INSTM Bulletin
Marine and Freshwater Sciences

Vol. 49, 2024, 129–142

Research Paper

Use of meiofauna to assess the environmental status of Bizerte lagoon (Tunisia) with a focus on the taxonomic and functional diversity of free-living nematodes

Mohamed ALLOUCHE*[1,2], **Sirine JEBALI**[1], **Amel HANNACHI**[1], **Sahar ISHAK**[1], **Hamouda BEYREM**[1], **Fehmi BOUFAHJA**[3], **Mohamed DELLALI**[1]

[1] University of Carthage. Faculty of Sciences of Bizerte. LR01ES14 Laboratory of Environment Biomonitoring, Coastal Ecology and Ecotoxicology Unit, Zarzouna 7021, Tunisia.

[2] University of Jendouba. Higher Institute of Biotechnology of Beja. Biology Department, 9000, BP: 382, Tunisia

[3] Imam Mohammad Ibn Saud Islamic University (IMSIU). College of Science. Biology Department, Riyadh, 11623, Saudi Arabia.

Correspondence: mohamed.allouche@fsb.ucar.tn / allouchemom2@gmail.com

Received: 05/04/2024; **Accepted**: 19/07/2024; **Published**: 21/11/2024

Abstract: The Bizerte lagoon, Tunisia, undergoes notable environmental shifts caused by both natural factors and human activities, disrupting its natural dynamics. Meiofaunal bioindicators, focusing on the diversity and functional traits of free-living marine nematodes, were used in biomonitoring at 5 characteristic lagoon sites. The analysis of meiofaunal abundance and specific diversity of nematodes revealed that the "M. Ab" station exhibits the highest overall abundance of various meiobenthic taxa, while the "MG" and "M.B" stations show comparatively lower abundances.

The specific inventory of nematodes highlighted the dominance of certain species at specific stations, suggesting an adaptation to particular types of pollution, providing them with greater opportunities for multiplication in these environments. Furthermore, the observed functional variations underscore the complexity of ecological interactions within these ecosystems. They also attest to the adaptive capabilities of nematodes to environmental conditions characteristic of each station in the Bizerte Lagoon. This study offers important insights for environmental management and the preservation of biodiversity in these sensitive environments.

Keywords: Meiofauna; Nematodes; Meiofaunal abundance; Environmental pollution; Functional traits; Bizerte Lagoon; Tunisia.

---

1. Introduction

For several decades, pollution has been one of the most serious problems facing our modern world (Nivedita *et al.*, 2022).The pollution of marine environmentsworldwide is more than ever the main concern (Thushari & Senevirathna, 2020; Kvamsdal *et al.,* 2023). Significant contamination of hydrosystems by various chemical substances (heavy metals, persistent organic pollutants, etc.) can lead to acidification, eutrophication, degradation of biodiversity, and disruption of the equilibrium of these ecosystems.

Ecotoxicological risk assessment requires the development of tools for early detection, hence the importance of biomonitoring. Biomonitoring is based on the measurement of parameters reflecting the toxicity of contaminants and enabling the identification of compounds responsible for observed biological effects. The bioindicators are one of the tools most often used in biomonitoring. These are organisms that, through their presence or absence, abundance, or rarity, can be used to assess the degree of environmental pollution. Various studies already pointed out that these organisms respond to sudden changes in important combinations of factors (Steyaert *et al.,* 2007; Moreno *et al.*, 2008).

Environmental shifts in Tunisia's Bizerte lagoon result from the interplay of natural and human factors, impacting its ecosystems. Pollutants, depending on their contamination levels and bioavailability, can adversely affect various biological levels, from cells to individuals, populations, and communities. (Hannachi *et al.,* 2022).

Studies carried out over many years have highlighted the environmental risks and damage threatening the Bizerte lagoon, including hydrocarbons and heavy metal pollution due to the proximity of several industries such as the Bizerte cement plant and the Elfouleth steel plant. In this context, this study aims to evaluate and update the database of environmental conditions at various stations in the Bizerte lagoon, based on both biotic and abiotic data.

The bioindicators considered in this study were mainly marine free-living nematodes, whose importance in the functioning of benthic ecosystems and their relevance as bioindicators of environmental conditions have already been highlighted by numerous studies (Aïssa, 1991; Fichet & Miramand, 1996; Beyrem & Aïssa, 2000; Schratzberger *et al,* 2000; Guo *et al.,* 2001; Mahmoudi *et al.,* 2002; Mahmoudi *et al.,* 2003; Ferris & Bongers, 2006). Other meiofaunal bioindicators were also considered, such as copepods, polychaetes, oligochaetes and amphipods.

2. Materials and Methods

2.1. Sediment collection

Sediment samples were collected on July 5, 2023, from five stations representative of the various anthropogenic pressures. The Menzel Jemil station, noted, as "M.J" (37.221672N, 9.934730E), receives discharges from urban runoff and sewers. The Chaara station noted as "CH" (37.248546N, 9.856975E) and the Menzel Abderahmane station noted as "M.Ab" (37.232211N, 9.861177E) are influenced by the passage of commercial cargo ships through the Bizerte canal and cement plant activity. Maghrawa station noted as "MG" (37.184088N, 9.929782E) influenced by agricultural inputs. Finally, the Menzel Bourguiba station noted as "M.B" (37.140800N, 9.820312E) is influenced by water inflows from Ichkeul Lake and receives industrial effluents from the "El Fouledh" steel plant.

The sediment was sampled following the method reported by Mahmoudi et al., (2007) using several hand cores (surface of 10 cm^2, inner diameter of 3.6 cm) to collect the first 5 cm at 50 cm water depth, known to host 90% of the meiobenthos (Mahmoudi et al., 2007). The collected sediments were stored in hermetically sealed jars.

