EGYPTIAN JOURNAL OF AQUATIC RESEARCH ISSN: 1687-4285
VOL. 33 NO. 3, 2007: 88-102

USING THE NEMATODE TO HARPACTICOID COPEPOD RATIO AS A MONITORING TOOL OF BENTHIC ORGANIC POLLUTION

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Keywords: Meiofauna, Total organic matter%, Grain size, sand fraction, silt fraction, abundance, self remediation

ABSTRACT

The current work discusses the use of nematode to copepod ratio in relation to determine the degree of organic loading at the Eastern Harbor, Alexandria after the cessation of sewage dumping at the bay by the end of 2001. Sediment samples were collected by Van Veen garb sampler from twelve stations in the area on six occasions between August 2002 and June 2003. Nematode and harpacticoid copepod were sorted, counted, and the ratio was calculated as well as total organic matter, mean grain size, sand fraction and silt fraction were measured. Results revealed significant variations in mean total abundance of nematode and harpacticoid over dates and among stations. Harpacticoid abundance increased with time from 2002 to 2003. According to the nematode to copepod ratio, the stations at the Eastern Harbor were divided into five groups: group of infinity ratio >1000, high ratio group (ratio<1000 and >100), two groups of mild ratio (<100>10 and (<10>1) and one undefined ratio's group (0/0). Sites of two extremes ratio might suffer from pollution due to absence or near absence of harpacticoid or even the absence of nematode and harpacticoid. Data of nematode to copepod ratio was normally distributed with the lowest frequency for the two extreme groups indicating that sediment at the Eastern Harbor is enhanced and getting ride of organic pollution with time and tended to self remediation. However, sites that have infinity or undefined ratios are not good indicator of organic pollution probably due to presence of other effective environmental variables or due to presence of other pollutants such as hydrocarbon oil and heavy metals.

1. INTRODUCTION

The Eastern Harbor (E.H) of Alexandria (Fig.1) plays an important role as fishing docks, water sporting center and as area of thousands of submerged archaeological artifacts representing significant era of the ancient Alexandria. The marine environment of the E.H. suffered from receiving considerable amount of waste effluents (mainly raw sewage) since 1976. The amount of this discharge has increased six times since 1985 (Aboul Kassim, 1987; Said and Maiza, 1987; Zaghloul, 1988; Zaghloul and Halim, 1992; Labib, 1994; Nessim, 1994). These effluents have led to a considerable increase in the level of the nutrients and heavy metals in the area compared with their outside counterparts in the neritic water of the Mediterranean (El Sayed and El Sayed, 1980; El Nady, 1981). In many sites the surface sediment has turned to anoxic environment leading to disappearance of most of its benthic fauna and flora. Since 1993, the Eastern Harbor went through another stage where most of sewage outfalls inside the Harbor were closed. Most of municipal wastewater that discharge into the sea from other outfalls along Alexandria shore line was stopped except for Qait-bey and El Silsila outfalls. The discharge from Qait-bey and El Silsila outfalls has been gradually decreased until their complete cessation by the end of 2001. As a result, temporal variation of sediment water content that proposed the displacement was observed as those surface sediments with their combined organic matter followed water circulation pattern in E.H. (Jammo, 2004). Anoxic bed sediment phenomenon in the Eastern zone of the E.H. was found to be existing during two seasons (summer and autumn) and an apparent temporal movement of anoxic surface sediments with time towards the Harbor outside direction was noticed (Jammo, 2004).

Raffaelli and Mason (1981) first proposed the use of the nematode to copepod ratio as a tool for biomonitoring. The validity of this technique subsequently became the subject of discussion among meiobenthologists, with some (e.g. Coull *et al.,* 1981) arguing that it was an over generalization and others (e.g. Warwick, 1981) suggesting modifications to improve its utility. Lambshead (1984) cast doubt on the usefulness of the ratio by pointing out the difficulties in separating the effects of pollution on the ratio from the effect of other environmental variables. This nematode to copepod ratio was examined in relation to metal pollution (Lee *et al*., 2001).

The meiofauna, unlike the macrofauna, spend their entire life cycle within sedimentary environment. As a result of direct benthic recruitment and short time generation meiofauna are more responsive to the input of a pollutant to the sedimentary environment than the macrofauna (Coull and Chandler, 1992). Direct benthic recruitment implies that the effects of pollution on the community structure are not masked by recruitment of individuals from outside the impacted area. Short generation times of majority of meiofaunal species have the advantage that all stages in life cycle are exposed to the pollutant (Coull and Chandler, 1992), which results in a short response time by the community, to a pollution event. Mitwally and Awads (2005) gave the first published information on the benthic meiofauna of this unique environment (Eastern Harbor). Mitwally *et al.* (2007) compared between polluted and non polluted sites by using nematode to copepod ratio.

