Chapter

Marine Free-Living Nematodes as Tools for Environmental Pollution Assessment: A Special Focus on Emerging Contaminants Impact in the Tunisian Lagoon Ecosystems

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Abstract

Coastal ecosystems are exposed to pollution by various contaminants due to several anthropogenic activities. Numerous pollutants, such as pesticides, drugs, metals, Polycyclic Aromatic Hydrocarbons (PAHs), Brominated flame retardants (BFRs), and Microplastics (MPs), transported in the water column tend to persist in the sediments. Among the Tunisian coastal areas, the Bizerte and Ghar El Melh lagoons are exposed to several pollutants resulting from different activities, such as agriculture, urbanization, and industrialization. Consequently, sediments are intensely dirtied by a wide range of pollutants. Due to their relatively short life cycles and high turnover rates, free-living nematodes reacted quickly to environmental changes. This most dominant meiobenthic taxon, has been mainly exploited as indicator of disturbance because of its ubiquity, high abundance, and taxonomic diversity. In this current chapter, we cited the different environmental pollutants effects and show the importance of nematodes as bio-indicator species in environmental monitoring.

Keywords: Tunisian Lagoon ecosystems, free-living nematodes, emerging contaminants (ECs), risk assessment, experimental approach

1. Introduction

Emerging contaminants (ECs), including drugs, polycyclic aromatic hydrocarbons (PAHs), metals, pesticides, brominated flame retardants (BFRs), and microplastics (MPs), resulting from the rise of industrial production and anthropogenic activities around lagoon ecosystems are transported toward the water column and adsorbed on sediments particles [1]. Coastal lagoons are considered to be distinct systems rather than adjoining ones [2]. As interfaces between land and sea, they exhibit high primary and secondary productions that promote the development of extensive fisheries and aquaculture [3]. As semi-enclosed systems, coastal lagoons are strongly influenced by freshwater input [4] and are usually impacted by agricultural, industrial, and tourism activities [5]. These unique features allow lagoon waters to acquire significantly different characteristics compared to the nearby seawater, which leads to greater diversity in the biological communities in these ecosystems. In Tunisia, the Bizerte and Ghar El Melh lagoons are exposed to several contaminants resulting from different activities, such as agriculture, urbanization, and industrialization [6].

Free-living marine nematodes sheltering the marine sediment matrices can accumulate these chemicals and then transfer them to higher trophic levels through the food chain causing widespread contamination of the trophic chain [7]. These species constitute the dominant meiobenthic group in marine areas. They are characterized by their high abundance (up to 20 million individuals per m^2) [8] and species richness (approx. 8000 species) [9], their ubiquity and holobenthic lifestyle [10], and their small size (1–5 mm in average length), which make them easily manipulated in laboratory studies [11]. Nematodes have also high fecundity and metabolic rates and their short generation time, less than a year [12], which allow fast experimental outcomes (e.g., a month) laboratory assays based on the rapid responses to pollutants [13–17].

The objective of this chapter is to describe previous studies and to show the ecological risks of emerging contaminants in two different Tunisian lagoon ecosystems " Bizerte and Ghar El Melh lagoons" by focusing on the responses of benthic meiofauna more particularly marine nematodes to experimental various environmental pollutants exposure.

2. Materials and methods

2.1 Bizerte and Ghar el Meleh lagoons

In Tunisia, lagoon milieus cover a total space of 1100 km^2 and are distributed over the entire Mediterranean coastline [18]. These lagoons are of high ecological and economic importance but are experiencing rising anthropogenic pressure, being exposed to various types of environmental degradation resulting from agricultural, manufacturing, and touristic activities [19]. Among its many lagoons, two are the best known and are located in northern Tunisia (**Figure 1**):

The Ghar El Melh is a shallow coastal lagoon, which is isolated by a narrow-vegetated sand strip from the Mediterranean Sea. It is located in the southern Mediterranean Sea on the northeastern coast of Tunisia (37°06–37°10 N and 10°08′–10°15 E) and is influenced by regional water circulation patterns [20]. It covers a surface area of about 3000 ha, including two small sub-lagoons, El Ouafi Lagoon to which it is permanently connected, and Sidi Ali El Meki Lagoon from which it is separated by embankments. The Ghar El Melh is linked to the Mediterranean Sea via a permanent channel. The lagoon displays different levels of salinity with the highest registered in the lagoon still areas. Freshwater inflows are cyclical, restricted in summer, and tall in winter, occasionally with the existence of exceptional floods generating a link between the lagoon and the Mejerda River. The benthic vegetation is dominated by *R. cirrhosa* that extensively covers (80–100%) the lagoon bottom in summer [21]. The benthic fauna has important biodiversity, such as the presence of molluscs, crustaceans, and fishes (26 species) [22].