2.2. Meiobenthic descriptors

The levigation-decantation-sieving method, as described by Vitiello and Dinet (1979), was used to extract the meiofauna. The meiobenthos is defined as any organism that passes through a 1 mm mesh sieve and is retained by a 40 µm mesh sieve. The contents collected by the 40 µm sieve were then fixed in 4% formalin solution (Schratzberger *et al.,* 2004) and a few drops of Rose-Bengal were finally added (0.2 g·L^{-1}) (Elarbaoui *et al.,* 2015).

One hundred individuals were randomly picked from each station sample under a dissecting microscope, transferred in 21% glycerol, and mounted on microscope slides for taxonomic identification based on morphological features (Seinhorst *et al*., 1959). The identification of genera and species was based on the keys provided by Platt and Warwick (1983, 1988), Warwick *et al*. (1998), as well as the Nemys database available on the website developed and regularly updated by experts in meiobenthological nematology from Ghent University in Belgium Ghent (Bezarra *et al*. 2021).

The nematode's functional traits categories considered in this study were (1) the amphid shapes according to the Semprucci *et al*. (2018) classification: circular (Cr), spiral (Sp), pouch (Pk), and indistinct (Id) ; (2) the tails shapes according to Thistle *et al.,*(1996) the classification: conical (co), clavate/conico-cylindrical (cla), short/round (s/r), and elongated/filiform (e/f) ; (3) trophic groups according to Wieser (1953) classification: epigrowth feeders (2 A), selective deposit feeders (1 A), non-selective deposit feeders (1B), and omnivores/predators (2B); life history (c-p scale) according to Bongers et al., (1991, 1995) classification, from c-p = 1 (i.e. good colonizers: short life cycle, high reproduction rates, tolerant to various types of stress) to c-p = 5 (i.e. resilient: long life-cycles, few offspring, sensitive to stress).

2.3. Statistical data processing

Community metrics for nematofauna in each station were analyzed using PRIMER 5.0 software (Clarke, 1993; Clarke and Warwick, 2001). Abundance (N), taxonomic richness (S), Margalef's species richness (d), diversity (Shannon-Weaner index) (H'), and evenness (Pielou) (J') were calculated. Normality tests (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett test) were conducted, and $\log_{10}(x + 1)$ raw data transformation was applied (Clarke, 1993). One-way ANOVA, with post-hoc Tukey's HSD tests, was employed to analyze overall differences and multiple pairwise comparisons among treatments using STATISTICA (v5.1) software. Non-metric multidimensional scaling (nMDS) plots were generated from species and functional traits abundance data, employing square root transformation and Bray-Curtis similarity measures (Bray and Curtis, 1957). SIMPER analysis (Clarke, 1993) allowed the assessment of the contribution of each species and functional trait to the average dissimilarity between treatments.

3. Results

3.1. Meiobenthic abundance

The nematode counts revealed significant variations between stations. The "M.Ab" station stands out with a high abundance of 1980 ± 120.66 individuals, closely followed by "CH" with 1328 ± 119.62 individuals and "M.J" with 1007 ± 50.40 individuals. In contrast, "MG" and "M.B" exhibit lower numbers (675.66 ± 86.40 and 246 ± 118.60 individuals, respectively) (Figure 1).

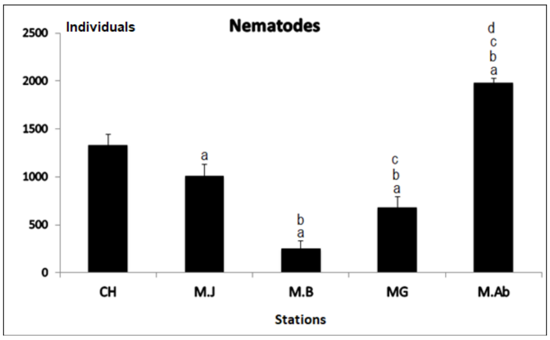

Figure 1. Comparative analysis of nematode abundance at different stations; CH: Charaa, M.Ab: Menzel Abdrahmene, MG: Maghrawa, M.B: Menzel Bourguiba M.J: Menzel Jemil.

The copepod counts reveal that the "MG" station has the highest copepod abundance (149 ± 9 ind). Statistical comparisons were significant between "MG" and all other stations, and between "M.B" and all other stations (Figure 2).

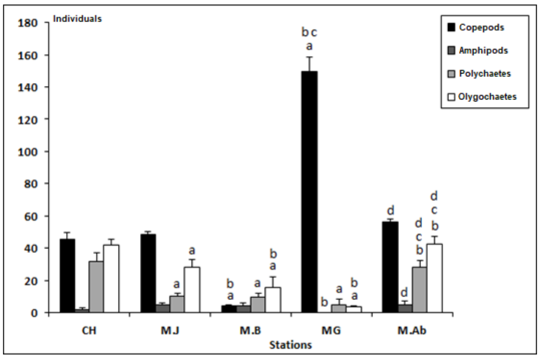

Figure 2. Comparative analysis of copepods, amphipods, polychaetes, and oligochaetes abundance at different stations (see Figure. 1 for the meaning of the abbreviations coding).

The amphipod counts showed that the stations "M.Ab" (5 ± 1 ind and "M.J" (5 ± 3 ind) exhibit the assemblages with the highest numbers. Statistical comparisons were significant only for “MG” vs. “M.J” and “MG” vs. “M.Ab” (Figure 2). Based on polychaete counts, it could be seen that the "CH" station (32 ± 5 ind), closely followed by the "M.Ab" station (28 ± 5 ind), exhibits the highest abundances. Statistical comparisons were significant between “M.Ab” and all the remaining stations except for the “CH” station (Figure 2).

