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Evaluation of Macrobenthic Invertebrate Biodiversity and Microbial Load with Relation to the Sediment Analysis in the Rosetta Branch, River Nile, Egypt

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ABSTRACT

Bottom fauna could be used as bioindicators for water quality status in various freshwater systems such as the Rosetta branch, Egypt. This branch receives several domestic, agricultural and industrial wastes. Such wastes could affect various aquatic taxa including macrobenthic invertebrates. The current investigation aimed to evaluate the water's physicochemical characteristics, microbial load and macrobenthic invertebrate biodiversity with relation to sediment analysis in the Rosetta branch, Egypt. A total of seven stations were investigated during two seasons (winter and summer) along the Rosetta branch. The sediment types in the evaluated stations were distributed as muddy gravelly sand (at EL-Rahawy and Dosouk stations), muddy sand and sandy mud (at EL-Qanater station). The water's physicochemical characteristics were evaluated. The numbers of the characterized animal species varied among the evaluated Rosetta stations. During the winter season, a total of five animal groups (Annelida, Arthropoda, Ostracoda, Mollusca and Free-living Nematodes) were detected. Concerning the second season, only three animal groups (Annelida, Arthropoda and Mollusca) were documented. The species equitability, richness and diversity index were calculated in each evaluated station. The microbial load of each sediment sample was evaluated. The fecal streptococci were high, indicating that the Rosetta branch is polluted by sewage. The results could be used for the inference of the biodiversity levels in this Egyptian ecological area. In addition, it could be used to design some informative plans for good management of biological resources in the future.

INTRODUCTION

Freshwater availability and purity represent a global concern. Concerning Egypt, the River Nile is considered the main primary freshwater supply for nearly all drinking and irrigation purposes. The Egyptian River Nile environment was changed due to population increase, upstream dam projects and water use by neighboring nations, **(Hussein *et al*., 2021; Abdel-Satar *et al.*, 2022).**

Rosetta branch is considered as the main freshwater stream extending northwards for about 236 km on the western boundary of the Nile Delta from Egypt's Delta Barrage **(Tahoun *et al.,* 2021)**. The previous authors confirmed that the Rosetta branch received several domestic, agricultural and industrial wastes. Such wastes could affect various aquatic taxa, including benthic invertebrates, zooplankton, bacteria and flora. Thus, identifying the biological features of this water body is vital.

The structure of macrobenthos assemblage in water bodies like Rosetta branch is complex and strongly affected by certain location processes and condition. Additionally, it is influenced by interspecific interactions **(Badawy *et al.,* 2013; El Sayed *et al*., 2020; Abdel-Satar *et al*., 2022)**.

The sediment analysis (at the bottom of the water column) plays a significant role in the river system's pollution scheme. It reflects the current water system quality and can be used to determine the presence of pollutants.

As known, the grain size distribution of the bottom sediments plays a vital role in the distribution of the bottom fauna and flora in the aquatic ecosystem e.g. benthic invertebrate, insects and attached benthic algae.

The biological importance of the benthic invertebrates was signified in various ecological roles, including nutrient recycling and decomposition **(Wahab *et al*., 2018; Abdel-Satar *et al*., 2022)**.

Distribution of bottom fauna in a certain water body arise from several environmental factors including the geological history of the area **(Carter *et al.*, 1980; Shurin, 2000)**.

Fisheries productivity in the natural water bodies is correlated with the abundance of certain natural feeding **(Elsebaie *et al*., 2014; Saad & Elsebaie, 2020)** including the benthic invertebrate **(Abdel-Gawad & Mola, 2015)**. Such biological values are revealed from its ability to construct the link between the unavailable nutrients and valuable nutrients resources in many fish and shellfish **(Idowu & Ugwumba, 2005)**.

Exploring and the management of macrobenthic invertebrates in the Rosetta branch have received slight attention. Information about morphological analysis, hydrodynamic status **(Holanda *et al.,* 2011)**, water flow **(El-Naggar *et al.*, 2016)**, water quality, the impact of pollution **(Shreadah *et al.*, 2012)** on fisheries productivity **(Khalil, 1998)** and the calculation of correlation between water Physico-chemical analysis **(El-Shorbagi, 2015)**, sediment analysis and animal taxa biodiversity of water bodies including Rosetta branch are considered a basic principle for good management of the benthic invertebrates' biological resources **(El-Adawy *et al.*, 2013; Donia & Bahgat, 2016; Elhaddad & Al-Zyoud, 2017)**.

Detection of the total and fecal coliforms groups, as well as the fecal streptococci group in the sediment could be used as bioindicators for estimating the pollution levels in a certain area **(Haroon, *et al*., 2020; Afify, *et al*., 2019; Goher *et al*., 2021)**.

The microbial load of the Rosetta branch is affected by receiving a several types of discharged wastes **(Tahoun, *et al*., 2021)**. Short-term of low-level of pollutants discharges may meet water quality criteria, but long-term discharges may result in high loads of pollutants accumulating in the sediments.

The current investigation aimed to evaluate the macrobenthic invertebrate biodiversity and microbial load associated with the sediment analysis in the Rosetta branch, the River Nile, Egypt.

MATERIALS AND METHODS

A total of seven Rosetta branch stations (Fig. 1) were estimated during winter and summer 2018.

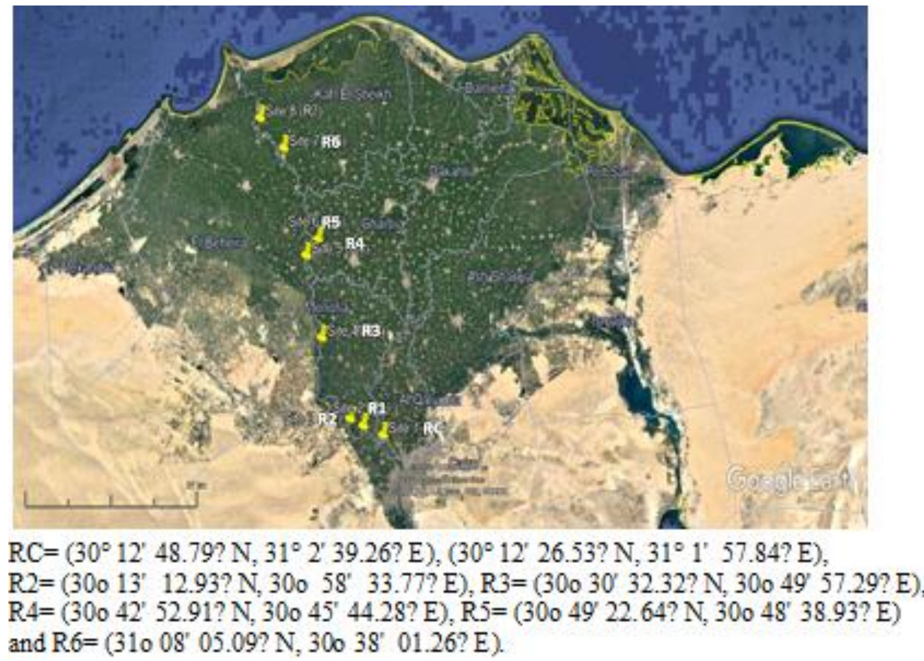


Fig.1. Map of the evaluated Rosetta branch stations. RC= Al-Qanater Al-Khiria, R1= El-Rahawy drain, R2= Al-qata, R3= Tamalay, R4= Kom Hamada, R5= Kafer Al-Zayat and R6= Dosouq.

Sediment analysis

The sediment samples were collected from the upper layer of each station (top of 15 cm of the bottom layer) using Ekman Grab bottom sampler ($275cm^2$).

The stones and/or plant pieces were removed from the dried samples using a 2mm sieve. From each sample, 0.5gm sediment was digested as described by **Kouadia and Trefry (1987)**.

The grain sizes were assessed using the dry sieving method of **Folk (1974)**. The sediment organic matter was determined according to **Hanna (1965)**. The carbonate contents of sediment samples were determined as described in the study of **Dean (1974)**.

The organic carbon values were measured as described in the study of **Bengtsson and Enell (1986)**.

Water analysis

Water physicochemical parameters (transparency, total dissolved solids, biological oxygen demand, NO_2, NO_3, NH_4, PO_4, Si_2, bicarbonate and dissolved oxygen) were estimated as described in **APHA (2005)**. The water pH, temperature and conductivity (EC, μScm^{-1}) were in-situ evaluated by the Hydrolab model (Multi Set 430i WTW). In addition, the transparency values were evaluated by a white/black Secchi Disk (25cm in diameter).