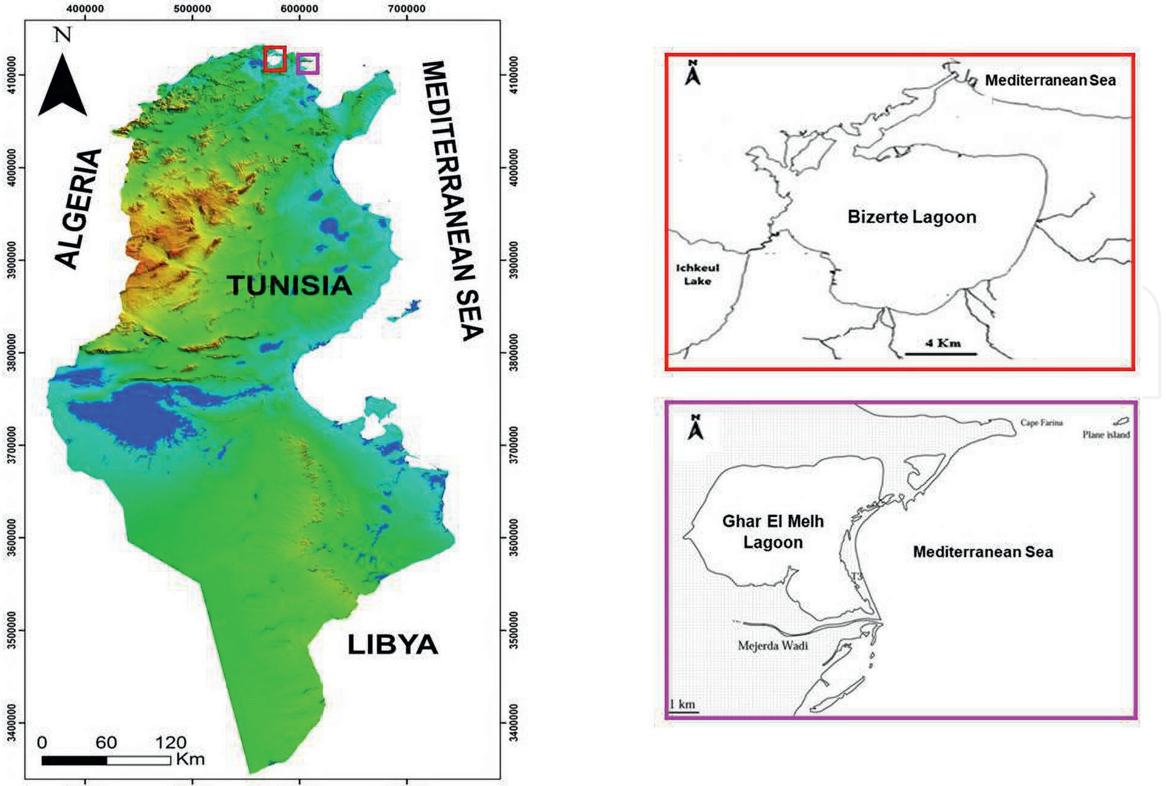

Figure 1.
Location of the Bizerte and Ghar ElMelh lagoons (Northern Tunisia).

The Bizerte lagoon is situated in the north of Tunisia in the latitudinal and longitudinal extensions of 37°8′–37°16′N and 9°46′ and 9°56′E. It has a 150 km^2 complete surface, an 8 m mean depth, and a 380 km^2 catchment area. It is connected with the Mediterranean Sea through a channel 12 m deep and communicates in the south with the Lake of Ichkeul (140 km^2) by the Tinja river. This ecosystem has great biodiversity (30 teleosts and eight elasmobranch fish species have been described) and its socio-commercial revenues in the animals' sale (mussel and oyster farming) [23]. Unfortunately, it receives freshwater inputs via eight rivers [24], urban (bounded by six cities) and industrial activity discharges as well as the products of agricultural activity. In addition, this lagoon is exposed to high biotic and abiotic variations [25].

2.2 Experimental nematodes study

Free-living marine nematodes are known to be well suited as bioindicators for monitoring studies in marine environments and bioassays (**Figure 2**) [26]. These worms are ubiquitous and occupy an important link in the food chain, feeding on microalgae and bacteria and, in turn, being preyed upon by macrobenthic predators, such as polychaetes, crabs, and fishes [27]. They are expected to be highly susceptible to sediment-associated pollutants because they live and feed on the sediment [8].

Technically, sediments containing meiofauna were collected from a coastal site in the Tunisian lagoon. Before being enriched with ECs, the sediment was arranged using the method of Austen et al. [28]. Then, large substrate particles (>63 mm) were removed by wet sieving, and appropriate concentrations of ECs were supplemented with sediment (100 g dry weight; dw). The microcosms used in this experiment were based on the original design of Austen et al. [28] and consisted of 570 mL glass bottles.