The oligochaete counts revealed that "M.Ab" station has the most numerous copepod assemblage (43 ± 5 ind). It was closely followed by the “CH” station (42 ± 4 ind). Statistical analysis revealed discernible differences between all stations, except for the comparisons "CH" vs. "M. Ab" and "MG" vs. "M.B" (Figure 2).

3.2. Nematode diversity

The species numbers recorded at the various stations investigated were considerably close to one another. Furthermore, none of the statistical comparisons between the number of species, the Shannon index (H'), Margalef's species richness (d), and Pielou's equitability (J') of each station showed any significant difference (Figure 3).

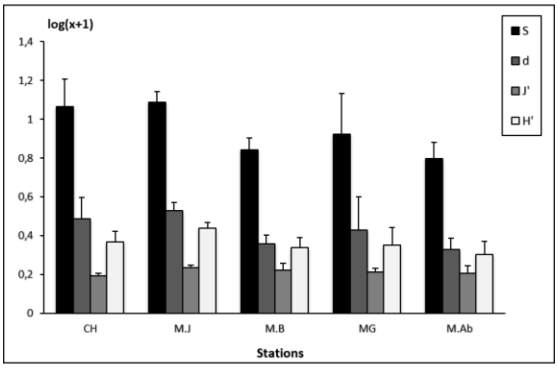

Figure 3. Comparative analysis of nematode diversity at different stations (see Figure. 1 for the meaning of the abbreviations coding).

3.3. Specific inventory

The nematodes collected from the sampled stations belong to 7 orders and 15 families. The latter were represented by 31 genera comprising 34 species. The most diverse families were Oncholaimidae (5 species), Xyalidae (5 species), and Cyatholaimidae (4 species) (Table 1).

The species *Marylinnia stekoveni* and *Oncholaimus campylocercoides* were present at all stations. The numbers of these cosmopolitan taxa fluctuated depending on the station. *M. stekoveni* dominated at the stations "M.Ab" and "M.B", accounting for 59.43 ± 12.45% and 52.74 ± 13.19% of nematode communities, respectively.

Conversely, *M. stekoveni* was very poorly represented at the "CH" and "MG" stations, accounting for only 1.35 ± 1.54% and 1.38 ± 2.4% of the population, respectively. The same results were also obtained for *O. campylocercoides*, which more or less dominated the "CH" station (39.9 ± 5.25%) and was poorly represented at the "MG" station (2.22 ± 2.27%).

Table 1. Relative abundances (%) of free-living nematode species and corresponding functional traits identified at 5 different stations in Bizerte lagoon, Tunisia. CH: Charaa, M.Ab: Menzel Abdrahmene, MG: Maghrawa, M.B: Menzel Bourguiba M.J: Menzel Jemil. Colonizer-Persistent (C-P) scores; tail shape: conical (co); elongated/filiform (e/f); clavate (cla); amphid shape: circular (Cr); poket-like(Pk); spiral (Sp); indistinct (Id); trophic groups : selective deposit-feeders (1 A); epistrate-feeders (2 A); non-selective deposit-feeders (1 B); omnivores-carnivores (2 B).

Genres & species	C-P	Tail	Amph	Troph	CH	M.J	M.B	MG	M.Ab
Ascolaimus sp.	2	co	cr	1B	0.33 ± 0.58	0	0,66±1,15	0	0
Odontophora villoti	2	co	REL	1B	0	0	3,84±3,77	0	0
Dorylaimopsis sp.	2	cla	sp	2A	1,36±2,36	5,57±1,17	0,83±1,44	0	0
Paracomesoma dubium	2	cla	sp	2A	1,21±1,07	47,06±8,80	0	0	0
Sabatieria splendens	2	cla	sp	1B	1,68 ±2,91	0	0	0	0
Chromadora nudicapitata	2	co	id	2A	0	0	0	2,88±3,23	0
Chromadorina sp.	2	co	id	2A	0	0	0	5,10±4,50	0
Prochromadorella longicaudata	2	co	id	2A	0,81±1,41	0	0,83±1,44	0	8,11±7,17
Longicyatholaimus longicaudatus	3	e/f	sp	2A	0	1,24±1,23	2,29±2,52	0	0
Cyatholaimus prinzi	3	co	sp	2A	2,04±2,04	0,88±0,76	0	0	0
Marylynnia stekoveni	3	e/f	sp	2A	1,35±1,54	13,24±4,22	52,74±13,19	1,38±2,40	59,43±12,45
Marylynnia sp.	3	e/f	sp	2A	0	2,17±1,93	7,58±11,44	0	2±3,46
Synonchiella edax	3	cla	sp	2B	0,33±0,58	3,06±1,57	0	1,46±1,27	0
Spirinia parasitifera	2	cla	sp	1B	0,27±0,47	0	0	5 ±3,12	1,32±1,14
Calamicrolaimus honestus	3	co	sp	2A	0	0	0	0	6,39±2,33
Anticoma acuminata	2	e/f	pk	1A	0,94±1,01	0	0	0	0
Anticoma eberthi	2	e/f	pk	1A	0,33 ±0,58	0	0	0	0
Metoncholaimus pristiurus	3	cla	pk	2B	44,20±3,77	0,89±0,77	2,55±2,87	0	0
Oncholaimus campylocercoïdes	4	cla	pk	2B	39,90±5,25	5,17±2,11	27,97±1,83	2,22±2,27	5,32±2,91
Oncholemellus calvadocicus	4	cla	pk	2B	0	0,41±0,71	0	0	0
Pontonema subtilis	4	cla	pk	2B	0	0	0		
Viscosia cobbi	3	e/f	pk	2B	1,42±1,67	0	0	0	0
Enoploides sp.	5	cla	Id	2B	0	0	0	1,41±2,45	0
Mesacanthion sp.	5	cla	Id	2B	0	0	0	0	0
Metalinhomeus numidicus	2	e/f	cr	1B	0,27±0,47	3,93±3,41	0	0,70±1,22	0,65±1,13
Terschellingia longicaudata	3	e/f	cr	1A	0	12,94±4,21	0,66±1,15	0	0
Terschellingia sp.	3	e/f	cr	1A	0	1,31±1,26	0	0	0
Daptonema trabeculosum	2	cla	cr	1B	1,62±0,69	1,67±1,93	0	2,17±2,12	0
Paramonohystera pilosa	2	cla	cr	1B	0,27±0,47	0	0	0	0
Steineria sp.	2	cla	cr	1B	0	0,41±0,71	0	0	0
Stylotheristus sp.	3	e/f	cr	1A	0,33±0,58	0	0	0	0
Theristus flevensis	2	co	cr	1B	0,61 ±0,54	0	0	63,34±12,70	0
Leptolaimus sp.	1	cla	cr	1B	0	0	0	0,70±1,22	0
Diodontolaimus sp.	2	co	REL	1B	0,95±0,11	0	0	0	0
Rhabditis sp.	1	co	Id	1B	0	0	0	0,70±1,22	0