The objective of this study was to use the nematode to copepod ratio as a monitoring too; to organic enrichment pollution and to determine to what extent the sediment of Eastern Harbor has recovered from pollution.

2. MATERIALS AND METHODS

2.1. Study area

The Eastern Harbor of Alexandria (Fig.1) is a semi-closed basin connected to the sea by two openings. El Boughaz opening is located at the western side (about 300m width) and El Silsila is located at the east. The Harbor is located at 29° 53°' -29°' 54' E (longitude) and 31° 12' -31° 13' N (latitudes). The Harbor surface area is about 2.53×10^{6} m^{3} with an average depth of 6m and a water volume of about 15.2×10^{6} m^{3}. The Harbor is shallow along its southern margin and deeper towards the openings. The Harbor bottom slopes down gradually north wards reaching maximum depth at the central area behind El Boughaz opening.

2.1.1. Sampling stations and field work

Twelve stations (Fig.1) were sampled bimonthly from August 2002 to June 2003 using the Van Veen garb. Two sub samples were taken with 60 cm^{3} syringe barrels with the needle and base cut off (area 4.9 cm^{2}) from each station. One sub sample was analyzed for sedimentological parameters and the other for meiofauna. Meiofaunal samples were kept in the refrigerator at 4 °C until investigation.

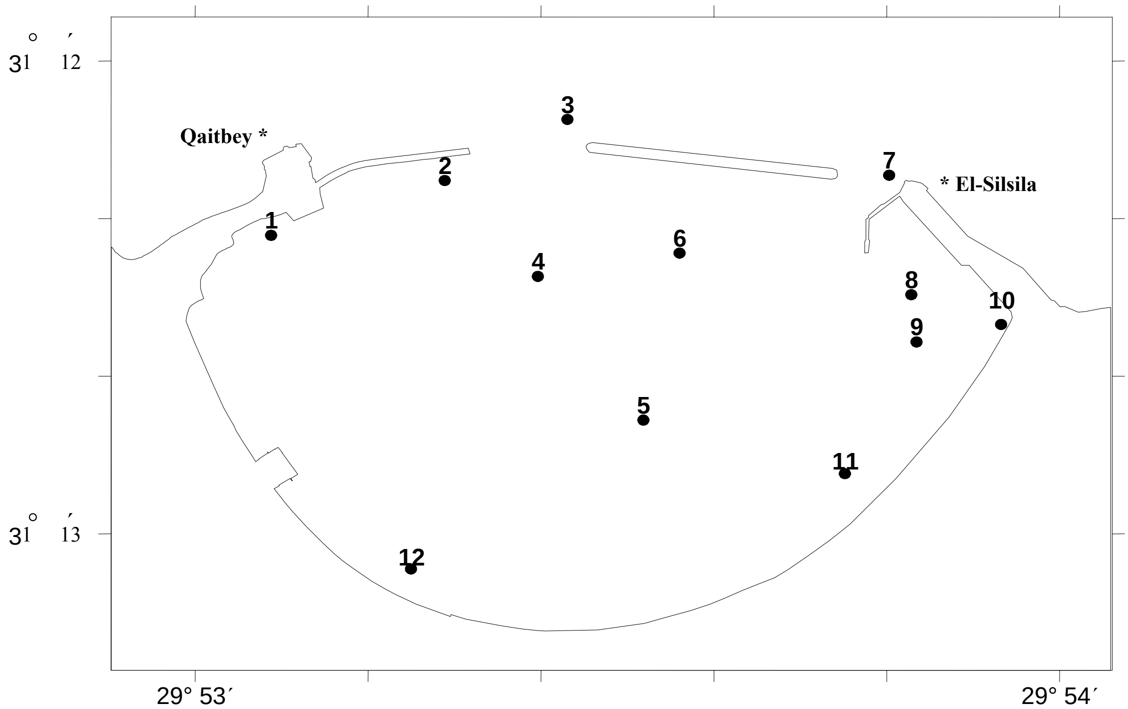

Fig. (1): Map shows the studied stations at the Eastern Harbor between 29° 53́- 29° 54́ E (longitude) and 31° 12́ -31° 13́ N (Latitude).

