Chapter

# New Approach for the Evaluation of Ecological Quality in the Mediterranean Coastal Ecosystems, Case Study of Bizerte Lagoon: Marine Nematodes Functional Traits Assessment

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## Abstract

Marine ecosystems have great economic and ecological value, as they provide good services and habitats for a variety of organisms. However, the marine environment is under anthropogenic stressors. The Mediterranean basin is one of the most threatened ecosystems, where urban and industrial waste is becoming a growing risk for coastal marine habitats integrity. The Bizerte lagoon represents a major coastal lagoon and is an example of such an aquatic environment continuously exposed to pollutants. Marine nematodes are the most diverse metazoans and represent an excellent model for the environmental monitoring because they can be easily sampled and maintained under experimental conditions. Nematode communities are investigated for the analysis of taxonomic diversity and ecological indices. Currently, we present here to evaluate the ecological quality based on the description of nematode assemblages using biological traits and functional groups. This relatively new approach allows obtaining insight into the status of marine coastal ecosystems.

**Keywords:** Mediterranean basin, Nematodes, Ecological quality, Chemicals pollutants, Ecological indices, Biological traits

## 1. Introduction

Aquatic environments and in particular coastal ecosystems are exposed to a variety of contaminants derived from human activities. The majority of pollutants is removed from the water column and accumulated in marine sediment. Among these chemical compounds, we can list pesticides commonly used worldwide as pest control agents in agriculture [1], persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins realized from industrial sources and maritime traffic [2], heavy metals originating from discharges from the metallurgical industry [3], for human health applications [4], and microplastic particles by the direct release of textile fibers or by cleaning products [5, 6]. All these compounds can enter marine ecosystems through a variety of routes, including urban (parking lots and residential areas) and agricultural (treated agricultural areas) runoff, washout or spray drift, or of contaminated sediments. Once these pollutants arrive in aquatic ecosystems, they can persist for a few months to several years [7, 8].

Like most coastal areas of the Mediterranean Sea, the Bizerte lagoon in northern Tunisia is a coastal ecosystem exposed to pollutants resulting from agriculture, urbanization, industrialization, as well as pressures from maritime and commercial ports. Marine sediments are therefore highly contaminated with a wide range of chemicals. Thereby, free marine nematodes are considered a useful tool to assess the presence of impact in marine ecosystems since they spent their life in the benthic ecosystems inhabiting the first ten centimeters of marine sediments [9] therefore directly exposed to pollutants. Nematodes are ubiquitous (the abundance ranges from 11 to 24 million individuals per square meter), occupy a key role in the benthic food webs [10], play an important role in the ecosystems functioning [11] through sediment aeration [12], and the mineralization of organic matter [13]. Additionally, due to their short life cycles, rapid metabolic rates, benthic larval stages, and rapid responses to environmental changes, nematodes are considered an ideal model for laboratory experiments [14, 15], and are classified excellent indicators for biomonitoring activities [9].

This chapter aims to describe the results of previous studies using traditional monitoring tools (analysis of the taxonomic structure of nematodes and determination of ecological indices) to assess the environmental quality and to describe the effectiveness of the new multivariate analysis approach (creation of functional groups of nematodes bases on biological traits) in order to provide clearer and more informative data on the state of the Mediterranean coastal ecosystem.

## 2. Materials and methods

### 2.1 Sampling site

Sediments samples are collected in the upper layer from Bizerte lagoon (NE, Tunisia) using Plexiglas hand-cores and placed in a bucket (**Figure 1**). Only the first 10 cm were sampled like that the presence of 90% of the nematode is located from 1 to 2 cm from the surface [16].

### 2.2 Sediment contamination and nematodes study

Sediment samples used for the experiment of chemical enrichment were alternately frozen (−20°C) and thawed three times in order to eliminate all fauna according to [17] before adding the selected concentrations of contaminant used [18]. Particles larger than 63 μm were removed by wet sieving and selected concentrations of chemical compounds were added to 100 g of Dry weight (DW) sediment. 2-l-glass bottles were used as microcosms in the experiment [17]. The control microcosm (C) consisted of non-treated sediments containing 200 g of natural sediments and 100 g of defaunated sediments in 1 L of filtered water (1 μm filtration).