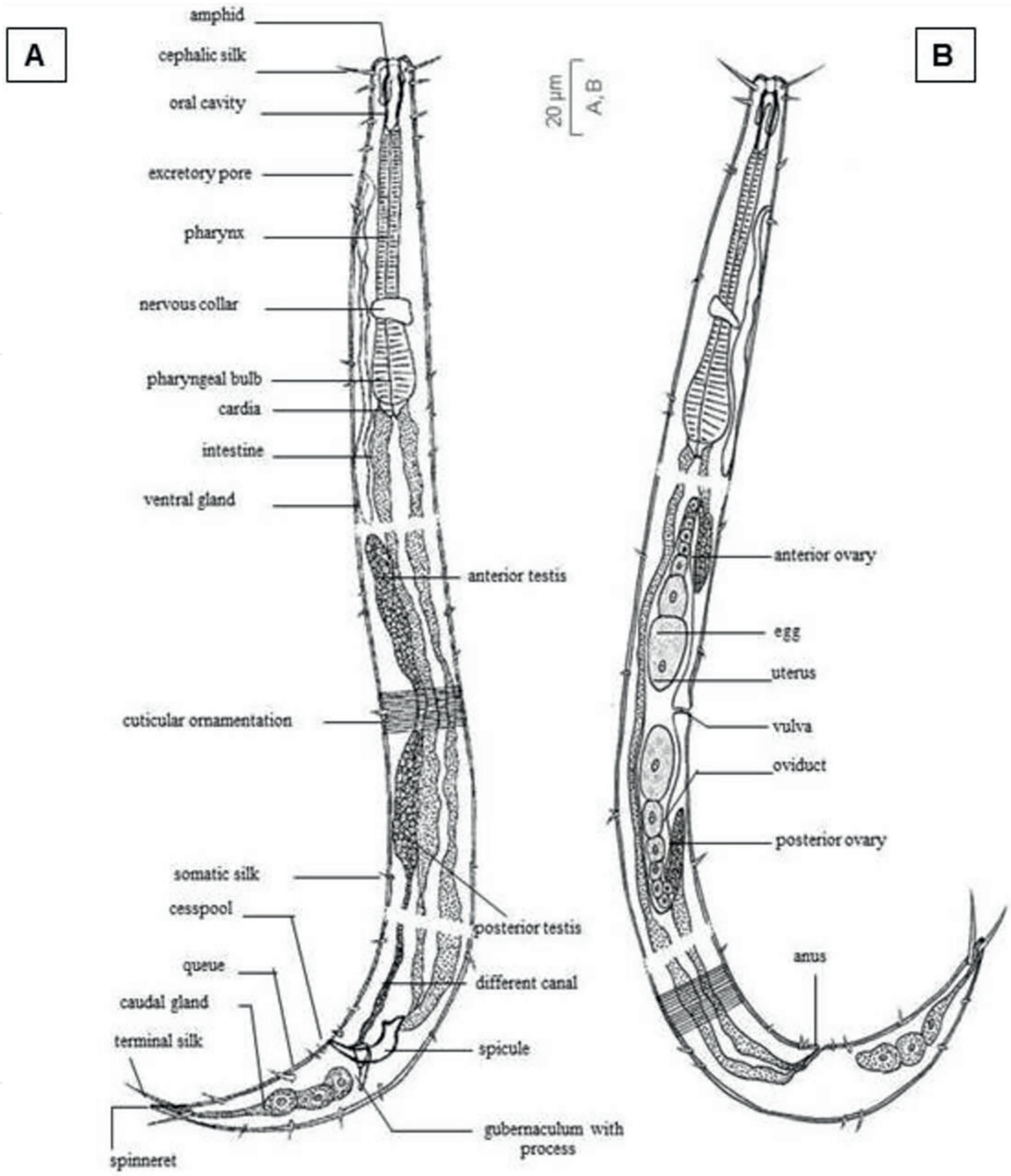

Figure 2.
*General organization of nematodes species"*Odontophora villoti" *(Axonolaimidae); A: male; B: gravid female.*

Treated microcosms were occupied by 300 g of homogenized sediments (200 g of natural sediment + 100 g of contaminated sediment) topped up with filtered natural water (0.1 mm). The control microcosm consisted of not treated and azoic sediments. Treatments were set up, each with minimum of three replications (control [(C)] and "treated by ECs" microcosm [29]. During the 30 days of the experiment, each microcosm was constantly aerated with an oxygen pump (**Figure 3**) [13].

At the end of the experiment, the sediment samples were fixed in a 4% buffered formalin solution. Nematodes were extracted by centrifuging with Ludox-TM three times and stained with Rose Bengal ($0.2\ g\ l^{-1}$) for one day [30]. The nematofauna taxa were counted on a stereomicroscope (50×, Wild Heerbrugg M5A Model), and a