2.1.2. Laboratory work

Meiofaunal samples were firstly anaesthetized with magnesium chloride (7%) for examination of live organisms and then stained with rose Bengal and left overnight. Meiofauna was extracted from sediment by swirl and decantation and collected on a 63µm sieve (Huys *et al.,* 1996; and Mitwally, 1999). Nematode and harpacticoid copepod were sorted and counted under stereomicroscope. The quantitative abundance was calculated as number of individuals per unit area of sediment (individuals 10/cm^2).

The sieving technique was applied to the sediments which lacking fractions smaller than 0.063µm according to Folk (1974). The pipette analysis was used for fine fraction (more than 0.063µm) using the technique described by Krumbein and Pettijohn (1938). The content of organic carbon (TOC) was determined by the direct method described by El Wakeel and Riely (1957). The method is based on the oxidation of the dry sample and titration of the consumed oxidant. A factor of 1.8 proposed by Trask (1939) was used to calculate the total organic matter.

2.1.3. Statistical analyses

Nematode to harpacticoid-copepod ratio (N/C ratio) was calculated by dividing the number of nematodes in a sample by the number of copepods. Where there were no copepods present in a sample the number of nematodes present was used to represent the ratio. Where nematodes were absent but copepod present the ratio was zero and zero was also used where both groups were absent (Lee *et al*., 2001).

All data analyses were performed using SYSTAT (1998) software. All nematodes, harpacticoid and their ratio data were log transformed (log *n*+0.1) to fit the normal distribution. Two way ANOVA was performed on log transformed data to test for significant differences, at α level = 0.05, over dates and among stations as well as the date and station interaction was tested. The number of data totaled 72.

Pearson correlation was applied to evaluate the relationship between sedimentological and biological variables. Also multiple regression analysis was performed to assess the linear relation ship between independent sedimentological and dependent data of nematode, copepod abundance and N/C ratio. Factor analysis (PCA) was performed six times. The first analysis is named "All data analyses" and was applied to assess the effect of sedimentological factors with nematode, copepod abundances, and N/C ratio. The other five analyses were performed on different nematode to copepod ratio to visualize the most important sedimentological factor controlling the ratio at each group and were named infinity group, group 2, 3, 4 and undefined group.

3. RESULTS

Figure (2&3) shows the abundance of nematodes, hapacticoid and nematode to copepod ratio during periods of 2002 and 2003 (A, B, C) and among twelve stations. Due to wide range of variations among data, log scale data was used.

During 2002, nematode abundance ranged from zero individuals 10/cm^2 to 21469 individuals 10/cm^2 respectively in August (st. 6 &11) and in October (st.1). Harpacticoid had the same minimum abundance (zero individuals 10/cm^2) at st.11 during August and maximum of 5934 individuals 10/cm^2 at st.5 during October. Nematode to copepod ratio followed the same pattern of nematodes (Fig. 2C). Samples of 2003 revealed that nematode and harpacticoid abundances were, by an order of magnitude, higher than during 2002 (Fig.3A, B) with few exceptions. On the other side, the nematode to copepod ratio had lower maximum values during 2003 than during 2002 with the same minimum (Fig.3C).

Results from two way ANOVA (Table 1) based on log transformed data (log *n*+0.1) and its *posteriori* Tukey tests revealed significant differences over dates (seasons) and among stations for mean abundance of nematodes, harpacticoid and nematode to copepod ratio (P = 0.00,0.00 (nematodes), 0.00,0.02 (harpacticoid), and 0.0.5, 0.04 (ratio) respectively for seasons and stations). The *posteriori* significant differences in mean nematode abundance was in April 2003, October 2002 and at stations 3, 6, 8, and 9 whereas for the rest of underline means no differences. Mean harpacticoid abundance was significantly different in August and December 2002 as well as in June 2003. Moreover, stations 3, 10, and 11 had significant different harpacticoid abundance. Although two ways ANOVA revealed significant variation in mean data of nematode to copepod ratio, the *posteriori* Tukey test could not reveal the dates or sites of variations.

Results from nematode to copepod ratio were classified into five groups according to their values and frequency. Fig. (4A) shows the classification of nematode to copepod ratio according to their values and frequencies. This figure revealed five groups representing the normal distribution of data. The two extremes of infinity ratio >1000 and undefined ratio had the lowest frequency (7& 6 data, respectively). The ratio's peak was at group 3 (ratio <100>10) with data frequency 26. The ratio at the other two groups were gathered from <1000>100 with 19 data (Group2) to <10>1 with 14 data (group4).