Macrobenthic invertebrate biodiversity

The sample collection, preparation and preservation were carried out as described in the work of **El-Shabrawy *et al.* (2008)**, with minor modifications. The samples were preserved in glass jars with 6% formalin solution. Characterization and classification of the macrobenthic animals were carried out using stereomicroscope according to **Pennak (1953)**, **Wirth and Stone (1963)**, **Brinkhurst (1966)**, **Brinkhurst and Jamison (1971)** and **Brown (1980)**.

Bacteriological analysis

The microbial contents were evaluated in the sediment samples. Each sediment sample (10g) was transferred to a bottle containing sterile peptone water. After shaking the bottles for 60 minutes, some serial dilutions were carried out. The dry weights of the soil at 105°C were calculated.

Total and faecal coliforms bacteria numbers were detected by the most probable number (MPN) technique **(Afify, *et al*., 2019**). Durham fermentation tubes were provided for all tubes. For faecal coliforms, the inoculated tubes were incubated at 35±0.5°C for 48 hours and at 44.5°C (in a water bath) for 24 hours. The formation of acid and gas denotes a positive outcome. Positive tubes were streaked on Eosin Methylene Blue (EMB) agar plates with sterile loops and incubated at 37°C for 24 hours before microscopic examination **(APHA, 2005)**.

The MPN technique was used to count the faecal streptococci bacteria **(Tahoun *et al.*,2021).**

Turbidity and sedimentation were observed on the bottom of the positive tubes within 48h at 37°C.

Statistical analyses

Data were analyzed using Microsoft excel. The equitability, richness, diversity index, similarity index and correlations between the environmental variables were calculated using PAST **(Qyvind *et al*., 2001)**. The Pearson correlation coefficient was used to select the affected variables. Probability values ≤ 0.05 were considered significant values.

RESULTS

Evaluation of the sediment samples

The sediment samples mainly consist of three fractions, gravel, sand and mud. These fractions vary in percentages and were distributed (Table 1 & Figs. 2, 3) through the evaluated stations (RC, R1, R2, R3, R4, R5 and R6).

Gravel fraction is concentrated at the Dosouk station (R6) during the winter season. It's related to the stable places in the River, while the lowest value was detected at the Kom Hamada station (R4), with an average of 9.87%.

Regarding the second season (summer), the gravel sediments were concentrated also at Dosouk station (R6), but the lowest value was detected at EL-Qanater (RC station) with an average of 13.79%.

The sand fraction was dominant during the two seasons and increased at Al-Qata (R2 station) in both seasons (winter and summer). On the other hand, it decreased in the Kom Hamada (R4 station) during the two evaluated seasons. The average values were 57.02% and 52.95 in the winter and summer seasons, respectively.

The maximum value of mud sediment in the branch was recorded at Al-Qata (R2) station in the two seasons, while the minimum value was detected at Kom Hamada (R4 station), with average values of 33.46 and 33.13% in winter and summer, respectively.

The results showed that, the gravel fractions are formed of granules (< 2mm) and shells, (the gravel was increased in the summer season). The mud fraction was stable during the two seasons, while the sand decreased during summer, affected by a high amount of water and increasing speed current (Table1 & Fig. 2).

Measuring the mean size (Mz)

The mean size pattern of the sediment samples is presented in Fig. (4). The samples were characterized as mud and fine sand (in south stations), changing to coarse sand and very fine sand northward.

Measuring of uniformity (sorting)

Regarding sorting distribution, the pattern of the sediment samples is presented in Fig. (5). The sediment samples in the evaluated branch stations are poorly sorted

Table 1. The distribution of gravel, sand and mud percentages in the Rosetta Branch stations

Station	Winter				Summer			
	Gravel	**Sand**	**Mud**	**Name**	**Gravel**	**Sand**	**Mud**	**Name**
RC	1.3	44.6	55.54	Sandy Mud	0.65	55.13	44.22	Muddy Sand
R1	19.37	69.72	11.78	Muddy Gravelly Sand	25.93	68.72	4.36	Muddy Gravelly Sand
R2	1.456	97.19	1.4	Sand	3.97	95.15	0.88	Sand
R3	5.95	89.78	4.27	Sand	5.95	89.78	4.27	Sand
R4	0.89	15.76	82.93	Sandy Mud	1.13	13.99	84.88	Sandy Mud
R5	8.45	37.6	54.5	Gravelly Sandy Mud	11.34	24.15	64.52	Gravelly Sandy Mud
R6	31.7	44.5	23.8	Muddy Gravelly Sand	47.53	23.7	28.77	Sandy Muddy Gravel
Average	9.87	57.02	33.46		13.79	52.95	33.13	

southward and moderately sorted at the middle stations. Moreover, the north stations are poorly and very poorly sorted.

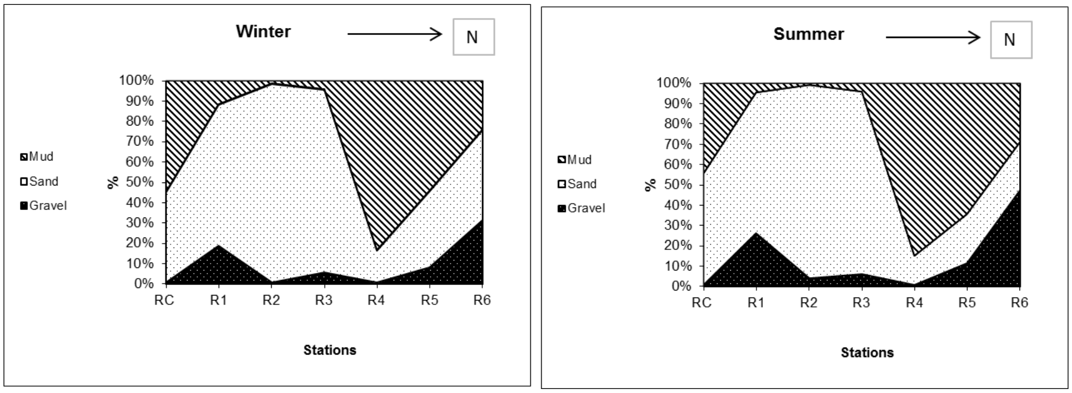


Fig. 2. The horizontal distributions of the evaluated Rosetta branch station sediment samples for the two seasons, winter and summer

Sediment types

The sediment types were distributed as sandy mud (during the winter), changing to muddy sand (during the summer) at the RC station. In addition, it was muddy gravelly sand (during the two seasons at the R2 station) at the R2 and R3 stations (during the two seasons). On the other hand, the sediment was identified as sandy mud at the R4 station. The gravelly sandy mud was detected at the R5 station. R6 station was muddy gravelly sand during winter and changed to sandy muddy gravel during the summer (Table 1).

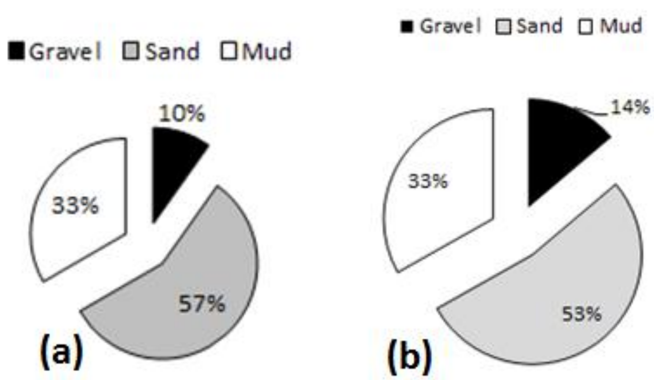


Fig. 3. The percentages of the gravel, sand and mud in the two seasons of winter (a) and summer (b)

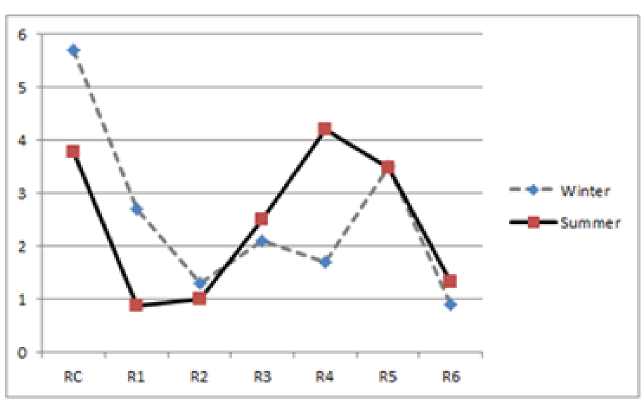


Fig. 4. Exploring the mean grain size values in different Rosetta branch stations (RC, R1, R2, R3, R4, R5 and R6)