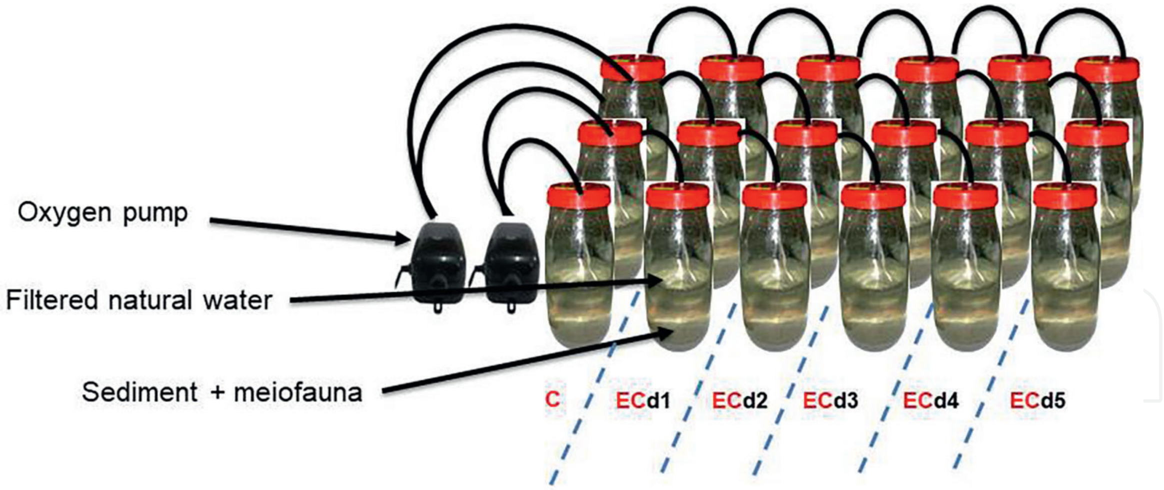

Figure 3.
*The experimental design. [C]: control; [***EC***d1,* **EC***d2,* **EC***d3,* **EC***d4,* **EC***d5]: increasing doses of emerging contaminant.*

maximum of 100 individuals/replicates were randomly taken. Animals were slowly evaporated in anhydrous glycerol, mounted on slides under an oil immersion objective (100×). The Platt and [31–33] pictorial guides and NeMys database [34] were used to species identify, respectively.

2.3 Biological parameters analysis

Nematodes are the most diverse and numerically dominant metazoans in aquatic ecosystems, and, because of their rare ability to survive in extremely polluted conditions, they are usually the only persistent taxon in heavily polluted/disturbed habitats [35]. To assess the effect of chemical pollutants on the benthic fauna, the research work has focused on studying ecological indices of nematodes, such as the spatial or temporal diversity of a taxonomic group (Shannon–Wiener diversity (H')), the distribution of the relative species abundances (Pielou's evenness (J')), the species number present (Margalef's species richness (d)), Maturity Index [36] (MI; based on the ecological characteristics and reproductive strategies of nematodes), and Index of Trophic Diversity [37] (ITD; expressed in an index calculated on the basis of the percentage of each feeding type) were studied.

In addition, the functional and morphological attributes of each species (i.e., trophic diet, tail shape, amphid shape, and life history) were considered. Trophic diet was categorized based on the characteristics of the buccal cavity [38], as epigrowth feeders (2A), selective deposit feeders (1A), non-selective deposit feeders (1B), and omnivores/predators (2B). The tail shape was illustrated into four types: conical (co), clavate/conico-cylindrical (cla), short/round (s/r), and elongated/filiform (e/f) [39]. The Amphid shape was distinguished into eight categories based on the shape of the amphidial fovea, of which four categories were used in our study—circular (Cr), spiral (Sp), pocket (Pk), and indistinct (Id) [8]. The life strategy (c–p scale) was estimated on a scale of c–p=1 (good colonizers: short life cycle, great reproduction rates, resistant to various types of stress) to c–p=5 (good persisters: lengthy life cycles, limited offspring, sensitive to stress), analogous to K/r-strategists, following [36, 40, 41]. The adult length was assigned to four groups (<1 mm, 1–2 mm, 2–4 mm, and >4 mm) [10].

Supplementary studies were conducted for the analysis of bacterial abundance. Density (per gram) was calculated for each sediment sample. Aliquots of 100 μL were successively diluted in PBS and then displaced on duplicate bacterial agar plates (Difco), which were later incubated at 37°C (overnight). Using the plaque method, 30 to 300 colonies were counted and the amounts were expressed as colony-forming units per gram of sediment (CFU g^{-1}) [14].

2.4 Statistical data analysis

Statistical analyses were executed using the Plymouth Routines in Multivariate Ecological Research (Primer v5.0) software package [42, 43]. For each microcosm treatment, all univariate indices were considered—abundance, species number (S), Shannon-Wiener diversity index (H'), Margalef's species richness (d), and Pielou's evenness (J'). Data were first tested to fulfill parametric requirements, Gaussian normality, and homogeneity of variances. Two tests were necessary: The Kolmogorov–Smirnov test to evaluate the first condition and the Bartlett to check the second condition. Once our data approximated normality, one-way ANOVA (1-ANOVA) was useful to determine the total significant difference between conditions. For multiple comparisons, the test of Tukey's HSD was applied (Statistica version 5.1).