Fig. (4B) shows the comparisons between mean nematode to copepod ratio and means sedimentological variables at each ratio group (previous classification, Fig.3A) and log scale data was used. Results revealed that content of organic matter was less than 10 by an order of magnitude and the highest values were at groups 4 and 5. Sand fraction was slightly less than 100 with no observed differences among groups. Silt fraction fluctuated between 1 and 10 by an order of magnitude and the highest fraction was associated with infinity and 4 groups. Mean grain size values revealed dominance of fine and very fine grained sand and decreased from infinity group 1 to the undefined group 5. However, simple correlation (Pearson) and multiple regression analyses could not reveal significant or linear relationship between ratio and sedimentological variables. Therefore, those results were not being represented here.

Factor analysis visualized the relationships between nematode to copepod ratio and sedimentological variables (Fig. 5A). On PC1, positive loadings of ratio, nematode, mean grain size, and TOM% as well as silt fraction were revealed. On PC2, high positive loadings of ratio, nematodes, mean grain size and moderate positive loading of TOM% were detected but the loading of silt fraction was negative indicating that the relationship between ratio, nematodes and silt fraction differed in sign and magnitude. Harpacticoid copepod loaded positively with sand and negatively with TOM% as well as silt fraction on PC1 and PC2, indicating that harpacticoid abundance flourish in sandy sites with moderate or low organic content. Figs. 5B, C, D, and E revealed that relationship between ratio, among different groups, fluctuated between positive and negative loadings with mean grain size i.e., the relationship differed in sign and magnitude indicating the mean grain size is the limiting factor for nematode to copepod ratio.

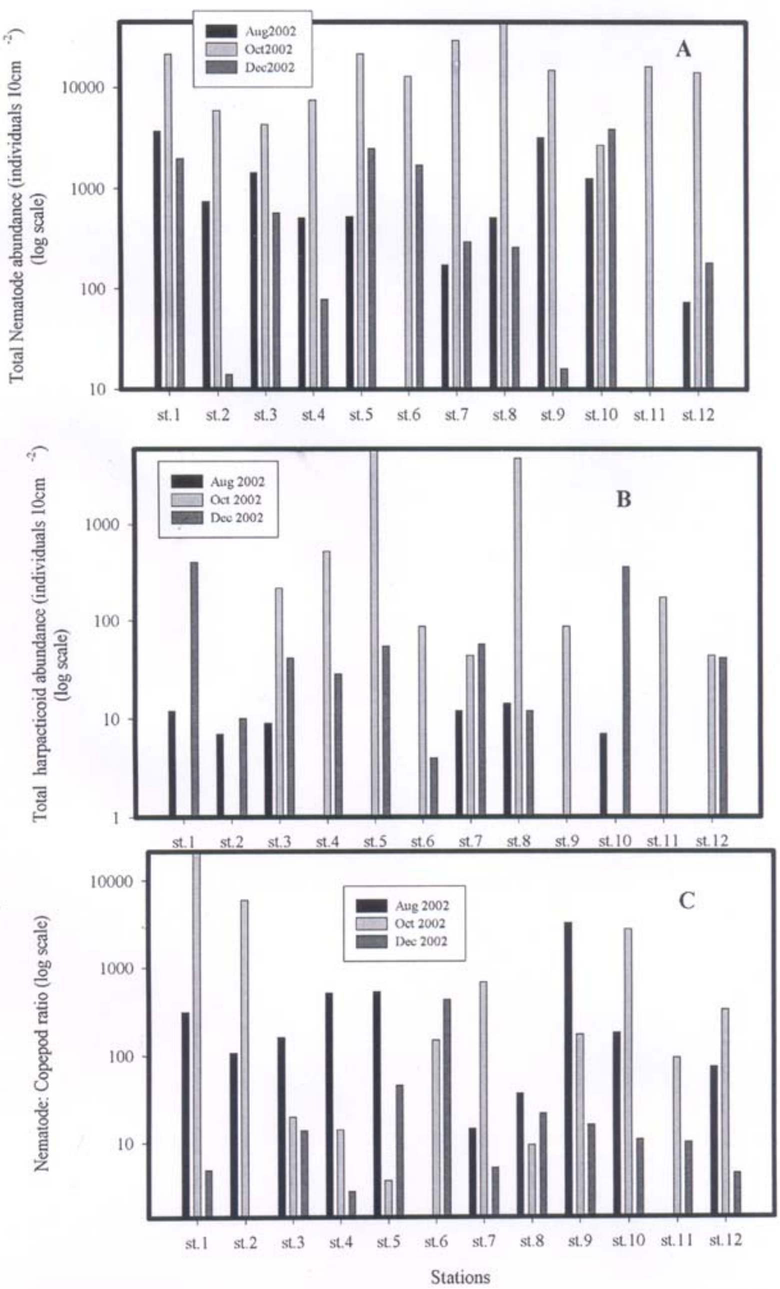

Fig.2: Distribution of log data of total nematode abundance and total harpacticoid abundance (individulas 10cm $^{-2}$) as well as nematode to copepod ratio at twelve stations during 2002. Abbreviations: Aug= August, Oct= October, Dec= December and st.= stations.