The specific inventory revealed that other opportunistic species were only able to survive to a limited extent. *Theristus flevensis*, for example, was highly represented at the "MG" station (63.34 ± 12.7%), whereas it was absent from most of the other stations. The same status may be applied to *Paracomesoma dubium*, which was absent from most stations but dominates at "M.J" (47.06 ± 8.8%).

The specific inventory also revealed that certain species are characteristic of certain stations. For example, *Vicosia cobbi, Anticoma accuminata,* and *Anticoma eberthi* were sampled only at the "CH" station, with very low numbers: only 1.42 ± 1.167%, 0.94 ± 1.01% and 0.33 ± 0.58% of the inventory, respectively. Similarly, *Calomicrolaimus honestus* was found only at the "M.Ab" station (6.39 ± 2.33%), and the species *Chromadora nudicaputatus*, *Chromadorina* sp. *Enoploides* sp. *Leptolaimus* sp. *Pontonema subtilis* and *Rhabditis* sp. were sampled only at the "MG" station.

3.4. Multivariate analysis

The results of MDS ordination of the nematode replicas showed significant differences in the specific the composition of the different communities (stress = 0.1), especially in the "MG" station. Replicates for this nematofauna were placed further away from all the others.

It's also worth noting that "CH" is somewhat out of step with the closer group formed by the replicates relating to stations "M. J", "M. B" and "M.Ab". (Figure 4).

Figure 4. Non-metric multidimensional scaling (nMDS) 2D plot based on square-root transformed nematode species abundances at different stations in Bizerte lagoon, Tunisia

The dissimilarity values revealed significant differences in the specific composition of the considered stations. The highest dissimilarities were found between station "MG" and the rest of the stations (> 94%), with a maximum dissimilarity of 96.43% noticed between "MG" and "M.B" (Table 2).

The SIMPER outcomes showed that the presence of *T. flevensis* highly contributed to the taxonomic difference between "MG" and "M.J", "M.B" and "M.Ab". On the other hand, the absence of *Metoncholaimus pristiurus* from "MG" contributed most to dissimilarity with "CH". Other species also contributed to the dissimilarity of the "MG" station with the rest of the stations, hence, *M. stekovni* contributed to dissimilarity with "M.Ab" and "M.B", while *P. dubium* contributed to dissimilarity with "M.J".

Table 2. Dissimilarity percentages (bold values) between “MG” and the other stations and results of Similarity Percentage analysis (SIMPER) based on square-root transformed data.(see Table. 1 for the meaning of the abbreviations coding).

	MG vs. CH (95,64 %)	**MG vs. M.J (94,95%)**
Species	*Metoncholaimus pristiurus* (32.13 %) *Oncholaimus campylocercoides* (28,22 %) -	*Paracomesoma dubium* (30,85 %) *Theristus flevensis* (25,08 %) *Terschellingia longicaudata* (8,51 %)
Trophic groups	2B	1B
Tail shapes	co	Co
Amphid shapes	Pk	Sp
Life histories	cp2	cp3
	MG vs. M.B (96,43%)	**MG vs. M.Ab (94,92%)**
Species	*Theristus flevensis* (32,47%) *Marylinnia stekoveni* (26,66 %)	*Theristus flevensis* (34,17 %) *Marylinnia stekoveni* (33,38 %)
Trophic groups	1B	2A
Tail shapes	Co	e/f
Amphid shapes	Cr	Sp
Life histories	c-p2	c-p3

3.5. Functional trait analysis

The analysis of the 4 functional traits considered in this study, namely, trophic groups, amphid and tail shapes, and life history showed fluctuations depending on the considered station.

3.5.1 Trophic groups

The stations "M.J", "M.B", and "M.Ab" showed the predominance of the epistrate herbivores (2A), constituting more than 65% of the nematode composition. In contrast, "CH" was dominated by the omnivore-carnivores (2B), while "M.B" had a prevalence of non-selective deposit feeders (1B). MDS ordination confirmed distinct nematode assemblages, with "M.J", "M.B", and "M.Ab" grouped separately from "CH" and "MG", indicating clear dissimilarities between replicates (figure 5).

3.5.2 Amphid shapes

The stations "M.J", "M.B", and "M.Ab" exhibit a predominant spiral amphid shape (sp) exceeding 60%. In contrast, "CH" is characterized by a prevalence of the pocket amphid shape (pk), while "MG" showed the dominance of the circular amphid shape (cr). MDS ordination analysis confirmed distinct amphid characteristics, grouping replicates from "M.J", "M.B", and "M.Ab" separately from "CH" and "MG", indicating clear disparities between replicates (figure 5).