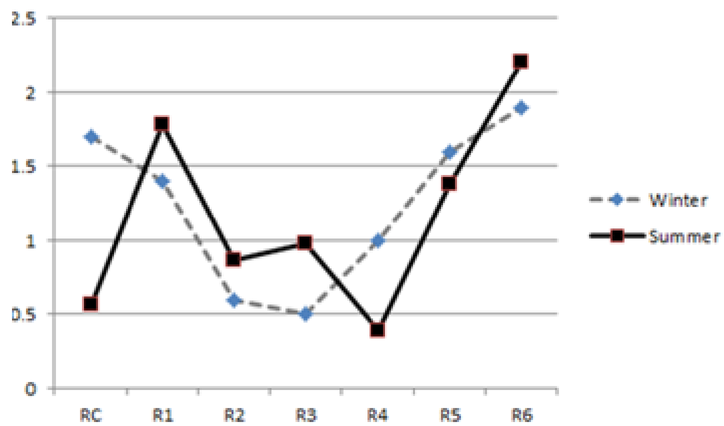


Fig. 5. The changes in the sorting degrees in different Rosetta branch stations (RC, R1, R2, R3, R4, R5 and R6)

Measuring the organic matter

The organic matter values in the evaluated Rosetta branch stations increased in the winter season (with an average of 5.18%). On the other hand, it decreased in the summer season (with an average of 4.99%). The horizontal distribution of organic matter is presented in Fig. (6a).

Water physicochemical parameters

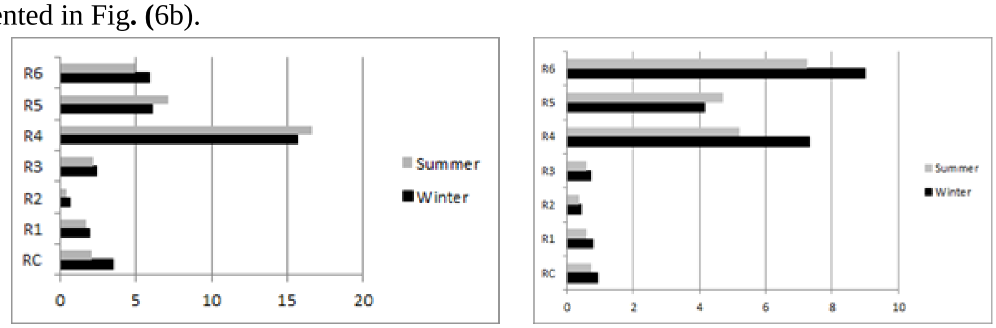
Some water physicochemical parameters (pH, temperature, conductivity, transparency, total dissolved solids, biological oxygen demand, NO_2, NO_3, NH_4, PO_4, Si_2 and dissolved oxygen) were determined and are shown in Table (2).

The mean values of the Temp, Trans., EC, TDS, pH, DO, BOD, NO_2, NO_3, NH_4, PO_4, Si_2 and HCO_3 in the winter season were 19.2 ^oC, 42.5(cm), 1014 (μScm^{-1}), 668.1(mg/l), 7.8, 2.2 (mg/l), 48.4 (mg/l), 12.3 (μg/l), 101.8 (μg/l), 9194 (μg/l), 737.8 (μg/l), 5.3 (μg/l) and 293.9 (μg/l), respectively.

Concerning the summer season, the mean values of the Temp, Trans., EC, TDS, pH, DO, BOD, NO_2, NO_3, NH_4, PO_4, Si_2 and HCO_3 were 24.1^oC, 39.4(cm), 771.3 (μScm-1), 508.3(mg/l), 7.9, 3.7 (mg/l), 31.4 (mg/l), 19.4 (μg/l), 53.5 (μg/l), 3636 (μg/l), 452.9 (μg/l), 4.1 (μg/l) and 250.2 (μg/l), respectively

Measuring the organic carbon

The average organic carbon concentration percentage in the winter was 3.33%. This value decreased in the summer season (2.78%). The horizontal distribution of organic carbon is presented in Fig**. (**6b).



(a) **(b)**

Fig. 6.The horizontal distribution of the organic matter (a) and organic carbon (b) values in each estimated Rosetta branch station (RC, R1, R2, R3, R4, R5 and R6)

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The temperature ranged from 17.7 to 24.7^{o}C. A highly significant difference was detected between the two seasons in the temperature degrees ($P < 0.01$).

Regarding water transparency, results showed a remarkable decrease in the transparency values after El Rahawy Drain, with a highly significant difference between stations ($P < 0.01$). It reached the minimum value of 5cm during winter. These deterioration effects of the water quality were dispersed along the extent of the branch until the Tamaly (R3 station). It was indicated by low transparency (15cm) at this station.

Transparency was positively correlated (Table 3) with DO (r=+0.81) and pH (r=+0.58). While transparency was negatively correlated with each of the EC (r= -0.7), TDS (r= -0.71), BOD (r= -0.73), NH_4 (r= -0.7), PO_4 (r= -0.61), Si_2 (r= -0.7) and HCO_3 (r= -0.64).

Both the EC and the total dissolved solids values were highly variable among the evaluated stations and/or seasons.

The EC recorded the highest value of 1508 $\mu s\ cm^{-1}$ at the R2 station in the winter season.

The lowest value (525$\mu S\ cm^{-1}$) was recorded in the summer season. Furthermore, the results showed high positive correlation values between the EC and each of the TDS (r= 0.99), BOD (r= 0.78), NH_4 (r= 0.92), PO_4 (r= 0.85) and si_2 (r= 0.78) and HCO_3 (r= 0.92).

The TDS values were varied between 336.5 (at the R1 station, summer season) & 995.28mg/ l (at the R2 station, winter season).

The pH of the Rosetta branch was directed to the alkaline side with highly significant differences between the stations. The lowest pH value (7.4) was detected at the R2 station. These results are confirmed by a highly negatively correlation (Table 3) between each of the BOD (r=-0.83), NH_4 (r=-0.54) and Si_2 (r=-0.81). On the other hand, the pH was positively correlated with the DO (r=0.73).

The dissolved oxygen (DO) values showed a highly significant difference between the stations and seasons ($P< 0.01$). Dissolved oxygen was depleted completely after the discharge point of the El-Rahawy Drain, especially in winter, and this effect was dispersed along the extent of the branch until the station of Kom Hamada. These results were confirmed by the highly negative correlation between DO and each of the BOD (r= -0.9), NH_4 (r= -0.82), PO_4 (r= -0.62), Si_2 (r= -0.8) and the alkalinity (r= -0.71).

The BOD values ranged from 4.1(at the summer) to 134.2 mg/l (at the winter). The BOD was positively correlated with each of the NH_4 (r= 0.81), PO_4 (r= 0.58), Si_2 (r= 0.95) and HCO_3 (r=0.67). The levels of BOD values showed a highly significant difference between sites.

Carbonates concentration depleted completely in all evaluated stations. The concentrations of bicarbonates varied from 186.25 mg/l (summer season) to 391.01 mg/l (winter season). The concentration ranges of the Nutrient salts (essential compounds for aquatic organisms) including NO_2 (0- 37.76 mg/l), NO_3 (5- 242.47 mg/l), NH_4 (321.45-17496 mg/l), PO_4 (7.08- 1562.02 mg/l), and Si_2 (1.3 - 16.1 mg/l) are fluctuated from station to station along the Rosetta branch.

Table 2. Analysis of water physicochemical parameters

Season	Winter			Summer		
Parameters	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Temp(°C)	17.7	20.9	19.2	23.6	24.7	24.1
Trans. (cm)	5	130	42.5	10	90	39.4
EC (µScm^{-1})	546	1508	1014	525	961	771.3
TDS (mg/l)	350	995.3	668.1	336.5	644.7	508.3
pH	7.4	8.2	7.8	7.7	8.2	7.9
DO (mg/l)	0	5.5	2.2	1.6	7	3.7
BOD (mg/l)	5.1	134.2	48.4	4.1	79.8	31.4
NO_2 (µg/l)	0	37.8	12.3	8.4	30.7	19.4
NO_3 (µg/l)	5	242.5	101.8	19.3	121	53.5
NH_4 (µg/l)	551	17496	9194	321.5	7124	3636
PO_4 (µg/l)	7.1	1562	737.8	25	711	452.9
Si_2 (mg/l)	1.3	16.1	5.3	1.3	10.4	4.1
HCO_3 (mg/l)	217	391	293.9	186.3	271.2	250.2

Min= Minimum; Max= Maximum; TDS = Total Dissolved Solids; BOD= Biological Oxygen Demand, and Trans= Transparency.