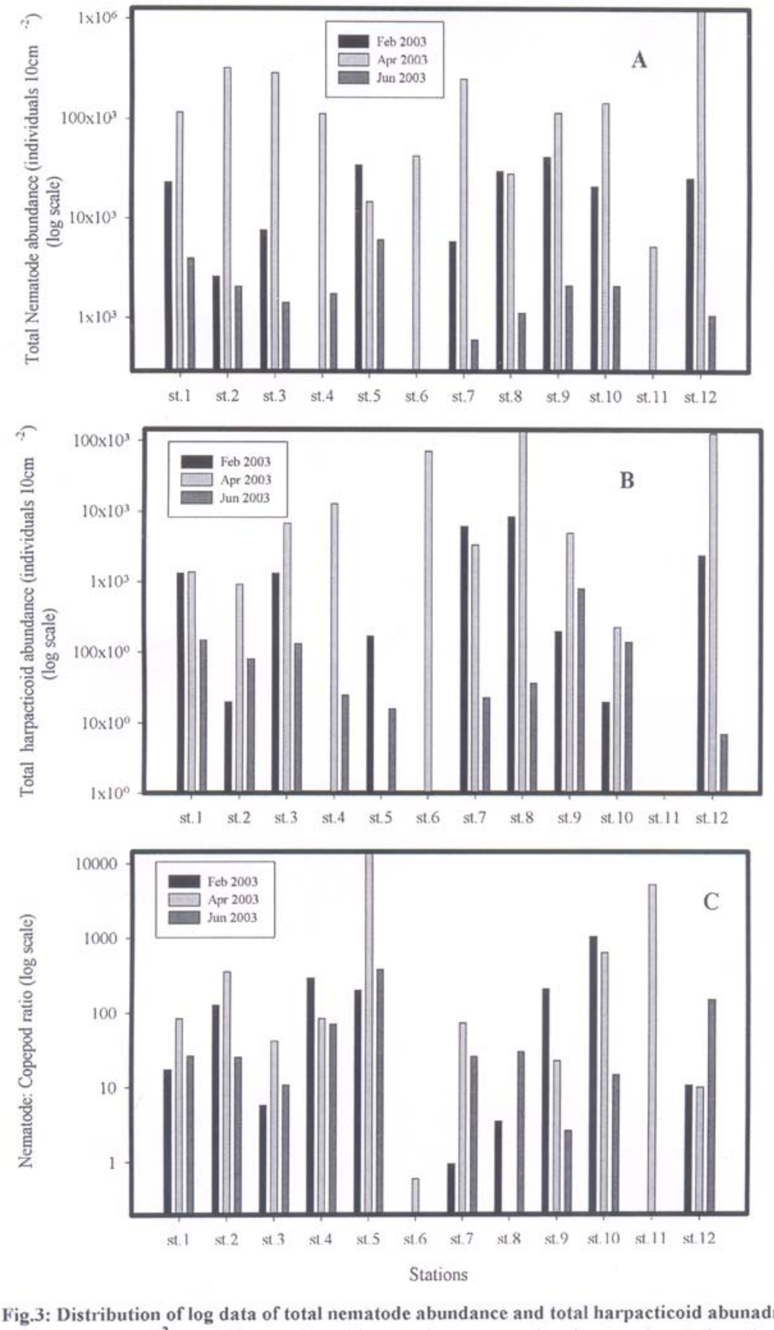

Fig.3: Distribution of log data of total nematode abundance and total harpacticoid abunadnce (individuals 10cm $^{-2}$) as well as log data of nematode to copepod ratio at twelve stations during 2003. abbreviations: Feb= February, Apr= April, Jun= June, and st= stations.