For multivariate analyses: a non-metric Multi-Dimensional Scaling ordination (nMDS) by the Bray-Curtis matrix of similarity (square-root transformed abundance) were performed to detect a possible trend in the distribution of treatment, depending on the responses of nematode taxa or those of biological traits after ECs exposure. Hierarchical cluster analyses (CLUSTER) were used to confirm the results provided by the nMDS. The analysis of Similarity (ANOSIM) was considered to test the dissimilarities significance eventually noted between the nematode assemblages or each biological trait. Finally, The SIMPER analysis was useful in determining the contribution (cumulative contribution of 70%) of each species or functional group to the mean dissimilarity between treatments.

To detect the different relationships between nematode taxa and functional groups, PAST v3.26 software was used to perform correspondence analysis (CA) using two-dimensional plots. Also, the principal component analysis (PCA) (performed after transforming the data into log (x + 1)) is associated with the Pearson correlation (adopted via XLSTAT. 2019) to determine the targeted relationships.

3. Results and discussion

Emerging pollutants (ECs) from industrial production or agricultural runoff enter coastal waters and cause negative impacts on benthic organisms. Meiobenthic organisms, particularly free-living marine nematodes, because of their short life cycles and rapid metabolic rates, are considered ideal for laboratory experiments [13–17]. Many studies have investigated the impact of various ECs on these animals from two famous Tunisian ecosystems "Bizerte and Ghar el Meleh lagoons":

3.1 Ghar el Meleh nematodes response

3.1.1 Drugs effects

Four 17-β-estradiol concentrations (0.15, 0.31, 0.62, and 1.24 ppm) were tested in an experimental microcosm, and effects on the nematode community from the Ghar el Meleh lagoon were examined after 30 days. Significant differences were noted between the control nematode assemblages and those from 17-β-estradiol treatments. Total abundance, Shannon–Wiener index, and evenness were affected by 17-β-estradiol contamination, but species richness was unaffected. The species named; *Chromadorina metulata* and *Ascolaimus elongatus* were eliminated and seemed to be intolerant to estradiol. *Kraspedonema octogoniata* reduced at all doses could be categorized as estradiol sensitive. *Spirinia gerlachi* augmented at all doses seemed to be an opportunistic species [44]. Another experimental study was carried out to determine the effects of endocrine disruptors "Estradiol Benzoate (hereafter EB)" (0.43, 4.3, 8.6, and 12.9 ng l^{-1}) for 30 days. A significant increase in nematode abundances was registered after the EB introduction. In contrast, a decrease in nematodes species diversity has been shown. A clear structural separation of the enriched replicates with EB from controls based on species lists using the nMDS ordination method. A predominance of non-selective deposit feeders and a decline of epistrate feeders were registered [45]. Meiobenthic nematodes were also exposed in experimental microcosms to a drug for COVID-19 treatment "ivermectin" (1.8 ng.g^{-1}, 9 ng.g^{-1}, and 18 ng.g^{-1}) for 10 days. A great reduction in abundance and diversity indices was recorded. The functional types represented by—nonselective deposit feeders and nematodes with circular or indistinct amphids, were affected while these of epistrate feeders and nematodes with rounded or elongated loop amphids, took advantage of ivermectin doses [46].

3.1.2 Polycyclic aromatic hydrocarbons (PAHs) effects

The effects of two lubricating oils on nematode assemblages were examined. Sediment was treated with mineral oil (Mobil 20 W-50), a synthetic lubricant (Mobil 0 W 40), and the same two lubricants after usage in a vehicle, and effects were examined after 35 days. Total nematode abundance, species richness, and number of species diminished significantly. The evenness was affected only in used mineral lubricant compared to the control. *Daptonema trabeculosum* was removed in all treatments and seemed to be a sensitive species. *Spirinia gerlachi* augmented in mineral lubricant ("clean" and used), was reduced in all synthetic lubricant. *Terschellingia longicaudata* augmented only in synthetic lubricant treatments ("clean" and used) seemed to be a "resistant synthetic-oil species" [47]. A microcosm experiment was carried out to study the effect of diesel on a free-living nematode community. Sediments were polluted by diesel (0.5–20 mg kg^{-1} dry weight (dw)), and effects were inspected after 90 days. Community structure, diversity, and species richness were modified significantly. The responses of nematode species to the diesel treatments were varied: *Chaetonema* sp. was eliminated and seemed to be intolerant species to diesel contamination; *Pomponema* sp. and *Oncholaimus campylocercois* were significantly affected but they were not eliminated, these species were considered as "diesel-sensitive"; *Hypodontolaimus colesi*, *Daptonema trabeculosum*, and *Daptonema fallax* that significantly augmented and appeared to be "opportunistic" species [48].

3.1.3 Metals effects

The lead and zinc influence, individually and in mixtures on marine nematodes were investigated after 1-month treatment. Results from the multiple comparison tests showed significant differences between nematode assemblages from controls and those from the treated microcosm. The diversity and species richness decreased significantly in the treated microcosms. Multivariate analyses showed that the differential response occurred in all treatments but the communities from microcosms contaminated with lead and zinc separately were much more strongly affected. *Calomicrolaimus honestus* was eliminated and seemed to be intolerant species, whereas *Oncholaimus campylocercoides* increased significantly at low and medium lead contamination, and at all zinc doses seemed "opportunistic" [49].