Evaluation of the macrobenthic invertebrate resources

A total of 24 macrobenthic invertebrate species were detected along the Rosetta branch in the two seasons (winter and summer). The recorded animals in the Rosetta branch during the two seasons (winter and summer) are displayed in Table (4).

Seasonal variations

Winter season

During the winter season, five animal groups (Annelida, Arthropoda, Ostracoda, Mollusca and Free-living Nematodes) were detected, characterized and documented. A total of 18 macrobenthic invertebrate species were detected throughout the season along the Rosetta branch.

The total numbers and averages of the characterized animal species (belonging to each documented macrobenthic invertebrate group) in each evaluated ecological station were calculated. The percentages of the different animals within each of the evaluated Rosetta branch station (RC, R1, R2, R3, R4, R5 and R6) were presented in Fig. (7a). Annelida taxa were detected in only three Rosetta stations (RC, R3 and R4). They constitute 35%, 98% and 96% of the recorded animals in the RC, R3 and R4, respectively.The highest number of Arthropoda (8320 org.m^{-2}) taxa was calculated in the RC Rosetta station (44%). On the other hand, Nymph of *perithemis* sp. constituted the lowest Arthropoda taxa numbers (16 org.m^{-2} in R6 station). Besides, this group constitutes 50%, 5% and 3% of the total detected animals in R1, R5 and R6 stations, respectively.

Table 3. Correlation coefficient values among the chemical parameters

	T	Tr	EC	TDS	pH	DO	BOD	NO_2	NO_3	NH_4	PO_4	Si_2	Alk.	CO_3
Trans	-0.2													
EC	-0.5	-0.7												
TDS	-0.4	-0.7	0.99											
pH	-0.1	0.58	-0.5	-0.5										
DO	0.2	0.81	-0.8	-0.8	0.73									
BOD	-0.1	-0.7	0.78	0.78	-0.8	-0.9								
NO_2	0.13	0.23	-0.4	-0.4	0.77	0.58	0.71							
NO_3	-0.5	0.22	0.02	0.02	0.39	0.16	-0.3	0.48						
NH_4	-0.5	-0.7	0.92	0.91	-0.5	-0.8	0.81	-0.6	-0.2					
PO_4	-0.3	-0.6	0.85	0.84	-0.3	-0.6	0.58	-0.1	-0.2	0.81				
Si_2	-0.1	-0.7	0.78	0.79	-0.8	-0.8	0.95	-0.6	-0.3	0.75	0.55			
Alk	-0.5	-0.6	0.92	0.92	-0.5	-0.7	0.6 -	-0.2	0.01	0.85	0.88	0.67		
CO_3	0.37	0.39	-0.6	-0.6	0.41	0.55	-0.6	0.33	-0.1	-0.5	-0.5	-0.4	-0.6	
HCO_3	-0.5	-0.6	0.92	0.92	-0.5	-0.7	0.67	-0.2	0.01	0.85	0.88	0.67	1	-0.6

T= Temperature; Tr= Transparency; TDS = Total Dissolved Solids; BOD= Biological Oxygen Demand, and Trans= Transparency.

Only 1% of the recorded animal groups were Arthropoda in both R3 and R4 stations. No, animals were recorded in the R2 station except *procladius* larvae (Arthropoda) during this season. The Ostracoda **(**presented by *Cypreides torosa*) was recorded in only three stations. The percentages of these taxa were 1%, 16% and 11% in R4, R5 and R6 stations respectively.

Concerning free-living Nematodes, a total of 3200 (16%) and 48 org.m^{-2} (4%) was calculated in RC and R6 stations, respectively. No free-living Nematodes were observed in the other Rosetta stations.

Correlations among the sediment analysis and the macrobenthos (winter season)

Correlation coefficient values between the sediment parameters and the macrobenthos during the winter season were calculated. A strong positive correlation (R= 0.766) was calculate between the bottom fauna and the mud concentrations across the Rosetta branch stations. On the other hand, a moderate negative correlation (R= -0.594) was calculated between the bottom fauna and both of the sand and gravel across the Rosetta branch stations. A positive correlation (R= 0.7346) was calculated between the bottom fauna and the organic matter concentrations across the Rosetta branch stations. Although technically a positive correlation (R=228) was calculate between the bottom fauna and the organic carbon.

As presented in in Table (5), the calculated correlation values were 0.2149 (weak positive relationship), -0.6395 (negative correlation) and 0.5327 (positive correlation) between (Mollusca and gravel), (Mollusca and sand) and (Mollusca and mud) respectively.

A positive correlation (R = 0.25) between the organic matter percentages and abundances of the Mollusca group in different the evaluated stations in the winter (*P*-Value is 0.59). Also, the correlation between organic carbon and the Mollusca group was 0.44 (*P*-value is 0.31).

Summer season

A total of three macrobenthic invertebrate groups (Annelida, Arthropoda and Mollusca) were characterized and documented in the branch stations (summer season). The total numbers and averages of characterized animal species (belonging to each documented animal group) in each evaluated station were evaluated. No, animals were recorded in the R1 station during this season.

The percentages of the different animals within each the evaluated Rosetta branch station was presented in **Figure (7b)**.

Annelida taxa were detected in only four Rosetta stations. It constitutes 50%, 9%, 6.6% and 49% of the recorded animals in the R2, R3, R4 and R6 respectively.

The highest number (33824 org.m^{-2}) of Arthropoda taxa was recorded in the R3 station. It constitutes 90.96%, 23.1% and 35.7 of the recorded animals in the R3, R5 and R6 respectively. The *Chironomus* Larvae constituted the lowest Arthropoda numbers (96 org.m^{-2} in the R5 station).

Neither Ostracoda nor free-living Nematodes taxa were detected in all investigated stations in this season. No macrobenthic invertebrate groups were recorded in the RC stations except Mollusca. The highest number of Mollusca (896 org.m$^{-2)}$ were detected in the R4 station. It constitutes 100%, 50%, 93.33, 76.92% and 15.28% of the recorded animal groups in the RC, R2, R4, R5 and R6 stations respectively (**Figure 7b)**.

Correlations among the sediment analysis and the macrobenthos (summer season)

Correlation coefficient values between the sediment parameters and the bottom fauna during the summer season were calculated and presented in Table (6).

These values were 0.0923 (weak weaker relationship), -0.8014 (negative correlation) and 0.7634 (strong positive correlation) between (Mollusca and gravel), (Mollusca and sand) and (Mollusca and mud) respectively (at $p < 0.05$).

A positive correlation (R = 0.6532) between the organic matter percentages and abundances of the Mollusca group in the different evaluated stations in winter (*P*-Value is 0.11). Also, the correlation between organic carbon and the Mollusca group was 0.678. (*P*-value is 0.094).

Species equitability, richness, and diversity index

The macrobenthic invertebrate species **equitability**, species Richness (marglef), and Shannon diversity indices in each the evaluated station were assessed in each the estimated season **(Figure 8a, b and c).**The estimated values varied among the evaluated stations. The equitability values were presented in **Figure (8a)**. All equitability values in the summer season were higher than detected in the winter in all the evaluated stations except R1 station.The species Richness (marglef) values were presented in **Figure (8b)**. These values ranged between 0 (R2) to 1.109 (R6) in the first season (winter).

Regarding the second season, these values ranged between 0 (R1) to 0.995 (R5).

Concerning the Shannon diversity **(Figure 8c**), the values ranged from 0 (R2) to 1.571(R6) in the winter season. The Shannon diversity values were ranged from 0 (R1) to 1.84 (R5) in the summer season.