Table (1): Results from Two way ANOVA and its *posteriori* Tukey tests based on log transformed data (*n*+0.1) of total abundance of nematode, harpacticoid copepod (individuals 10/cm^2) and nematode: copepod ratio (N:C). Bold values indicate significant variation in measured variables over dates and among stations. The underline indicates no significant differences in mean data. Abbreviations: df= degree of freedom, F= F ratio, *P*= probability value at the significant α level=0.05, ns= not significant, Ap= April, Au= August, De= December, Fe= February, Ju= June, Oc= October, numbers from 1 up to12 = stations 1 up to station 12.

Factor	df	F	*P*	Tukey test											
Nematode															
Season	5	13.64	**0.00**	Ap003		Au002		De002		Fe003		Ju003		Oc002	
Station	11	5.22	**0.00**	4	5	7	8	6	9	3	10	11	12	1	2
Season*Station	55	5.64	1.00	ns											
Harpacticoid															
Season	5	7.45	**0.00**	Ap003		Fe003		Oc002		Au002		De002		Ju003	
Station	11	2.42	**0.02**	1	2	4	5	6	3	10	11	7	8	9	12
Season*Station	55	0.25	1.00												
N:C ratio				ns											
Season	5	2.45	0.05	Ap003		Au002		De002		Fe003		Ju003		Oc002	
Station	11	2.11	0.04	1	2	3	4	5	7	9	10	12	11	6	8
Season*Station	55	0.45	0.98	ns											

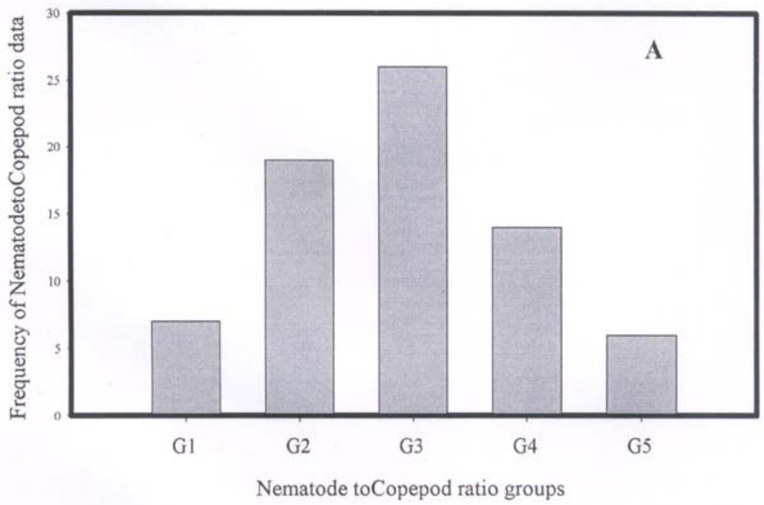

Fig.4(A): Hostogram frequency of nematode to copepod ratio data at each group (G).

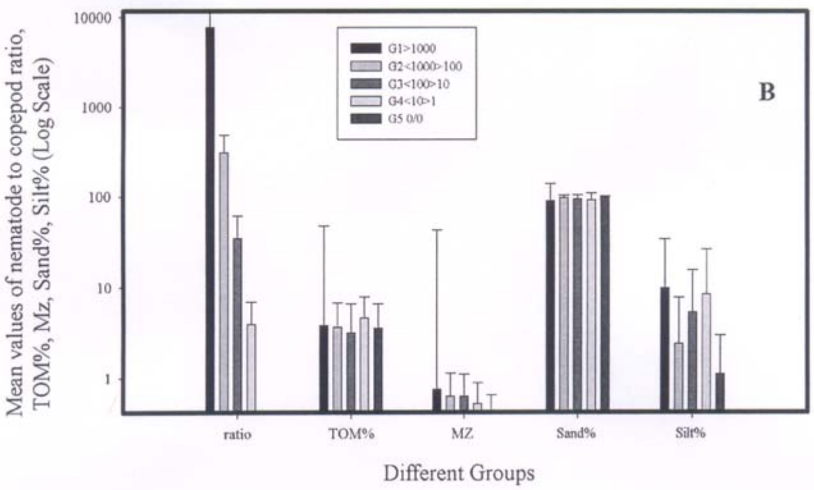

Fig. 4(B): Distribution of nematode to copepod ratio in relation to measured sedimentological variables at each group. Abbreviations: Mz= mean grain size and TOM%= total organic matter.