3.5.3 Tail shapes

The "CH" and "M.J" stations exhibit a dominant clavate/conico-cylindrical (cla) tail shape, exceeding 60%. Conversely, the "M.B" and "M.Ab" stations are characterized by an absolute dominance (more than 60%) of the elongated/filiform tail shape (e/f). The station "MG" is dominated (over 60%) by nematodes with conical tails (co). The results given in nMDS ordination confirm distinctions in tail shapes among stations, notably placing "MG" replicates apart from the others (figure 5).

3.5.4 Life cycles

The "M.J" and "MG" stations are dominated (over 60%) of species with short life cycles (c-p2). Conversely, the "M.B" and "M.Ab" stations are mostly populated by species with longer life cycles (c-p3). The station "CH" has particularly the highest proportion of long-lived species (c-p3, ~50% of the community; c-p4, ~40%). The nMDS ordination analysis further confirmed the dissimilarity in terms of species life cycles among stations, grouping "MG" and "M.J" replicas separately from "CH", "M.B", and "M.Ab" replic (figure 5).

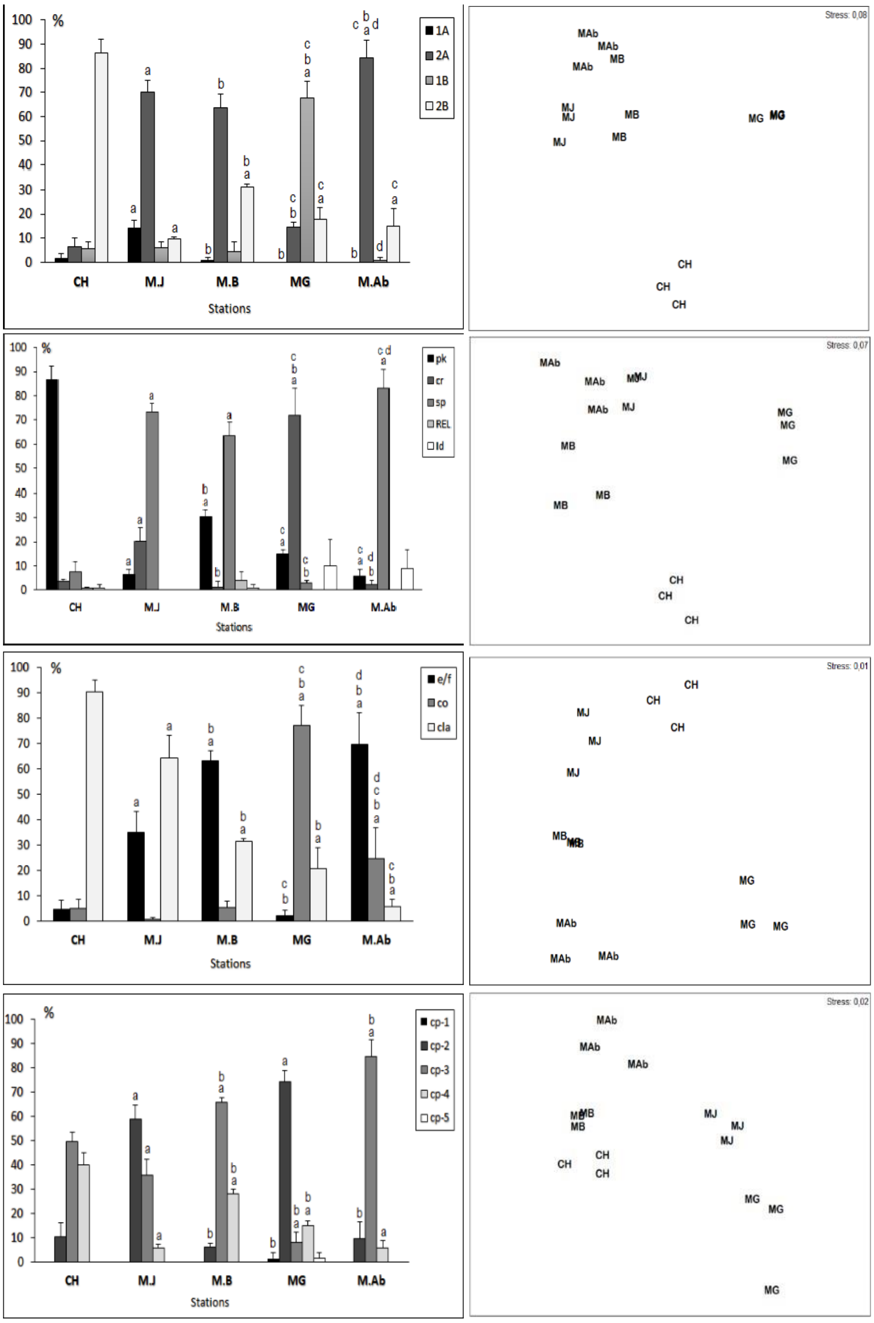

Figure 5. Non-metric multidimensional scaling (nMDS) 2D plots (right) and graphical summary (left) based on abundances of functional groups of nematode assemblages at different stations

4. Discussion

The main objective of this work was to highlight the influence of environmental variations on the meiobenthic community distribution across five stations in Bizerte lagoon: Chaara (CH), Menzel Jemil (M.J), Maghrawa (MG), Menzel Bourguiba (M.B) and Menzel Abdrahmene (M.Ab). Our findings were based on the abundance of the overall meiofauna and the specific diversity and functional traits of free-living marine nematodes to determine the quality of the environment and the magnitude of anthropogenic impact on it.