The effects of mercury contamination (low, 0.084 ppm; medium, 0.167 ppm; and high, 0.334 ppm) on a free-living nematode community were examined after 60 days. The majority of univariate indices decreased significantly with increasing levels of mercury. The responses of nematode species were varied: *Araeolaimus bioculatus* was eliminated at all mercury doses; *Marylynnia stekhoveni* augmented at low and medium treatments was performed to be an "opportunistic species," whereas *Prochromadorella neapolitana*, which amplified at all concentrations, seemed to be a "mercury-resistant species" [50].

Nematodes were subjected to cobalt and/or zinc enrichment in a microcosm experiment for 30 days. Nematode abundance, diversity, and taxonomic structure were significantly altered. Using multivariate analyses, the data showed that nematodes assemblages from treated microcosms with zinc alone were much more negatively affected compared with those exposed to cobalt alone. The nematode species' responses to the cobalt and zinc treatments were different. *Oncholaimellus mediterraneus*, *Oncholaimus campylocercoides*, and *Neochromadora trichophora* were significantly affected by cobalt contamination. *Hypodontolaimus colesi* was eliminated and seemed to be an intolerant species versus zinc [51].

The ecotoxicity of a chromium-enriched superfood, *Spirulina platensis*, on the nematodes was investigated after 1 month of exposure. The abundance, taxonomic structure, and the nematode's functional diversity showed significant changes between the *Spirulina* and *Spirulina* + chromium groups. The lowest taxonomic

			Ghar el Meleh lagoon	
ECs	**Sensitive species**	**Resistant species**	**Opportunistic species**	**Functional traits response**
17-β-estradiol	*Kraspedonema octogoniata*		*Spirinia gerlachi*	NS
Ivermectin				Nematodes with circular or indistinct amphids were the most affected
Lubricating oils	*Daptonema trabeculosum*	*Terschellingia longicaudata*		NS
Diesel	*Chaetonema sp*		*Daptonema trabeculosum*	NS
Lead + zinc	*Calomicrolaimus honestus*		*Oncholaimus campylocercoides*	NS
Mercury	*Araeolaimus bioculatus*	*Prochromadorella neapolitana*	*Marylynnia stekhoveni*	NS
Permethrin	*Daptonema trabeculosum*	*Oncholaimus campylocercoides*		NS

Table 1.
Ghar ElMelh lagoon nematodes response to ECs. NS (not studied).

and morpho-functional diversity were observed in the highest concentration of *S. platensis* (50% DW). The nematode species' responses differed depending on their functional traits. *Spirulina* supplemented with chromium induced high toxicity for nematodes species, whereas, the *Spirulina*/chromium combinations toxicity was lower suggesting mutual neutralization between these two components [52].

3.1.4 Pesticides effects

The nematode response to permethrin contamination [P1: low (5 mg kg^{-1}), P2: medium (25 mg kg^{-1}), and P3: high (250 mg kg^{-1})] was examined in a microcosm experiment and the effects were evaluated after 30 days. The univariate and multivariate analyses showed significant variances between nematode assemblages from control assemblage and those from permethrin treatments. Total nematode abundance (I), Shannon-Weaner index (H'), species richness (d), evenness (J'), and number of species (S) reduced significantly. The nematode community responses were varied: *Oncholaimus campylocercoides, Theristus pertenuis, Araeolaimus bioculatus,* and *Calomicrolaimus honestus* amplified in all doses, appeared to be "permethrin-resistant species." *Daptonema trabeculosum* was eliminated and appeared "permethrin-sensitive species" (**Table 1**) [53].

3.2 Bizerte lagoon nematodes response

3.2.1 Drugs effects

Free-living marine nematodes from Bizerte lagoon were exposed to the penicillin G (D1: 3 mg.L^{-1}, D2: 30 mg.L^{-1}, D3: 300 mg.L^{-1}, D4: 600 mg.L^{-1}, and D5: 700 mg.L^{-1}) in microcosm experiment for 30 days. Results showed significant differences between nematode assemblages from control assemblage and those treatments. Univariate measures, containing diversity (H'), species richness (d), equitability (J'), and a number of species (S) diminished significantly with increasing levels of antibiotic treatment. Results of multivariate analyses showed that the nematode's response was varied: *Kraspedonema octogoniata* and *Paracomesoma dubium* were eliminated at all doses tested and seemed to be sensitive species; *Oncholaimus campylocercoides* survived even the highest dose of D5, may be classified as "opportunistic" species, whereas, *Nannolaimoides decoratus* that showed a positive response at the highest concentration, seems to be "penicillin G resistant" species [12]. In terms of feeding responses, Microvores (M), Deposit feeders (DF), and Ciliate consumers (CF), most abundant in the control microcosm, were very much affected and their abundance decreased significantly in response to antibiotic contamination. Epistrate Feeders (EF) seem unaffected by the treatment but an abundance of optional Predators (FP) and exclusive Predators (Pr) showed a significant increase in dominance compared to the control [54]. In addition, the trophic index was significantly reduced in all microcosms treated whereas the trophic ratio 1B/2A appears to be insignificant [55].