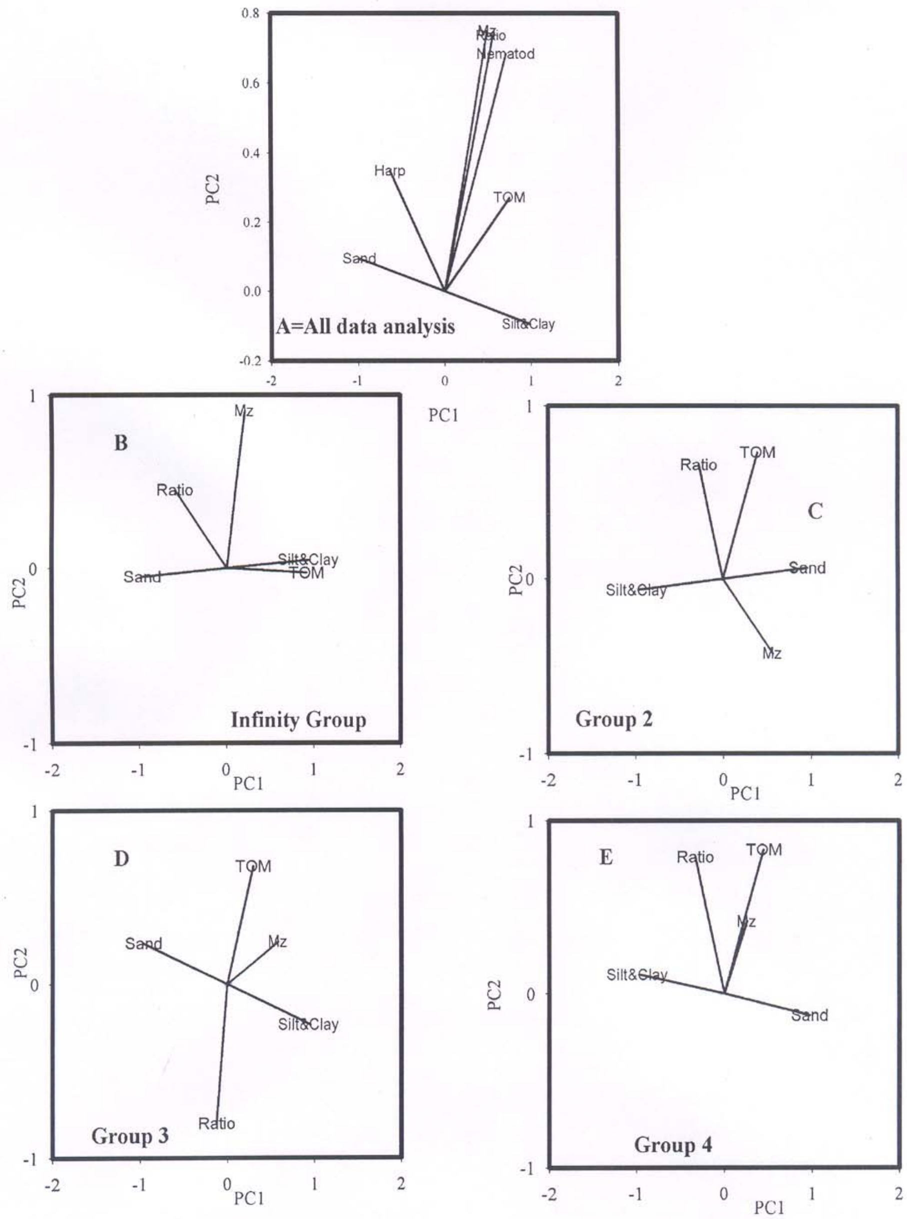

Fig.5: Factor analysis. 5A: All data analysis= the relationship between nematode to copepod ratio, total organic matter (%TOM), mean grain size (Mz), sand fraction (%) and silt-clay fraction (%) comined over dates and among stations. Figs 5B,C,D, E: the above relationships at different groups.

4. DISCUSSION

Higher abundances of nematodes and harpacticoid–copepod during 2003 than during 2002 indicated that the sediment situation at Eastern Harbor is in progress getting ride of highly organic load. Harpacticoid–copepod is very sensitive to abnormal conditions (high load of pollution, prevalence of silt sediment). Copepods were more common on coarse sand and rare on polluted beaches (Raffaelli, 1982).

The present results revealed that there were six stations were highly organically polluted with infinity ratio (>1000) whereas two sites were highly polluted with undefined ratio (0/0). Raffaelli and Mason (1981) proposed that ratios from clean beaches were always less than 100 even for muddy sites whilst sites with ratios exceeding 100 were polluted. Sites of infinity ratio were characterized by high nematode abundance and absence or near absence of harpacticoid copepod. Nematodes presumably utilizing large amounts of organic materials associated with organic pollution whereas harpacticoid appeared generally sensitive to environmental stress (Raffaelli and Mason, 1981 and McIntyre, 1977) or may be absent from very fine sediment with median grain size of 150µm or less (Lambshead, 1984). Sutherland *et al*. (2007) concluded that the category of infinity ratio is the only that suggests a sharp cut off for copepod tolerance to occur at this point.