The results showed that the "M.Ab" station had the highest overall abundance of the various meiobenthic taxa, as well as a fairly significant nematode diversity. These results suggest there are more favorable and probably more stable environmental conditions at that location than at the other stations. In contrast, the "MG" and "M.B" stations showed lower abundances, coupled with relatively high variability. The meiofaunal abundance pattern also suggests that nematodes prefer less stressful environments, while copepods are more abundant under conditions of moderated stress intensity, as observed at the "MG" station. Finally, amphipods and polychaetes showed higher abundances at less stressed stations, such as "M.Ab" and "M.J".

Shannon's diversity index (H') revealed a distinct trend, with the "M. J" station showing the highest diversity (H' = 1.74 bits), followed by "CH" (H' = 1.33 bits), "M.G" (H' = 1.27 bits), "M.B" (H' = 1.19 bits) and "M.Ab" (H' = 1.09 bits). These results highlighted the close relationship between nematode diversity and environmental stress intensities, with the "M.J" station displaying the highest diversity.

The specific nematode inventory associates the predominance of certain species in specific stations. Thus, species such as *T. flevensis*, dominant in the "MG" station, or *P. dubium*, dominant in the "M.J" station, were characteristic of the station. Previous work has shown that these 2 taxa were tolerant of global pollution of harbor areas (Ridal & Ingels 2021). These results therefore suggest a certain correlation between the environmental conditions of the considered stations and the species' sensitivity to stress. The inventory also revealed more cosmopolitan species such as *M. stekoveni* and *O. campylocercoides*, which dominate in the more stable stations. Previous studies have shown that, over a long period, high granulometry causes a proliferation of the trophic group 2A (genus: *Marylynnia*) due to increased bacterial activity (Semprucci et al., 2013; Boufahja et al., 2016). Similarly, it has already been shown that *O. campylocercoides* can tolerate hydrocarbons (Elarbaoui *et al.,* 2015; Soto et al., 2017; Allouche *et al.,* 2020) and heavy metals (Mahmoudi *et al.,* 2005; Hermi *et al.,* 2009; Rosner *et al.,* 2023) and is probably adapted to harbor pollution. On the other hand, some species that were less competitive but more tolerant to pollution, such as T. flevensis and *P. dubium*, were able to survive in polluted sites.

The dissimilarity values clearly defined the "MG" station as the one with the most distinguished nematode community from the other stations surveyed (dissimilarity higher than 94%), mainly due to the presence of *T. flevensis* inventoried only at this station. Although this station is considered to be close to all sources of industrial pollution, it is nonetheless influenced by several lotic water flows, notably the Oued Gueniche, which carries pesticides from nearby agricultural activities. The results also suggest a dissimilarity between the "CH" station and the rest of the stations, due to the influence of the cement plant and its location at the mouth of the canal linking Bizerte lagoon to the Mediterranean Sea. The nMDS analysis confirms this dissimilarity, placing the "MG" replicates far away, and the "CH" replicas slightly distinguished from the other replicates.

Functional variations underline the complexity of ecological interactions within these ecosystems. The nMDS analysis confirms these differences, highlighting a clear separation between stations. The study conducted by Ferris and Bongers (2006) has shown that nematode trophic groups varied considerably depending on the environmental quality. Thus, some of the collection stations were dominated by epistrate herbivores (2A), while others were dominated by omnivores-carnivores (2B) or non-selective deposit feeders (1B). These feeding differences reflect without doubt the quantity and quality of food resources available in each location.

The "M.J" and "MG" stations are characterized by a predominance of species with short generation times, reflecting probably an unstable environment, while "M.B" and "M.Ab" sheltered longer life spans, reflecting relatively a more stable environment. Darby and Neher (2007) highlighted the importance of nematode life histories as bioindicative tools that mirror of environmental conditions. However, these findings prompt inquiries (1) the precarious stability of the "M.J" station, despite its apparent taxonomic diversity, renders it inhospitable to species incapable of quickly adapting to rapid surrounding environmental changes; (2) the surprising stability of the "M.B" station, despite it is exposed to heavy metals released by the "El Fouleth" steel plant, suggests the presence of a nematode community adapted to this type of contamination.

The amphid is the nematode's main chemosensory organ, and some amphid forms are known to be linked to a higher sensitivity to pollution than others. The results obtained for amphids revealed that the "M.B" station is more polluted, as it has the lowest percentage of species with the circular amphid (cr) shape, known to be related to over-pollution (Wakkaf *et al.,* 2020)

Nematode tail shapes reflect mobility and the distances they cover through locomotion. Diversity in tail shapes within the community signifies adaptations in response to sediment quality. The results showed that the clavate (cla) and conical (co) shapes were mainly dominant at the "CH" and "MG" stations, reflecting the need of these communities to explore their environments. Hedfi et al. (2023) indicate that the clavate (cla) and conical (co) tail types were probably related to better jumping efficiency.

5. Conclusion

To sum up, this study has enabled us to reach a better understanding level of the effects of environmental stress on meiobenthic nematodes. Ultimately, the investigation of the various nematode assemblages revealed that the dissimilarities observed were mainly due to the existence of possible functional interactions between worms and environmental conditions.

With more details, the results obtained indicated notable distinctions among the stations, where the "M.Ab" station stood out for more favorable environmental conditions, while 'MG' and 'M.B' were more under stress based on their lower abundances and high quantitative and qualitative variability.

The specific inventory associated with the predominance of certain species at particular stations, suggests a correlation between environmental conditions and the degree of sensitivity of these species. The dissimilarity values confirmed significant differences between stations, highlighting the influence of various factors such as agricultural pollutants at “MG” or the impact of the cement plant at “CH”.