The ecotoxicity of ciprofloxacin on the nematodes community was studied. Four ciprofloxacin doses [D1 (50 mg/g), D2 (100 mg/g), D3 (200 mg/g), and D4 (500 mg/g)] were applied, and responses were considered after 1 month. All univariate measures were modified significantly compared to those in the control assemblage. The non-parametric Multi-Dimensional Scaling based on species abundances (MDS) showed significant separation of the control assemblage from the treated populations. *Odontophora villoti* was reduced at all ciprofloxacin concentrations and considered "sensitive," whereas *Metoncholaimus pristiurus* was affected by moderate concentrations and was described as "opportunistic." *Paramonohystera pilosa*, whose abundance increased with antibiotic doses, appeared "resistant" [13]. The trophic structure of nematodes was modified in terms of relative abundance—the microvores (M), epigrowth feeders (EF), and ciliate consumers (CF) elevated in the control assemblage, were highly altered in response to contamination. Nevertheless, the deposit feeders (DF), optional predators (FP), and exclusive predators (Pr) showed a significant increase. In addition, ciprofloxacin leads to a significant reduction in bacterial density with the highest dose, which could explain the results obtained for the nematode trophic group's distribution [14]. The association of the two-dimensional (2D) non-metric multidimensional scaling (nMDS) plots and relative functional groups abundances revealed that all biological traits were affected. Amphid shape and feeding diet were the most affected and the tail shape was the closest biological trait to the generic distribution [15].

3.2.2 polycyclic aromatic hydrocarbons (PAHs) effects

The nematofauna were exposed to four treatments of three polycyclic aromatic hydrocarbons (PAHs), including one with chrysene (150 ppb), chrysene (150 ppb) plus fluoranthene (75 ppb), chrysene (150 ppb) and phenanthrene (15 ppb), and an uncontaminated reference during 30 days. Results showed that the diversity of nematodes differed based on hydrocarbon combinations. Nematodes populations in contaminated compartments differed from those in control. *Rhabditis* sp., *Calamicrolaimus honestus*, and *Oncholaimus campylocercoides* presented in all compartments and categorized as tolerant to PAHs. *Parasphaerolaimus paradoxus*, *Encheliidae* (sp.), *Trichotheristus mirabilis*, and *Theristus pertenuis* were considered sensitive because of their presence only in control compartments [56]. *Metoncholaimus* response studies after selection showed a marked increase in activity of catalase and glutathione S-transferase, and the response was more accentuated when zinc and permethrin were administered in combination [57]. In another study, *Oncholaimus campylocercoides* were cultured and exposed for 21 days to phenanthrene and chrysene. Toxicity has been shown with high levels of PAH fluorescence at the level of the spicules, mouth, and pharynx compared to the other organs [58].

Three increasing concentrations of BaP (i.e. 100, 200, and 300 ng/l) were used in the experiment for 30 days to determine the effect on nematode structure and functional traits. The results revealed a reduction in the abundance and significant changes were observed at the community level. The nematode populations were dominated at the start of the experiment and also after being exposed to BaP by *Odontophora villoti*, explicable through the presence of well-developed chemosensory organs (i.e., amphids), which potentially increased the avoidance reaction following exposure to this hydrocarbon. Moreover, changes in the activity of catalase 'CAT', glutathione S transferase 'GST', and ethoxyresorufin-O-deethylase 'EROD' were detected in *Oncholaimus campylocercoides*, paralleled by significant reductions in CAT activity compared to controls at concentrations of 25 ng/l BaP and associated with a significant increase in GST and EROD activities [59].

3.2.3 Metals effects

The nickel effects on nematode communities were examined. Sediments were contaminated with three concentrations [(250 ppm), (550 ppm), and (900 ppm)], and effects were studied after 1 month. Results showed significant differences between nematode assemblages from undisturbed controls and those from nickel treatments. Diversity and species richness indices diminished significantly with increasing nickel levels. The nematode species responses to the nickel treatments were varied: *Leptonemella aphanothecae* was removed and seemed to be sensitive species; *Daptonema normandicum*, *Neochromadora trichophora,* and *Odontophora armata* that were significantly augmented at 550 ppm appeared to be "opportunistic," whereas *Oncholaimus campylocercoides* and *Bathylaimus capacosus* that augmented at all doses used (250, 550, and 900 ppm) seemed to be "resistant" [60].

Nematodes were exposed to chromium concentrations (500 ppm, 800, and 1,300 ppm), and effects were studied after 4 weeks through an experimental microcosm. Results showed significant differences between univariate measures of control nematodes and those from treatment microcosms. *Leptonemella aphanothecae* species was eliminated at all doses tested and seemed to be sensitive; *Daptonema normandicum* and *Sabatieria longisetosa* that significantly augmented at 500 ppm appeared to be "opportunistic" at this dose, whereas the *Bathylaimus* species that augmented seemed to be "resistant" [61].