The present results (Table 2 and Fig.4) summarize that sites of infinity ratio had the coarsest mean grain size (0.74 µm ± 0.41) whereas sites of undefined ratio characterized by the finest mean grain size (0.41µm ± 0.23). At the same time, total organic content was 0.25% higher at the former than the latter. These results indicated that sediment at infinity group afford a suitable environment to nematode to consume the organic matter whereas the sediment at undefined ratio groups was too small to nematode to survive. Jammo (2004) concluded that anoxic bed sediment phenomenon was recorded in the Eastern zone of the Eastern Harbor during summer and autumn only. He commented also that an apparent temporal movement of anoxic surface sediment with time towards the Harbor outside direction was noticed. The temporal movement of anoxic sediment bed with time probably was the reason for repetition of undefined ratio (0/0) over dates (Table 2).

There are two explanations for the undefined ratio. Firstly; the sites of undefined ratios (st.6 and 11) probably were under stress of other pollutants, such as oil, that unlike sewage do not lead to an immediate increase in food supply and may also be toxic and lead to sharp drop in all meiofaunal taxa. This explanation agreed with Raffaelli and Mason (1981) and Mitwally *et al*. (2007) conclusion. Secondly; the sediment of undefined ratio sites had high silt fraction with black organic rich mud that blocked the sediment interstices and allow anoxic condition to appear. Sutherland *et al*. (1998; 2007) found that sites of undefined ratio located directly in areas of bio deposit mud that was characterized by a high proportion of clay and silt and occur when deposition is greater than re-suspension and consolidation processes. Mitwally and Awads (2005) found that relationship between total organic carbon and meiofaunal abundance differed in sign and magnitude and this relationship become strongly positive whenever oxygen content in the sediment was high. Therefore, the second explanation of Sutherland *et al*. (1998 and 2007) and Mitwally and Awads (2005) confirmed that two sites 6 and 11, at the current results, suffered from anoxic condition rather than being stressed by a different kind of pollutants. Then the current results revealed that sites of undefined ratio are under the combined effect of organic and non organic pollution such as hydrocarbon oil or heavy metals.

However, the normal distribution of nematode to copepod frequency data (Fig. 4A) revealed that the frequency of two extreme ratios was 13 data out of 72 indicating that the Eastern Harbor, in general, is in good shape and in progress getting ride of high load of organic pollutants and those under stress 13 recorded data were not big issue to worry about. This conclusion confirmed the idea of self remediation that was suggested by Jammo (2004). Self remediation of the Harbor's environment would be accomplished with time if human interface maintained limited. Idea of self remediation was resulted from the apparent temporal movement of anoxic beds that was mentioned earlier. This idea was confirmed by results of N/C ratio.

5. CONCLUSIONS

Sediment at the Eastern Harbor is enhanced with time due to higher abundances of nematodes and copepods as well as low nematode to copepod ratio during season of 2003 than during 2002. Areas of infinity ratio characterized by absence of harpacticoid, higher nematode abundance, the coarsest grain size, in relative to other groups, allowing probably higher utilizing of high organic matter by nematodes. On the other hand, areas of undefined group had zero records of both nematodes and harpacticoids revealing the high silt fraction and high organic matter that probably caused anoxic conditions or probably was due to effect of other pollutants such as hydrocarbon oil or heavy metals. Normal distribution of nematode to copepod ratio frequency data revealed that the Eastern Harbor is in good shape and most sites were recovered from pollution. Sediment at the Eastern Harbor is tended to self remediation with time.

ACKNOWLEDGMENTS

The author deeply thanks Prof. Hassan B. Awads for providing her with samples and sedimentological data. This work is funded by a CSI/UNESCO Project.

Table (2). Dates and stations of two extreme groups of nematodes to copepod ratio.

Areas of infinity ratio >1000		Areas of undefined ratio 0/0	
August 2002	St.9	August 2002	St.6
October 2002	St.1	August 2002	St.11
October 2002	St.2	February 2003	St.6
October 2002	St.10	February 2003	St.11
February 2003	St.10	June 2003	St.6
April 2003	St.5	June2003	St.11
April 2003	St.11		

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