The treated microcosms were constituted by 300 g of homogenized sediments (two-thirds of natural sediments and one-third of contaminated sediments) in 1 L of filtered water as reported in [19]. Overall, one control [(C)] and $n^{th}$-enriched

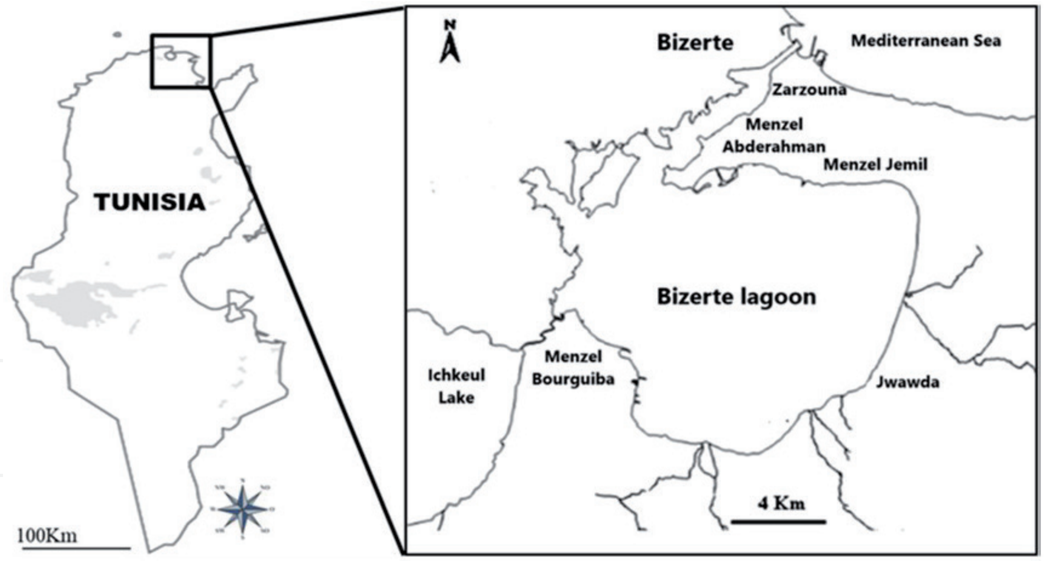


**Figure 1.**
*Location of the Bizerte lagoon (northern Tunisia).*

microcosms with contaminants were investigated [20]. Each treatment was replicated three times and the experiment was run for one month as reported by [21].

After generally 30 days, the experiment was conducted adding 4% buffered formalin solution in the experimental plots. Using the Ludox™ centrifugation technique and sieves (40 μm - 1 mm), the meiobenthic nematodes were extracted from the sediments and stained for 48 h with Rose Bengal [22]. Afterward, meiofaunal taxa were identified and counted under a stereomicroscope (50×, Wild Heerbrugg M5A Model), and a maximum of 100 nematodes/replicates were randomly collected. All specimens were placed in twenty percent of glycerol, evaporated to anhydrous glycerol, and mounted on slides [23]. The genus-level were identified according to the literature available [24] and NEMYS repository [25] using a Nikon microscope (Image Software NIS Elements Analysis Version 4.0 Nikon 4.00.07–build 787–64 bit).

### 2.3 Ecological indices analysis

The ecological diversity indices present tools that take into account both the number of species present and their relative abundance. In order to assess the effect of chemical pollutants on the benthic fauna, the former research work has focused on studying the univariate biodiversity indices of meiofaunal taxa as well as monitoring changes to the composition. The spatial or temporal diversity of a taxonomic group (Shannon Diversity), the distribution of the relative abundances of species (Equitability), the specific richness (the number of species present), Index of Maturity, and Trophic Diversity were studied. **Table 1** shows these indices in detail.

### 2.4 Functional traits analysis

Currently, the new research is based on the classification of nematodes into functional groups according to the diversity of their morphological attributes and life-history strategies that are related to ecological functions [31]. The combined analysis of functional traits was suggested for the first time by [32] in order to have more effective indications of the state of the environment. The assessment of functional diversity is based on the grouping of functionality measures into one using a multivariate approach that provides more informative ecological data than single

| Index | Formula | Using | Reference |
|---|---|---|---|
| Shannon Diversity | H′ = −Σ (pi ln pi) | Most commonly used, makes it possible to assess the spatial or temporal diversity of specific taxonomic group. | [26] |
| Equitability | J' = H′/ ln S | Equitability (J') provides details on the species/ genus/taxon relative contribution to the overall diversity. | [27] |
| Margalef Diversity | D = (S -1)/ln (N) | D is the raw number of species in a sample. It takes into account both the number of species and the total sample size. | [28] |
| Index of Maturity | MI = $\Sigma^n$ i = 1 (vi × fi) | MI is considered as a measure of the assemblage's life strategy. | [29] |
| Trophic Diversity | TD = Σθ2 | High TD values indicate the dominance of a single trophic guild compared the overall assemblages. | [30] |

**Table 1.**
*The ecological indices used in marine nematodes.*

analyzes. It is about making more reliable correlative relationships with environmental factors that can reveal additive relationships within communities.