The functional changes made clear the complexity of the ecological interactions within Bizerte lagoon, Tunisia. The life history of nematodes has particularly shown specific adaptations to the ambient conditions.

Acknowledgments:

This work was supported by a funding of the University of Carthage, Faculty of Science of Bizerte.

References

1. Aïssa, P. (1991). Ecologie des nématodes libres de la lagune de Bizerte. Dynamique et biocénotique [Thèse doctorat d'état, Univ. Tunis II Fac Sci Tunis]. 370p.
2. Allouche, M., Nasri, A., Harrath, A.H., Mansour, L., Beyrem, H., & Boufahja, F. (2020). Migratory behavior of free-living marine nematodes surrounded by sediments experimentally contaminated by mixtures of polycyclic aromatic hydrocarbons. *Journal of King Saud University-Science*, 32(2), 1339-1345. https://doi.org/10.1016/j.jksus.2019.11.025
3. Beyrem, H., & Aïssa, P. (2000). Les nématodes libres, organismes sentinelles de l’évolution des concentrations d’hydrocarbures dans la baie de Bizerte (Tunisie). *Cahiers de Biologie Marine*, 41(3), 329-342. https://doi.org/10.21411/CBM.A.E84EC367
4. Bezerra, T.N., Decraemer, W., Eisendle-Flockner, U., Hodda, M., Holovachov, O., Leduc, D., Miljutin, D., Mokievsky, V., Santiago, P.R., & Sharma, J. (2020). Nemys: World Database of Nematodes. Available online: http://nemys.ugent.be
5. Boufahja, F., Semprucci, F., & Beyrem, H. (2016). An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. *Ecological Indicators*, 60, 292-309. https://doi.org/10.1016/j.ecolind.2015.07.002
6. Bray, J.R., & Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological monographs*, 27(4), 326-349. https://doi.org/10.2307/1942268
7. Clarke, K.R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology-Progress Series*, 92, 205-219. https://doi.org/10.3354/meps092205
8. Clarke, K.R., & Warwick, R.M. (2001). Change in marine communities. An approach to statistical analysis and interpretation, 2, 1-168. 2nd Edition, Primer-E, Ltd., Plymouth Marine Laboratory, UK.
9. Darby, B.J., Neher, D.A., & Belnap, J. (2007). Soil nematode communities are ecologically more mature beneath late-than early successional stage biological soil crusts. *Applied Soil Ecology*, 35(1), 203-212. https://doi.org/10.1016/j.apsoil.2006.04.006
10. Elarbaoui, S., Richard, M., Boufahja, F., Mahmoudi, E., & Thomas-Guyonc, H. (2015). Effect of crude oil exposure and dispersant application on meiofauna: An intertidal mesocosm experiment. *Environmental science. Processes & impacts*, 17(5), 997-1004. https://doi.org/10.1039/c5em00051c
11. Ferris, H., & Bongers, T. (2006). Nematode indicators of organic enrichment. *Journal of Nematology*, 38(1), 3-12.
12. Fichet, D., & Miramand, P. (1996). Métaux lourds et méiofaune benthique: indicateurs de pollution des sédiments portuaires (premiers résultats). Actes du 11ème Colloque de l’Union des

Océanographes de France. J. Rech. Océanogr, 21, 82.

13. Guo, Y., Somerfield, P.J., Warwick, R.M., & Zhang, Z. (2001). Large-scale patterns in the community structure and biodiversity of free-living nematodes in the Bohai Sea, China. *Journal of the Marine Biological Association of the UK*. 81(5), 755-763. https://doi.org/10.1017/S0025315401004568
14. Hannachi, A., Nasri, A., Allouche, M., Aydi, A., Mezni, A., D'Agostino, F., Avellone, G., Gambi, C., & Beyrem, H. (2022). Diuron environmental levels effects on marine nematodes: Assessment of ecological indices, taxonomic diversity, and functional traits. *Chemosphere*, 287(part 3), 132262.https://doi.org/10.1016/j.chemosphere.2021.132262
15. Hedfi, A., Ishak, S., Al Hebs, A., Al-Hoshani, N., Ben Ali, M., Rudayni, H. A., & Boufahja, F. (2023). Separate and Simultaneous Effects of Silver Nanoparticles and Arsenic on the Taxonomic Diversity of Free-Living Marine Nematodes. *Diversity*, 15(7), 836. https://doi.org/10.3390/d15070836
16. Hermi, M., Mahmoudi, E., Beyrem, H., Aïssa, P., & Essid, N. (2009). Responses of a free-living marine nematode community to mercury contamination: results from microcosm experiments. *Archives of Environmental Contamination and Toxicology*, 56, 426-433. https://doi.org/10.1007/s00244-008-9217-3
17. Kvamsdal, S., Hopland, A.O., Li, Y., & Selle, S. (2023). Expert opinions on threats and impacts in the marine environment, *Marine Policy*, 147, 105382.https://doi.org/10.1016/j.marpol.2022.105382
18. Mahmoudi, E., Baccar, L., Beyrem, H., & Aïssa, P. (2002). Response of free living nematodes to the quality of waters and sediments at Bou Ghrara lagoon (Tunisia) during winter 2000. *Mediterranean Marine Science*, 3(2), 133-146. https://doi.org/10.12681/mms.252
19. Mahmoudi, E., Beyrem, H., & Aïssa, P. (2003). Les peuplements de nématodes libres, indicateurs du degré d'anthropisation des milieux lagunaires: Cas de la lagune de Bou Ghrara (Tunisie). *Vie et Milieu*. 53, 47-59.
20. Mahmoudi, E., Essid, N., Beyrem, H., Hedfi, A., Boufahja, F., Vitiello, P., & Aissa, P. (2005). Effects of hydrocarbon contamination on a free-living marine nematode community: results from microcosm experiments. *Marine Pollution Bulletin*, 50(11), 1197-1204. https://doi.org/10.1016/j.marpolbul.2005.04.018
21. Mahmoudi, E., Essid, E., Beyrem, H., Hedfi, A., Boufahja, F., Vitiello, P., & Aïssa, P. (2007). Individual and combined effects of lead and zinc on a free-living marine nematode community: Results from microcosm experiments. *Journal of Experimental Marine Biology and Ecology*, 343 (2), 217-226. https://doi.org/10.1016/j.jembe.2006.12.017
22. Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., & Fabiano, M. (2008). An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbor through the analysis of meiofauna and nematode assemblages. *Estuarine, Coastal and Shelf Science*, 77(4), 565-576. https://doi.org/10.1016/j.ecss.2007.10.016
23. Niveditha, S. K., Haridevi, C. K., Hardikar, R., & Ram, A. (2022). Phytoplankton assemblage and chlorophyll a along the salinity gradient in a hypoxic eutrophic tropical estuary-