Table 4. The recorded macrobenthic invertebrates in the Rosetta branch during the two seasons (winter and summer)

	Winter							Summer						
Taxa/ Stations	**Rc**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Rc**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
Annelida														
Limnodrilus huffmeisteri	P	A	A	P	P	A	A	A	A	P	P	P	A	P
L.udekemianus	P	A	A	P	P	A	A	A	A	A	A	A	A	P
Glossiphonia paludosa	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Branchiura sowerbyi	P	A	A	A	P	A	A	A	A	A	A	A	A	P
Arthropoda														
***Chironomus* Larvae**	P	A	A	A	P	A	A	A	A	A	P	A	P	P
***Chironomus* Pupa**	P	A	A	A	A	A	A	A	A	A	P	A	A	A
Ephydra sp.	A	P	A	A	A	P	P	A	A	A	A	A	A	A
Nymph of *perithemis* sp	A	P	A	P	A	A	p	A	A	A	A	A	A	A
***Procladius* larvae**	P	P	P	A	A	A	A	A	A	A	A	A	A	A
Ostracoda														
Cypreides torosa	A	A	A	A	P	P	P	A	A	A	A	A	A	A
Mollusca														
Pirenella conica	P	A	A	A	P	P	P	P	A	A	A	A	P	A
Bellamya unicolor	A	A	A	A	A	P	A	A	A	A	A	A	P	A
Biomphalaria alexandrina	A	A	A	A	A	A	P	A	A	A	A	A	A	A
Bulinus truncatus	A	A	A	A	A	A	P	A	A	A	A	P	P	A
Corbicula fluminea	P	P	A	A	A	A	P	P	A	A	A	A	A	A
Corbicula consobrina	A	A	A	A	A	A	A	P	A	A	A	A	P	A
Abra ovata	P	A	A	A	A	A	A	A	A	A	A	A	A	A
Melanoides tuberculata	A	A	A	P	A	A	A	P	A	A	A	P	P	P
Mutela rostrata	A	A	A	A	A	A	A	P	A	A	A	A	A	A
Gabbiella senaariensis	A	A	A	A	P	A	P	P	A	A	A	P	A	P
Gypraulus ehrenbergi	A	A	A	A	A	A	A	A	A	A	A	P	P	A
Helobdella conifera	A	A	A	A	A	A	A	A	A	A	A	A	A	P
Theodoxus niloticus	A	A	A	A	A	A	A	A	A	P	A	A	A	A
Free living Nematodes	P	A	A	A	A	A	P	A	A	A	A	A	A	A

Table 5. Correlation coefficient values (below diagonal) and *P*-value (Upper diagonal) between sediment parameters and bottom fauna during the winter season.

	Moll	Arth	Ost	FLN	Ann	G	S	M	OM	OC
Moll		0.67	0.15	0.66	0.66	0.64	0.12	0.22	0.6	0.32
Arth	0.2		0.52	0	0.92	0.46	0.65	0.45	0.81	0.54
Ost	0.6	-0.3		0.49	0.31	0.89	0.04	0.05	0.02	0.09
FLN	0.21	1	-0.32		0.98	0.49	0.69	0.5	0.75	0.53
Ann	-0.21	0.05	0.45	0.01		0.28	0.24	0.11	0.02	0.47
G	0.21	-0.34	-0.07	-0.31	-0.48		0.91	0.48	0.77	0.29
S	-0.64	-0.21	-0.78	-0.19	-0.51	-0.06		0	0.02	0.07
M	0.53	0.34	0.76	0.31	0.66	-0.32	-0.93		0.02	0.25
OM	0.25	-0.11	0.82	-0.15	0.83	-0.14	-0.84	0.84		0.06
OC	0.44	-0.28	0.69	-0.29	0.33	0.47	-0.72	0.5	0.74	

Moll: Mollusca, Arth= Arthropoda, Ost= Ostracoda, FLN= Free living Nematodes, Ann= Annelida, G=Gravel, S= Sand, M= Mud, OM= organic matter and OC= Organic carbon.

Table 6. Correlation coefficient values (below diagonal) and *P*-value (Upper diagonal) between sediment parameters and bottom fauna during the summer season.

	Moll	Arth	Ann	G	S	M	OM	OC
Moll		0.353	0.815	0.844	0.03	0.046	0.112	0.094
Arth	-0.42		0.029	0.738	0.285	0.385	0.624	0.509
Ann	-0.11	0.804		0.407	0.704	0.412	0.685	0.746
G	0.092	-0.16	0.375		0.542	0.598	0.746	0.223
S	-0.8	0.472	0.177	-0.28		0.013	0.038	0.009
M	0.763	-0.39	-0.37	-0.24	-0.86		0.011	0.141
OM	0.653	-0.23	-0.19	-0.15	-0.78	0.869		0.112
OC	0.679	-0.3	0.151	0.528	-0.88	0.616	0.653	

Moll= Mollusca Arth= Arthropoda, Ann= Annelida, G=Gravel, S= Sand, M= Mud, OM= organic matter and OC= Organic carbon.

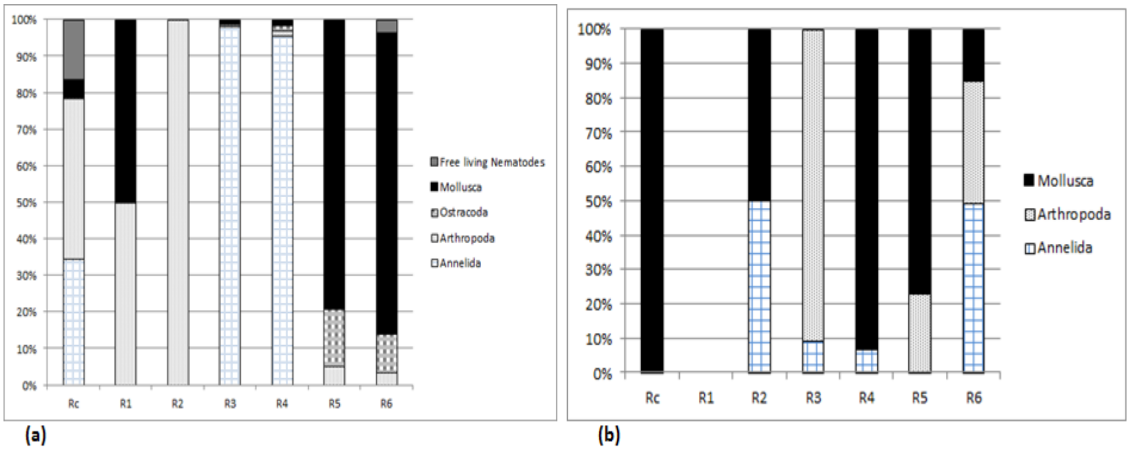

Fig.7. The relative contribution (%) of the descriptive groups to the total density of macrobenthic invertebrates recorded in each of the estimated Rosetta station during the winter (a) and the summer (b).

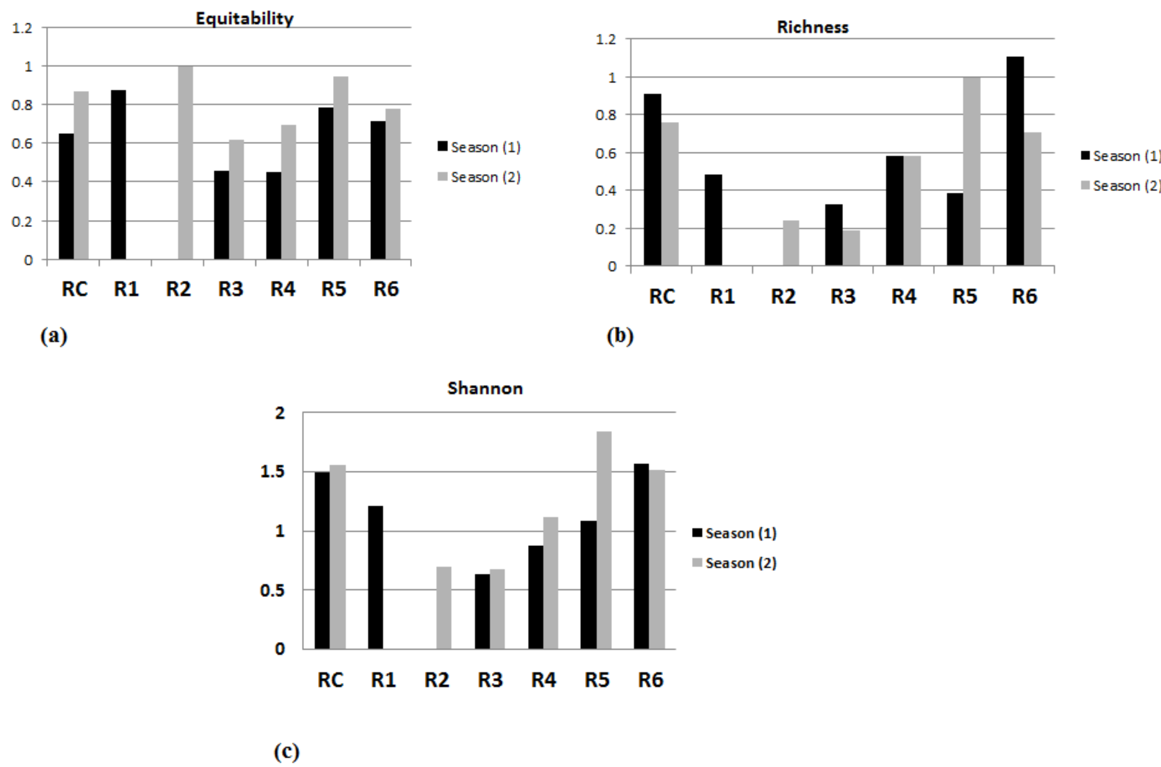

Fig. 8. The macrobenthic invertebrate species equitability (a), species richness (b), and Shannon diversity index (c) in the estimated Rosetta branch stations (RC, R1, R2, R3, R4, R5 and R6). Season 1 (winter season) and Season 2 (summer season).