3.2.4 Brominated flame retardants (BFRs) effects

The taxonomic and trophic response of marine nematodes to polybrominated diphenyl ether (BDE-47) was examined using four concentrations [(2.5 mg.kg^{-1}), (25 mg.kg^{-1}), (50 mg.kg^{-1}), and (100 mg.kg^{-1}] after 30 days after exposure. All univariate indices were significantly affected compared to the control. After grouping nematode species according to their trophic diversity, their abundance showed differential responses. A significant separation between the control microcosm and each treatment condition was registered using the non-metric multidimensional scaling analysis and cumulative k-dominance. The analyses of trophic groups' abundance showed the control microcosm was dominated by microvores, represented by *Terschellingia*. However, when treated with the highest concentration of BDE-47, the community was occupied by the facultative predators and epigrowth feeders represented by *Metoncholaimus pristiurus* and *Paracomesoma dubium*, respectively [17]. Nasri et al [16] showed also that BDE-47 decreased nematodes and bacterial abundance. The taxonomic structures as well as the relative abundances of each functional group were modified. Nevertheless, only three of the functional traits, adult length, feeding group, and amphid shape, showed a clear difference between the control nematodes assemblages and those treated with BDE-47. A positive correlation was registered between bacteria and the functional groups [1A, Cr, and ef], conversely, a negative correlation was recorded only with the "cla"-type tail shape.

3.2.5 Microplastics (MPs) effects

The ecotoxicity of heavy metals and polyvinyl chloride microplastics (cadmium (10 and 20 mg kg^{-1}), polyvinyl chloride (PVC) and its modified forms; PVC-DETA (PD) and PVC-TETA (PT) (20 and 40 mg kg^{-1}), separately and in mixtures on marine nematodes was investigated after exposure during one month. Results displayed that single treatments were toxic for free-living nematodes. The binary combinations of contaminants have a lesser toxic effect compared to their individual effects. This effect could be related to the high-capacity chelating ability of PVC and its polymers against cadmium [62].

The toxic mechanisms exerted by two lipid regulator agents, as well as their interactions with the polyvinyl chloride microplastic on marine nematodes, were examined in an experimental microcosm. Two concentrations of Atorvastatin and Simvastatin, (0.6 mg.kg^{-1} and 6 mg.kg^{-1}), as well as a single dosage of polyvinyl chloride microplastics at 20 mg.kg^{-1}, separately and their mixtures were used. Results showed a significant reduction in abundance in treatments compared to control. A significant decrease in epigrowth feeders (2A) abundance, which possesses conical (co) tails, and indistinct (id) amphideal foveas, reflected mainly in the decrease in abundance of the species *Prochromadorella longicaudata*. The exposure to microplastic affected only the omnivores-carnivores guild, while, the mixtures with drugs lead to synergic interactions that increased their toxic effects on marine nematode communities (**Table 2**) [63].

4. Conclusions

Due to their relatively short life cycles, high turnover rates, high abundance, taxonomic diversity [13–17], the lack of larval dispersion and their quick reactions to

			Bizerte lagoon	
ECs	**Sensitive species**	**Resistant species**	**Opportunistic species**	**Functional traits response**
Penicillin G	*Kraspedonema octogoniata*	*Nannolaimoides decoratus*	*Oncholaimus campylocercoides*	NS
Ciprofloxacin	*Odontophora villoti*	*Paramonohystera pilosa*	*Metoncholaimus pristiurus*	Amphid shape and feeding diet were the most affected
Chrysene, fluoranthene and phenanthrene	*Theristus pertenuis*	*Oncholaimus campylocercoides*		NS
BaP		*Odontophora villoti*		NS
Nickel	*Leptonemella aphanothecae*	*Oncholaimus campylocercoides*	*Odontophora armata*	NS
Chromium	*Leptonemella aphanothecae*	*Bathylaimus species*	*Daptonema normandicum*	NS
BDE-47	*Terschellingia species*	*Metoncholaimus pristiurus*		adult length, feeding group, and amphid shape were the most affected
PVC-Atorvastatin + Simvastatin	*Prochromadorella longicaudata*			An epigrowth feeders (2A) decrease (with conical (co) tails, and indistinct (id) amphideal foveas)

Table 2.
Bizerte lagoon nematodes response to ECs. NS (not studied).

environmental changes [16, 17] compared to more slowly macrofauna responds [64]. Nematodes, the dominant meiobenthic taxon (>50% in the littoral zones, [35]), was well chosen as indicators of ecological risk assessment of emerging contaminants and reliable models in ecotoxicology. The laboratory experiments results revealed that some nematodes can be considered sensitive species to a contaminant type studied. Others were found to be more resistant to all exposure levels, while a number were classified as opportunistic species since they proliferate only under conditions highly unfavorable to life.

Conflict of interest

The authors declare no conflict of interest.

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