Ulhas Estuary, West Coast of India. *Marine Pollution Bulletin*, 180, 113719. https://doi.org/10.1016/j.marpolbul.2022.113719

24. Platt, H.M., & Warwick, R.M. (1983). Free-living marine nematodes. Part I. British Enoploids. Cambridge University, London.
25. Platt, H.M., & Warwick, R.M. (1988). Free-living marine nematodes. Part II. British Chromadorids. Synopsis of the British fauna (New Series). No. 38, E.J. Brill/W. Backhuys, Leiden.
26. Ridall, A., & Ingels, J. (2021). Suitability of free-living marine nematodes as bioindicators: status and future considerations. *Frontiers in Marine Science*, 8, 685327. https://doi.org/10.3389/fmars.2021.685327
27. Rosner, A., Ballarin, L., Barnay-Verdier, S., Borisenko, I., Drago, L., Drobne, D., & Cambier, S. (2023). A broad-taxa approach as an important concept in ecotoxicological studies and pollution monitoring. *Biological Reviews*, 99(1), 131-176. https://doi.org/10.1111/brv.13015
28. Schratzberger, M., Rees, H.L., & Boyd, SE. (2000). Effects of simulated deposition of dredged material on the structure of nematode assemblages - the role of burial. *Marine Biology*, 136, 519-530. https://doi.org/10.1007/s002270050712
29. Schratzberger, M., Whomersley, P., Warr, K., Bolam, S.G., Rees, & H.L. (2004). Colonisation of various types of sediment by estuarine nematodes via lateral infaunal migration: A laboratory study. *Marine Biology*, 145, 69-78. https://doi.org/10.1007/s00227-004-1302-1
30. Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4(1), 67-69. https://doi.org/10.1163/187529259X00381
31. Semprucci, F., Moreno, M., Sbrocca, S., Rocchi, M., Albertelli, G., & Balsamo, M. (2013). The nematode assemblage as a tool for the assessment of marine ecological quality status: a case-study in the Central Adriatic Sea. *Mediterranean Marine Science*, 14(1), 48-57. https://doi.org/10.12681/mms.366
32. Semprucci, F., Balsamo., M, Appolloni, L., & Sandulli, R. (2018). Assessment of ecological quality status along the Apulian coasts (Eastern Mediterranean Sea) based on meiobenthic and nematode assemblages. *Marine Biodiversity*, 48, 105-115. https://doi.org/10.1007/s12526-017-0745-9
33. Soto, L.A., Salcedo, D.L., Arvizu, K., & Botello, A.V. (2017). Interannual patterns of the large free-living nematode assemblages in the Mexican Exclusive Economic Zone, NW Gulf of Mexico after the Deepwater Horizon oil spill. *Ecological Indicators*, 79, 371-381. https://doi.org/10.1016/j.ecolind.2017.03.058
34. Steyaert, M., Moodley, L., Nadong, T., Moens, T., Soetaert, K., & Vincx, M., (2007). Responses of intertidal nematodes to short-term anoxic events. *Journal of Experimental Marine Biology and Ecology*, 345(2), 175-184. https://doi.org/10.1016/j.jembe.2007.03.001
35. Thistle, D., Lambshead, P.J.D., & Sherman, K.M. (1995). Nematode tail-shape groups respond to environmental differences in the deep-sea. *Vie et Milieu*, 45, 107-115.
36. Thushari, G.G.N., & Senevirathna, J.D.M. (2020). Plastic pollution in the marine environment. *Heliyon*, 6(8), e04709. https://doi.org/10.1016/j.heliyon.2020.e04709

37. Vitiello, P., & Dinet, A. (1979). Définition et échantillonnage du méiobenthos. *Rapports et procès-verbaux des réunions Commission internationale pour l'exploration scientifique de la Mer Méditerranée*, 25, 279-283.
38. Wakkaf, T., Allouche, M., Harrath, A. H., Mansour, L., Alwasel, S., Ansari, K. G. M. T., & Boufahja, F. (2020). The individual and combined effects of cadmium, polyvinyl chloride (PVC) microplastics and their polyalkylamines modified forms on meiobenthic features in a microcosm. *Environmental Pollution*, 266, 115263. https://doi.org/10.1016/j.envpol.2020.115263
39. Warwick, R.M., Platt, H.M., & Somerfield, P.J. (1998). Free-living marine nematodes: Part III. Monohysterids. Synopsis of British fauna, No. 53.
40. Wieser, W. (1953). Sexual dimorphism of the Enchelidiidae (free-living marine nematodes) as a taxonomic problem. *Zoologischer Anzeiger*, 150(7/8), 152-170.