Clustering analysis

The Clustering analysis presented in **Figs. (9a and b)** described the similarity status among the evaluated Rosetta stations (RC, R1, R2, R3, R4, R5 and R6).

The dendrogram (**Fig. 9a)** reflected the similarity values among the evaluated stations within the winter season. RC is distantly related to the other Rosetta stations (R1, R2, R3, R4, R5 and R6).

The highest similarity values were detected between R1 and both R2 and R3. These values were highest than the similarity values between R1 and each of R4, R5, and R6.

The similarity between R2 and R6 is higher than calculated between R2 and R4. The similarity between R3 and R6 is higher than calculated between R3 and R5.

The same similarity value was calculated between R4 and both R5 and R6.

Regarding **Figure (9b)**, the constructed dendrogram reflected the similarity values among the evaluated Rosetta stations during the summer season. The highest similarity was observed between R1 and both R2 and R3. The R6 station is distantly related to the other evaluated stations. The similarity value between R4 and R5 is higher than the similarity between R4 and R3. The similarity between R2 and R6 is lower than calculated between R2 and R3.

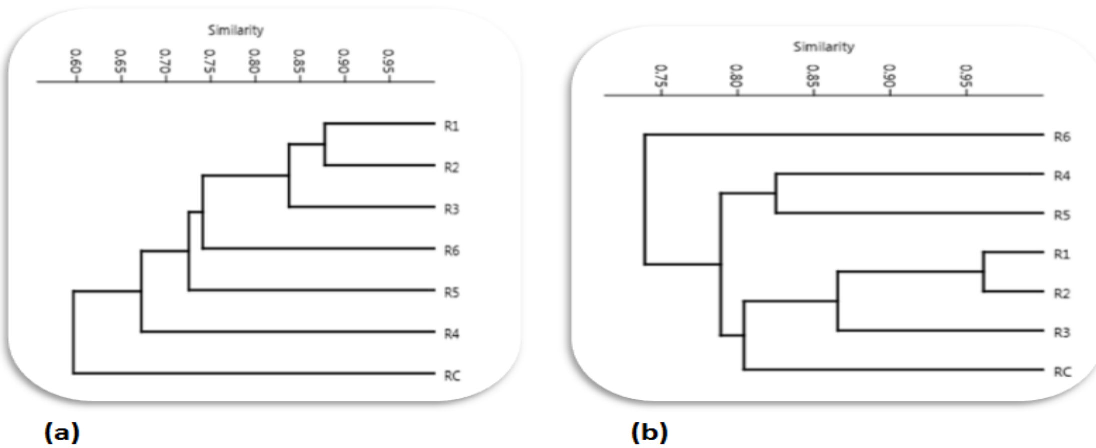

Fig.9. Clustering analysis among the estimated Rosetta stations (RC, R1, R2, R3, R4, R5 & R6) based on Macrobenthic invertebrate biodiversity. (a) winter season, (b) summer season

The similarity between R3 and both R4 and R5 is similar. The similarity value between RC and R1 is higher than the similarity between RC and R6.

Bacteriology

Enumeration of the bacterial indicators (faecal coliform, total coliform and faecal streptococci) at sediment samples that were collected from the Rosetta branch during the winter and summer, of 2018 were presented in Table (7).

Total coliform (TC), faecal coliform (FC), and faecal streptococci (FS) numbers during the winter were in the ranges of 11×10^2-210×10^3, 7×10^2-150×10^3, and 15×10^2- 240×10^3 CFU/g, respectively. On the other hand, during the summer the ranges were 9×10^2 - 240×10^3, 9×10^2 - 210×10^3, and 15×10^2 - 150×10^3 CFU/g for the TC, FC and FS, respectively.

The values of the TC and the FC during winter were arranged to descend as R1, R5, R3, R2, R4, R6, and RC respectively. Also, the TC and the FC values during the summer were arranged as R1, R5, R3, R2, R4, R6, and RC, respectively.

On the other hand, values of the FS during winter were arranged to descend as R1, R3, R2, R5, R4, R6, and RC respectively, while during the summer were (R1, R3), (R4, R6), R5, R2, RC respectively.

The Bacterial indicators of the sediment samples at the Rosetta branch during the winter and the summer seasons were presented in **Figs. (10a and b).**

Table 7. Variations of TC, FC and FS (CFU/g) of sediment samples collected from Rosetta branch.

	Winter			**Summer**		
Par. Station	**TC**	**FC**	**FS**	**TC**	**FC**	**FS**
RC	11×10^2	7×10^2	15×10^2	9×10^2	9×10^2	15×10^2
R1	210×10^3	150×10^3	240×10^3	240×10^3	210×10^3	150×10^3
R2	64×10^3	39×10^3	110×10^3	70×10^3	40×10^3	53×10^3
R3	70×10^3	70×10^3	150×10^3	75×10^3	43×10^3	150×10^3
R4	19×10^3	16×10^3	75×10^3	42×10^3	35×10^3	110×10^3
R5	150×10^3	93×10^3	110×10^3	120×10^3	120×10^3	93×10^3
R6	20×10^2	15×10^2	120×10^3	28×10^3	21×10^3	110×10^3
Mean	73728.6	52885.7	99785.7	82271.4	67128.6	95357.1
Maximum	210×10^3	150×10^3	240×10^3	240×10^3	210×10^3	150×10^3
Minimum	11×10^2	7×10^2	15×10^2	9×10^2	9×10^2	15×10^2
SD	79488.9	55152	82027.4	79260.9	73111.9	53243.7

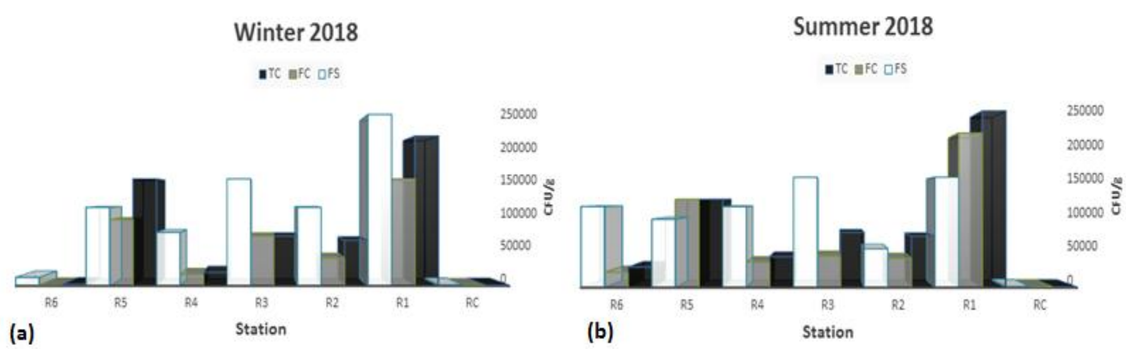

Fig.10. Bacterial indicators of the Rosetta branch sediment samples during the winter (a) and the summer (b) seasons

DISCUSSION

In the present study, the results were analyzed for characterization, documentation and evaluation of the macrobenthic invertebrate's biological resources in different seven stations along the Rosetta branch. Detecting the presence and/or absence of the tolerant and sensitive biological taxa reflects the pollution levels (**Badawy *et al*., 2013**) in certain habitats such as the evaluated stations.

Due to its distribution varies with space, time, and suitable habitations, macrobenthic invertebrates are considered suitable bioindicators of aquatic environmental changes **(Kumar et al. 2013)**. As known, the biological techniques for the evaluation of the water quality in freshwater resources have become common. Some of these techniques were documented due to their informative indication for long-term trends **(Kumar *et al*., 2013; Abdel-Satar *et al*., 2022).** We found that the evaluation of the sediment, water properties and levels fluctuations in the evaluated stations are considered informative factors **(El-Shabrawy *et al.*, 2008; Wahab *et al*., 2018)** influencing the macrobenthic invertebrate taxa richness and equitability in both the two seasons (winter and summer).