In the **Table 2**, Five functional attributes were proposed for nematodes: (a) Adult length (1–2 mm, 2–4 mm, and ˃ 4 mm) [32]; Amphid shape (indistinct (Id); slit-like (St), blister-like (Bs); longitudinal slit (Ls); spiral (SP); rounded or elongate loop (REL); pocket-like (Pk), and circular (Cr) [32]; Tail shape (short/ round (s/r), elongated/filiform (e/f), conical (co), clavate/ conical–cylindrical (cla)) [33]; Life history (c-p scores; from 1 to 5) evaluated through a scale from colonizers to persistent [29, 34]; and Feeding diet: (selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth-feeders (2A), and omnivores-carnivores (2B)) [35].

### 2.5 Statistical data analysis

Nematodes data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov, and Bartlett tests, respectively. The software PRIMER v5 (Plymouth Routines in Multivariate Ecological Research, version 5.1.8) will be used following the standard methods described by [36] for univariate and multivariate analysis of data [37]. For each condition, the univariate indices used: species numbers (S), species richness (d), and the Shannon diversity (H′) and Pielou's evenness (J') indices. Subsequently, means of all univariate indices examined using a one-way analysis of variance (1-ANOVA) among all microcosms. Multiple comparisons were performed using the Tukey HSD test (software Statistica version 5.1). Significant differences were considered when p-values were $< 0.05$.

For multivariate analysis of nematode communities, the non-metric Multi-Dimensional Scaling (nMDS) ordination measures applied on square-root transformed functional traits abundances and using Bray–Curtis similarity to represent how treatments or biological attributes are matched. The SIMPER analysis run to determine the contribution of each genus or functional group (cumulative contribution of 70%) to the average dissimilarity among treatments.

## 3. Results and discussion

Ecotoxicological studies using the traditional approach for aquatic ecosystem monitoring considered nematodes diversity and taxonomic composition. The most

| Genera | Functional traits | | | | |
|---|---|---|---|---|---|
| | 1A, 1B, 2A and 2B | C-p score | Amphid shape | Tail shape | Adult length |
| *Daptonema* | 1B | 2 | Cr | cla | 1–2 mm |
| *Metalinhomoeus* | 1B | 2 | Cr | e/f | 2–4 mm |
| *Sabatieria* | 1B | 2 | Sp | cla | 1–2 mm |
| *Ascolaimus* | 1B | 2 | Cr | co | 2–4 mm |
| *Theristus* | 1B | 2 | Cr | co | >1 mm |
| *Paramonohystera* | 1B | 2 | Cr | cla | 1–2 mm |
| *Promonhystera* | 1B | 2 | Cr | e/f | 1–2 mm |
| *Desmolaimus* | 1B | 2 | Cr | cla | 1–2 mm |
| *Odontophora* | 1B | 2 | Cr | co | 2–4 mm |
| *Steineria* | 1B | 2 | Cr | cla | 1–2 mm |
| *Terschellingia* | 1A | 3 | Cr | e/f | 2–4 mm |
| *Anticoma* | 1A | 2 | Pk | e/f | 1–2 mm |
| *Synonchiella* | 2B | 4 | Sp | e/f | 2–4 mm |
| *Viscosia* | 2B | 3 | Pk | cla | 1–2 mm |
| *Metoncholaimus* | 2B | 3 | Pk | cla | 2–4 mm |
| *Oncholaimus* | 2B | 4 | Pk | cla | 2–4 mm |
| *Oncholaimellus* | 2B | 4 | Pk | cla | > 4 mm |
| *Bathyeurystomina* | 2B | 4 | Pk | e/f | > 4 mm |
| *Marylynnia* | 2A | 3 | Sp | e/f | 1–2 mm |
| *Comesoma* | 2A | 3 | Sp | co | >1 mm |
| *Prochromadorella* | 2A | 2 | Id | co | 1–2 mm |
| *Cyatholaimus* | 2A | 3 | Sp | co | 2–4 mm |
| *Paracomesoma* | 2A | 2 | Sp | cla | 2–4 mm |
| *Calomicrolaimus* | 2A | 3 | Sp | co | 1–2 mm |
| *Cobbia* | 2A | 3 | Cr | e/f | >1 mm |
| *Desmodora* | 2A | 2 | REL | co | 1–2 mm |
| *Spirinia* | 2A | 3 | REL | co | 2–4 mm |

