highest zooplankton diversity was recorded in site 6, and all the species inhabiting this site have a high tendency to tolerate pollution.

Table 10 Diversity indices between sites based on data of zooplankton

Sites	Total species	Total individuals	Shannon	Species richness	Evenness	Simpson
1) El-Qanater	18	2539	2.11	2.17	0.73	0.84
2) El-Rahawy	19	914	2.04	2.64	0.69	0.80
3) Kata	20	1009	2.36	2.75	0.79	0.88
4) Tamalay	19	3362	1.61	2.22	0.55	0.65
5) Kom Hamada	21	2898	2.33	2.51	0.76	0.87
6) Kafer El-Zayat	21	3332	2.59	2.47	0.85	0.90

PCA between zooplankton and heavy metals

The principal component analysis (PCA) was conducted between heavy metals and zooplankton (Fig. 6). Cobalt showed a significant positive correlation (at the same direction) with the most dominant zooplankton groups and species, especially total zooplankton, total rotifer, total Protozoa, Keratella cochlearis, Bosmina longirostris, total Copepoda, total Cladocera, and total other taxa).

PCA diagram showed significant negative correlations for the dominant zooplankton with the other heavy metals (Al, Fe, Mn Ni, Zn).

On the vice versa, nematoda and the rotifer Brachionus angularis recorded a positive correlation with the other heavy metals except cobalt. These results indicated that these species can tolerate the high concentrations of heavy metals. The present findings agreed with (Saad et al. 2015).

Conclusion

In conclusion, pollution affects the distribution of zooplanktons as shown in El-Rahawy station that had the highest level of pollution, and on the other hand, recorded the lowest degree of diversity of zooplanktons. More stringent measures must be followed in dealing with the problem of pollution resulting from drainage and intense industrial activities in the Nile Delta region, as it is the

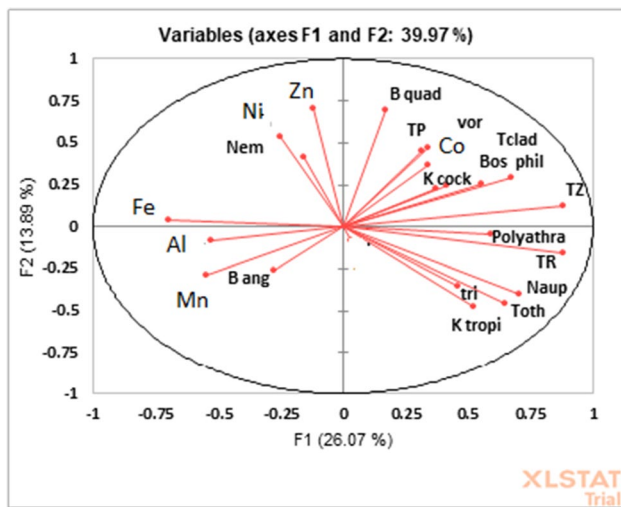


Fig. 6 Principal component analysis (PCA) diagram between zooplankton and heavy metals. Heavy metals (aluminum: Al, iron: Fe, manganese: Mn, zinc: Zn, nickel: Ni, and cobalt: Co) and the dominant zooplankton and their groups (total zooplankton: TZ, *Keratella cochlearis*: K.Coch, *Brachionus angularis*: B ang, *Bosmina longirostris*: Bos, nauplius larvae: Naup, nematoda: Nem, total cladocera: T clad, total rotifera: TR, total others: Toth, total protozoa: TP, Vorticella sp.: vor, *Polyathra vulgaris*: Polyathra, *Philodina sp.*: phil, *Brachionus quadridentatus*: B quad, *Keratella tropica*: K tropi, *Trichocerca elongata*: tri)

most crowded place in Egypt, and therefore, the impact of pollution will affect a larger percentage of the population.

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Author contributions The authors confirm contribution to the paper as follows: E-STER and SEE studied conception and design, OMA-E and HRAM conducted the data collection, MLY, HRAM, and OMA-E made the analysis and interpretation of results, MLY and HRAM made figures, and MLY drafted manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Declarations

Conflict of interest I declare that the authors have no competing interests as defined by Applied Water Science, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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