Regarding the winter season, five animal groups (Annelida, Arthropoda, Ostracoda, Mollusca and Free-living Nematodes) were characterized and documented. On the other hand, during the summer season, only three animal groups (Annelida, Arthropoda and Mollusca) were characterized. Neither Ostracoda nor free-living Nematodes taxa were detected in all the investigated stations. These three animal groups were also recorded by **Abdel-Satar *et al*. (2022)** in different Rosetta stations.

The organic matter in the sediment samples was evaluated in the present study because it is considered a suitable indication of the amount of food setting on the bottom of the water column **(Byers *et al*., 1978; Wahab *et al.*, 2018).** The correlations among the sediment types and macrobenthic invertebrates' abundances reflect the levels of such suitability.

Regarding the winter season, a strong positive correlation value (r= 0.766), was calculated between the bottom fauna and the Mud concentrations across the Rosetta branch stations. This finding means that the bottom fauna scores go with Mud concentration scores. A similar finding was confirmed (**Vivier and Cyrus, 1999)**. Also, positive correlation values were calculated between the bottom fauna taxa and each of the organic matter (r=0.734) and the organic carbon (r= 0.228). These results reflected the values of the organic matter in the sediment to the bottom fauna. Organic matter in the sediments is thought to provide food for deposit feeding and detrivores macrobenthos. There is usually higher species abundance in organically rich environments than in organically poor environments (**El-Shabrawy *et al.*, 2008; Oberndorfer and Lundholm, 2009).** So, the highest number of animals was recorded in the R4 station. On the other hand, the lowest number was detected in the R2 station. The organic content and type of sediments are known to have pronounced effects on the spatial distribution and community composition of benthos.

In the present study, the changes in the mean grain size in the different Rosetta branch stations were evaluated. The rate of sedimentation (deposition) of particulate matter is determined by the particle size, shape, density with respect to water density, and water viscosity**.**

The highest organic matter values were recorded in the R4 station. There are slit differences among the organic matter content in the two seasons. This finding may be due to the gradual accumulation of phytoplankton decomposition **(Payne, 1986)** from the previous seasons.

Regarding the summer season, the gravels were concentrated at the R6 station but the lowest value was detected at the RC station.

In addition, all the gravel sediments were granules (the gravel was increased in the summer). The sand sediment in the branch was increased at Al-Qata (R2 station) in both seasons (winter and summer). On the other hand, it decreased in Kom Hamada (R4 station) in the two evaluated seasons. The sediment in the evaluated branch stations is poorly sorted southward and moderately sorted at the middle stations. Also, the north stations are poorly and very poorly sorted. It's related to the slow motion to sediment at the bottom.

The organic matter values and the average percentage of organic carbon concentrations in the evaluated Rosetta branch stations increased in the winter while decreasing in the summer. It's related to the water movements' differentiation in the two evaluated seasons.

These variability may be due to seasonal variations. This conclusion was supported in some other studies in different ecological locations (**El-Shabrawy *et al*., 2008)** along the River Nile (**Abdel-Gawad and Mola, 2015; Abdel-Satar *et al.*, 2022).** Also, these variations could be due to the macrobenthic invertebrate's biodiversity levels being affected by nearby a group of environmental conditions, including abiotic and biotic factors. It could be influenced by the inter-relationship among such factors **(El-Shabrawy *et al*., 2008; Wahab *et al.*, 2018).**

Tahoun *et al.* (2021) reported that the Rosetta branch received several domestic, agricultural, and industrial wastes. Such wastes could affect various aquatic taxa including benthic invertebrates, zooplankton, bacteria and flora. So, identifying the biological features of this water body is vital. Due to the previous reasons, **El Sayed *et al*. (2020)** concluded that the furthermost resistant benthic invertebrates to pollution were often detected in this branch. They recommended the use of biotic indices, precisely which depend on bottom fauna in Egypt to reflect the water quality in a certain water body like the Nile River.

The gravel sediments in the winter are concentrated at the R6 station. It's related to the stable places in the River while the lowest value was detected at the R4 station.

In the present study, the Annelida group was represented by *Limnodrilus huffmeisteri, L. udekemianus, G. paludosa* and *B. sowerbyi* (in the winter season). The *G. paludosa* was not recorded in the summer season. The *Limnodrilus huffmeisteri* was the most dominant Annelida taxa in both two evaluated seasons. The highest average density values were 4763.42 and 644.571 Org. /m^2 were detected in the winter and summer seasons respectively. A similar observation was confirmed by **El-Shabrawy *et al*. (2008)** at EL-Qanater Fish farm (NIOF). Recently, **Abdel-Satar *et al.* (2022)** found that the *Limnodrilus* sp. was the greatest dominant taxa of phylum Annelida in different Rosetta branch stations.

Some animal species belonging to the Mollusca group were recorded in both the two evaluated seasons. This observation was confirmed by **Abdel-Satar *et al.* (2022)** in different Rosetta branch stations. Some of the Mollusca species were absent in certain seasons. For example, the *B. alexandrina* and *A. ovate* were recorded in the winter while absent in the summer season. The *C. consobrina*, *M. rostrata*, *G. ehrenbergi*, *H. conifer* and *T. niloticus* were absent in the winter while recorded in the summer season.

The calculated correlation values confirmed that the abundance of the Mollusca taxa was correlated to the abundance of muddy sediment.

In the present study, the Arthropoda taxa were presented by *Chironomus* Larvae, *Chironomus* Pupa, *Ephydra* sp., Nymph of *perithemis* sp. and *Procladius* larvae. All of them were recorded in the winter. On the other hand, the last three taxa were absent in the summer season. Chironomids (aquatic insects) are common inhabitants of polluted freshwater environments. So, it is encouraged for use as an indicator organism **(Seidman *et al,*. 1986).** As revealed by **Abdel-Satar *et al*. (2022)**, the *Chironomus* spp. was the supreme Arthropod taxa recorded at both Rosetta and Damietta branches.

In the present study, the Ostracoda as microscopic aquatic crustaceans, **(**presented by *Cypreides torosa*) was recorded in only three stations. The percentages of these taxa during the winter season were 1%, 16% and 11% in the R4, R5 and R6 stations respectively. *The C. torosa* is considered an euryhaline species, generally recorded in transitional environments as inland ponds, lagoons and estuaries **(Mischke *et al.*, 2019; Roberts *et al*., 2020; Can *et al*. 2021).** As demonstrated by **Can *et al*., (2021),** some Ostracoda species such as *Limnocythere inopinata* preferred a narrow temperature (from 11 to 14 °C) range. The Ostracoda species sensitivity and tolerance to pollution, and temperature changes were discussed. Also, the *C. torosa* sizes, abundance and distribution are affected by the environmental conditions, including pH, $CaCO_3$ concentrations and temperature **(Wrozyna *et al*., 2022)** degree fluctuations (vital control on the *C. torosa* life cycle), but relatively slight is explained about such controls.

Generally, the Ostracods were favorable models for inference of the effects of the paleoclimatic changes. They found that it could be preserved in the sediments. So, **Ostracoda** fossils are considered a good tool to discover the aquatic environmental factors during the past half a century **(Can *et al*., 2021; Wrozyna *et al.*, 2022).**

A total of 3200 and 48 Org. m^{-2} of the free-living Nematodes were recorded in the winter season in the RC and R6 stations, respectively. No free-living Nematodes were observed in the Rosetta stations during the summer season.

Soko and Gyedu-Ababio (2019) used free-living nematodes as bioindicators for pollution in different Incomati River sites (from June 2017 to April 2018, Southern Mozambique) that were strongly affected by anthropogenic activities.

The macrobenthic invertebrate species Equitability, species Richness (marglef), and diversity indices in each the estimated Rosetta branch station were evaluated. The estimated values varied among the evaluated stations during the two seasons.

Wahab *et al.* (2018) recorded a lot of aquatic plants in some Lake Nasser khors that saved appropriate substrate for the growth of *Dicrotendipes* species. Also, **Tahoun *et al.* (2021)** found that the Epiphytic microinvertebrates associated with the *Eichhornia crassipes* detected several taxa through the summer season. So, the biodiversity levels were affected by seasonal variations and/or the heavy load of pollution discharged to the Rosetta branch stations. To explore the effects of pollution on the water characteristics, the water physicochemical characteristics in the Rosetta branch were estimated.