**Table 2.**
*List and functional traits of nematodes genera identified in the Bizerte lagoon.*

widely used ecological index is species richness, as it gives a clear and simple indication of the species number present in a sample. However, this measure strongly depends on the sample size or the environment studied [38]. Other diversity indices such as Shannon-Wiener and Pielou regularity as well as Index of Maturity [29] and Trophic Diversity [30], have been proposed and are regularly used to describe nematode assemblages in different environmental conditions [37]. The main advantage of diversity indices over the richness is that they give a better picture of the dominance of the species by considering the relative abundance of each taxon. Several studies investigated the impact of various environmental pollutants on benthic ecosystems using nematodes diversity indices to investigate their toxicity and harmful concentrations.

The results of nematodes exposure to metals during one month such as chromium have shown significant differences between univariate indices measures (most were decreased significantly). The responses of nematode species are characterized by the disappearance such *Leptonemella aphanothecae* which shows its high sensitivity to chromium, and an increasing of *Bathylaimus* species showing their high resistance [39]. In the same context, treatment of nematodes with nickel showed significant differences between nematode assemblages from control microcosms and those from treatments. Most univariates measures, including diversity and species richness, decreased significantly with the increasing of the metal concentrations. Results from multivariate analyses of the species abundance demonstrated that nematodes responses were highly variable: *Leptonemella aphanothecae* was considered sensitive because disappeared at all nickel concentrations tested; *Daptonema normandicum*, *Neochromadora trichophora*, and *Odontophora armata* which significantly increased at the high nickel concentration appeared to be "opportunistic" species at this concentration whereas *Oncholaimus campylocercoides* and *Bathylaimus capacosus* which increased at all nickel concentration seemed to be "nickel-resistant" [40]. Experiments of pesticide exposure such as permethrin have demonstrated that univariate indices were significantly different. The multivariate analyses revealed that nematode species *Pselionema* sp., *Prochromadorella neapolitana*, and *Spirinia gerlachi* were eliminated at the low dose and seemed to be intolerant to permethrin; *Trichotheristus mirabilis* and *Xyala striata*, which increased with increasing contamination levels, seemed to be 'opportunistic' and/or 'resistant' species [41]. Results of glyphosate treatment showed that Shannon-Wiener Diversity was reduced in all treatments. Species such as *Marylynnia stekhoveni* and *Microlaimus cyatholaimoides* decreased in all treatments and appeared to be "glyphosate sensitive" whereas *Paramicrolaimus spirulifer*, *Paracomesoma dubium*, *Metacomesoma punctatum*, *Terschellingia longicaudata*, and *Daptonema hirsutum* seemed to be "glyphosate resistant" species [42].

Exposed nematode communities to environmental levels of pharmaceutics compounds were also investigated. Thus, penicillin G exposure has demonstrated that diversity (H′), species richness (d), equitability (J), and the number of species (S) were decreased significantly and *Kraspedonema octogoniata* and *Paracomesoma dubium* were seemed to be sensitive species; *Oncholaimus campylocercoides* as "opportunistic", whereas, *Nannolaimoides decoratus* is "penicillin G resistant" species [43]. In other studies, Ciprofloxacin exposure caused a decrease in diversity index and nematode species were responded differently: *Odontophora villoti* was considered "sensitive," whereas *Metoncholaimus pristiurus* as "opportunistic" and *Paramonohystera pilosa*, appeared "tolerant" [23]. The exposure with collagen has induced a reduction in diversity indices. Nematodes species such as *Ptycholaimellus ponticus*, *Theristus modicus*, and *Kraspedonema reflectans*, were classified as "collagen-sensitive", *Sigmophoranema rufum*, *Lauratonema hospitum*, *Enoploides spiculohamatus*, and *Trichotheristus mirabilis*, were "collagen-tolerant" species [44]. The response of nematodes to polybrominated diphenyl ether (BDE-47) was also studied, and the results showed that all univariate indices (Species number (S); Shannon diversity index (H′); Margalef's species richness (d) were decreased and Pielou's evenness (J')) were significantly modified, and the species of *Terschellingia* were considered "BDE-47 sensitive," whereas *Metoncholaimus pristiurus* and *Paracomesoma dubium*, were "BDE-47 tolerant" (**Table 3**) [19].