The water temperature is directly affected physical and chemical parameters, and animal and plant life in the freshwater **(El Sayed, 2015).** The temperature ranged between 17.7 -24.7^{o}C there is a highly significant difference between the two evaluated seasons. Usually, Temperature is negatively correlated with HCO_3 because the decrease in water temperature increases the solubility of CO_2 and later increases bicarbonate ions **(El Sayed *et al.*, 2020; Abdel-Satar *et al*., 2017).**

The water transparency reflects the depth of the photic area **(Goher *et al.*, 2018)** in the branch. The results showed a remarkable decrease in transparency values after the El Rahawy Drain with a significant difference among the stations ($p < 0.01$). It reached the minimum value of 5cm during winter (due to the discharge of heavily polluted effluent loaded with industrial, agriculture and domestic wastes). These deterioration effects of the water quality were dispersed along the extent of the branch until Tamaly (R3 station). It was indicated by low transparency (15cm) at this station.

Transparency is positively correlated with the DO and the pH. While transparency values were negatively correlated with each of the EC, TDS, BOD, NH_4, PO_4, Si_2, HCO_3. These results were in agreement with **(El Sayed *et al.*, 2020; El Sayed, 2011)**. They concluded that the transparency values were affected by the particulate content in the water from floating substances and suspended matter.

EC is a measure of the ability of an aqueous solution to carry an electric current and the dissolved ions are the conductors **(Elsayed *et al*, 2019).**

Both the EC and the Total Dissolved Solids values were highly variable among the evaluated stations and/or seasons. A positive correlation was calculated between these two parameters.

The EC recorded the highest value 1508 µs cm^{-1} at R2 in winter due to the intrusion of the drain's effluents into the lowered level water in the branch causing elevation of dissolved and suspended particles which increases the ability to convey electrical current. The lowest value (525µs cm^{-1}) was recorded in the summer due to the increase in water level during the flood period where EC and the water level are inversely related **(Islam *et al*., 2015).** Also, the results showed high positive correlation values between the EC and each of the TDS, BOD, NH_4, PO_4 and Si_2 and HCO_3. These results indicated that bicarbonate is considered a major anion in the freshwater. This finding was confirmed by **El Sayed (2011)** for the Rosetta branch. Whereas EC negatively correlated with the DO and the pH.

TDS values varied between 336.5 (R1 station, summer season) to 995.28 mg/l (R2 station, winter season). This finding may be due to the raising of water levels during the flood period **(Mohamed, 2008).**

The pH is the master control parameter in aquatic life for the biological and/or chemical transformation of water (**Haroon *et al.*, 2018).** The pH of the Rosetta branch lies on the alkaline side with highly significant differences between sites. The lowest pH value (7.4) was detected in the R2 station (winter season). This may be due to the lower activities of the phytoplankton. This finding may be due to the fungal and bacterial effects in the sediment. In these conditions, methane and hydrogen sulfide could be released. Also, the organic acids and other breakdown products could be constructed **(Goher, 2014)**. These results were confirmed by the negative correlation with the BOD, NH_4 and Si_2.

The results showed that the pH values were positively correlated with the DO (Dissolved oxygen). These results confirmed the effects of the photosynthetic activity on the raising the pH value **(Abdel Sater *et al*., 2017).**

DO is considered a vital parameter **(Mahmoud *et al*., 2008)** in the evaluation of the pollution levels in a certain water body.

DO values showed a highly significant difference between sites and seasons ($p< 0.01$). Dissolved oxygen was depleted completely after the discharge point of the El-Rahawy Drain, especially in winter season. This effect was dispersed along the extent of the branch until Kom Hamada. This finding may be attributed to the direct effects of the discharged effluent heavily loaded with organic and inorganic wastes. These results were confirmed by the highly negative correlation between DO and each of the BOD, NH_4, PO_4, Si_2 and Alk where the DO could be consumed via the oxidation of nitrogenous and organic matters **(El-Sayed *et al.*, 2020).** The maximum value of DO (7 mg/l) was recorded at the R1 station (upstream of El Rahawy Drain) during the summer season which may be attributed to the abundance of phytoplankton, since the photosynthetic process was regarded as the main source of oxygen in the aquatic environment **(Goher, 2021).**

The BOD values ranged from 4.1 (summer) to 134.2 mg/l (winter). BOD was positively correlated with each of the NH_4, PO_4, Si_2 and HCO_3.

The biological and chemical oxygen demands have great importance for assessment the of organic matter pollution **(Ali *et al.*, 2014)**. Concerning our results, the levels of BOD were higher than the internationally permissible levels and showed a highly significant difference between sites. This may be due to the presence of many polluted sources along the extent of Rosetta especially, in the El Rahawy Drain (R1 station) and Kafr El-Zayat (R5 station). These sources were heavily loaded with domestic, industrial and agricultural wastes containing high amounts of inorganic and organic constituents.

Both the carbonates and bicarbonates constituted main components of the alkalinity of water surface (**Hassouna *et al,*. 2019).**

Carbonate concentration was depleted completely in all the evaluated stations. The concentrations of bicarbonates varied from 186.25 mg/l (summer season) to 391.01 mg/l (winter season). This variability may be due to the bacterial decomposition of the organic substance, where bicarbonate and ammonia are the final products **(Moustafa *et al.*, 2010).**

The correlation values between the bicarbonates and each of the Mg, Ca, Cl and Alk were 0.93, 0.88, 0.92 and 0.79 respectively. These results were in agreement with El Sayed, (2015) results for the same branch.

The concentration ranges of the Nutrient **(Haroon *et al.*, 2018)** salts (essential compounds for aquatic organisms) including NO_2 (0- 37.76 mg/l), NO_3 (5- 242.47 mg/l), NH_3 (321.45-17496 mg/l), PO4 (7.08- 1562.02 mg/l), and Si_2 (1.29- 16.09 mg/l) are fluctuated from station to station along the Rosetta branch. These values were higher than those recorded by **Metawea (2009)** and **El-Sayed (2011).** On the other hand, the recorded values were near the **El-Sayed et al. (2020)** results. They characterized the Rosetta Branch as a drain of waste, especially during the drought period. This may be due to the probable harmful effects of the large quantities of the wastes (announced into the Rosetta branch escorted by low freshwater level).

The results showed that the highest numbers of all the bacterial indicators were recorded at the R1 station (affected by the El-Rahawy drain). As known, the Tala drain (Kafer Al-Zayat province) discharged its waste effluents in the R5 station. So, high levels of microbial loads were detected in the R5 station.

The microbial pollution of the Rosetta branch originated from numerous drains that discharged lot quantities of wastewater and domestic as well as agricultural and industrial wastes. For example, the El-Rahawy drain, El-Tahreer drain, Sabal drain, Tala drain, and Zawiet El-Bahr drain, are highly Rosetta branch affected sites. So, the high load of organic matter and pollutants that came to the Rosetta branch (Nile River) water from the El-Rahawy drain and others caused these highly microbial count values **(Tahoun *et al.*, 2021).**

It is noted that the counts of the bacterial indicators in the Rosetta branch sediment samples were higher than the water samples from each of the sampling stations. This finding was also confirmed by **Tahoun *et al.* (2021).** This could be attributed to that; the sediments mostly provide a proper natural environment for microbial existence and living **(Abd-Elfattah *et al*., 2021).**

CONCLUSION

The evaluated aquatic taxa could be affected by various domestic, agricultural, and industrial wastes. The Rosetta Branch is characterized as a drain of wastes especially in the drought period. So, annual documenting the biological aspects of this branch is essential.

The water physicochemical characteristics, microbial load, and macrobenthic invertebrate biodiversity were evaluated during two seasons (winter and summer). The sediment types in the evaluated Rosetta branch stations were distributed as muddy gravelly sand, muddy sand and sandy mud.

The Dissolved oxygen was depleted completely after the discharge point of the El-Rahawy Drain, especially in the winter season and this effect was dispersed along the extent of the branch until Kom Hamada. The bottom fauna including Annelida, Arthropoda, Ostracoda, Mollusca and Free-living Nematodes could be used as bioindicators for the water quality status in various freshwater systems such as the Rosetta branch, Egypt.

The numbers of the characterized animals varied among the evaluated stations. The fecal streptococci, were high, indicating that the Rosetta branch is polluted by sewage. The results could be used for inference of the biodiversity levels in such Egyptian ecological area. Also, it could be used to design some plans for good management of such biological resources in the future.

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