Currently, some studies have started using the new assessment approach (functional traits analyses) to address the effects of various contaminants on nematode species. Among these studies, many experiments were conducted to evaluate the impacts of ciprofloxacin on nematodes functional traits evolution. A change in the nematofauna structure was registered and characterized by low values of the

| Chemicals compounds | Ecological indices responses | Functional traits responses | References |
|---|---|---|---|
| Chromium | H′ ↓, D ↓, J' (ns), S (species number) ↓ | | [39] |
| Nickel | H′ ↓, D ↓, J' (ns), S ↓ | | [40] |
| Permethrin | H′ ↓, D ↓, J' ↓, S ↓ | | [41] |
| Glyphosate | H′ ↓, D ↓, J' ↓, S ↓ | | [42] |
| Penicillin G | H′ ↓, D ↓, J' ↓, S ↓ | | [43] |
| Collagen | H′ ↓, D ↓, J' ↓ | | [44] |
| Ciprofloxacin | H′ ↓, D ↓, J' ↑, S ↓ | Lower taxonomic diversity All Functional traits were modified especially the tail shape. | [23, 45] |
| BDE-47 | H′ ↓, D ↓, J' ↑, S ↓ | • Restructuration of nematodes biological traits<br>• Amphid shape was the most changed | [19, 46] |
| cd, PVC, and Mixture | cd (H′ ↓), PVC (H′ ↓), Mixture (H′ (ns)) | • Restructuration of nematodes biological traits<br>• Feeding diet and amphid shape and were the most modified | [47] |
| Separate and mixed PAHs (anthracene, pyrene, and benzo[a]pyrene) | D ↓ | Life history and feeding diet were the most modified | [48] |

**Table 3.**
*List of studies using ecological indices and functional traits approach of nematodes for assessment of ecosystem quality.*

taxonomic diversity. The nMDS second-stage ordination plots for matrices including nematode genera and biological traits showed that all attributes were modified and the tail shape was the closest to the generic structure [45]. Another study that treated the toxicity and the interactions between metals and plastic (Polyvinyl chloride) demonstrated that the single treatments was toxic for marine nematodes. However, the mixture of these pollutants has a lesser lethal impact compared to their separate effects. The nMDS second-stage ordination of inter-matrix rank correlations for matrices already mentioned showed that the proximate functional trait to the taxonomic responses was the amphid shape [47]. The response of meiobenthic nematode communities to the effect of the polybrominated diphenyl ether, BDE-47 has shown a decrease in taxonomic diversity and a modification in all biological trait abundance. Only three functional traits (body length, feeding group, and amphid shape), presented a clear difference between the untreated and treated microcosms. The nMDS second-stage ordination of inter-matrix rank correlations indicated that the amphideal shape was the most modified functional trait [46]. Finally, a study examining the single and binary PAHs (anthracene, pyrene, and benzo[a]pyrene) toxicity on marine nematodes have demonstrated that the single or mixtures treatments exhibited restructuring of trophic diversity with an increase of epigrowth-feeders abundance. The nMDS second stage ordination of inter-matrix rank correlations showed that the feeding diet and life history were the most modified functional traits after treatment with PAHs [48]. Although some studies have the approach proposed here to assess the environmental pollutants, there is still a lot of work to do to validate the use of the new approach to assess the quality of the environment influenced by different types of stress (**Table 3**).

## 4. Conclusions

The use of the approach based on the functional traits of marine free-living nematodes seems to be at present more relevant than the classical ecological index analysis methods used to detect changes quality of aquatic ecosystems. This new approach has made it possible to provide additional ecological information on the nematode responses and then on ecosystem functioning [32]. All functional traits included Adult length; Amphid shape; Tail shape; Life history; and Feeding type [9, 32–35] constituted a good approach to the determinate of the ecological status of the ecosystem.

## Conflict of interest

The authors declare no conflict of interest.



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