

RESEARCH ARTICLE

Is Changing in Feeding-Groups Nematode as an Indicator of an Ecosystem Disturbed by Ciprofloxacin/Bde-47 Combined Exposure?

Ahmed Nasri^{1,2}, Fouzi Bouleefah^{1,3}, Amel Hannachi¹, Ibrahem Bokharem³, Mohamed Allouche^{1,4}, Hamouda Beyrem¹, Ezzeddine Mahmoudi¹

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

²Ecology and Dynamics of Anthropized Systems Unit (UMR CNRS-UPJV-7058 EDYSAN), University of Picardie Jules Verne, Amiens, France.

³University of Ajdabia, Faculty of Science, Zoology Department, Libya.

⁴University of Jendouba, Institue of Biotechnology of Beja, Biology Department, BP: 382, Tunisia.

Received: 04 June 2024 Accepted: 18 June 2024 Published: 25 June 2024

Corresponding Author: Ahmed Nasri, University of Carthage, Faculty of Sciences of Bizerte, LR01ES14 Laboratory of Environment Biomonitoring, Coastal Ecology and Ecotoxicology Unit, 7021 Zarzouna, Tunisia.

Abstract

A laboratory bioassay was executed to examine the combined environmental concentrations ecotoxicity of two type emerging pollutants, on meiobenthic taxa especially, marine free- living nematodes. After 30 days' experiment incubation, the meiobenthic taxa average density, the specific structure as well as the biological traits of nematofauna presented significant differences between the control and mixture groups. All taxa abundance decreased significantly with all concentrations applied, particularly with the highest, M2. Only species number (S) and species richness (d) were affected significantly in the same compartment, while Shannon diversity (H') and Pielou's evenness (J') did not show any variation in nematodes. A taxonomic restructuring has been recorded in correlation with abundance decrease of species from trophic groups 1A: ie., Terchellengia Longicaudata, Terchellengia communis; 1B: ie., Odontophora villoti, Paramonohysteria pilosa, and also, to the abundance increase of species belonging to trophic groups 2A: represented by Paramonohysteria pilosa, and 2B: ie., Metoncholaimus pristiurus, Oncholaimellus mediterraneus. In addition, the relative abundances of each nematode biological trait assemblages were touched and revealed that, amphid shape, life history and feeding group, showed a strong difference among the control and the treated compartment, and only the first biological trait mentioned was the closest to the taxonomic species distribution.

Keywords: Ciprofloxacin/BDE-47 exposure, Meiobenthic taxa, Meiobenthic nematodes, Taxonomic structure, Biological traits.

1. Introduction

Coastal areas represent key interaction zones among inland waters and the sea, providing a wide range of ecosystem services, such as nutrient supply, climate regulation, and the food chain balance (Barbier, 2017). However, the very rapid economic growth of coastal cities in recent decades is the consequence of increased urbanization and industrialization, marine pollution, habitat degradation and altered food webs resulting into a degradation of these services (Todd et al., 2019). Coastal marine areas located near large cities have become sinks for many pollutants of various origins which have the property of being adsorbed by suspended particles in the water and then and collected in the sediments (Soclo et al., 2000). Among the different types of pollutants, antibiotics and brominated flame retardants (BFRs)

Citation: Ahmed Nasri, Fouzi Bouleefah, Amel Hannachi, *et al.* Is Changing in Feeding-Groups Nematode as an Indicator of an Ecosystem Disturbed by Ciprofloxacin/Bde-47 Combined Exposure?. Annals of Ecology and Environmental Science. 2024;6(2): 17-27.

©The Author(s) 2024. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

called which are classified as emerging contaminants and are almost ubiquitous in aquatic environments as well as characterized by durability, persistence, bioaccumulation along the food chain (Costa et al., 2008).

Antibiotics are defined as natural or synthetic molecules with antimicrobial action (Kemper, 2008). They are not totally metabolized in the body, and the residues are evacuated in the urine and the feces end up in the treatment plants (Zuccato et al., 2010). These pharmaceuticals have received great attention due to their high water solubility, persistence and highly dangers to living organisms (Tang et al., 2015). Due to their intensive use and low biodegradability, the antibiotics levels recorded in the sediments were in the order of mg/L (Bradley et al., 2017). Among antibiotics, Ciprofloxacin (CIP) is one of the most commonly used (Haddad et al., 2015). It is antimicrobial nature significantly resistant to degradation (Girardi et al., 2011). It has a strong adsorption affinity on sedimentary particles (Tolls, 2001).

Among the number of brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs) are widely used as additives in consumer products such as electronic equipment, machinery, and textiles to minimize the ignition of flammable materials (De Wit, 2002) and are produced commercially and have become emerging contaminants found in aquatic ecosystems (Costa et al., 2008). PBDEs enter marine environments via various routes and are detected at levels between ng/g to μ g/g in sediments (Parolini et al., 2012). Tetrabromodiphenyl ether (BDE-47) is a highly responsive congener in aquatic environments (Bramwell et al., 2014). It is a lipophilic compound resistant to degradation and adsorbed on sediment particles and bioaccumulating in the tissues of various organisms in the food chain (Noyes et al., 2010). In Tunisia, BDE-47 was detected in some species of Bizerte lagoon, such as clams and fish at levels of 50 ng/g lipid weight (lw) (El Megdiche et al., 2017) and 100 ng/g lw (Ben Ameur et al., 2013).

Since marine sediments constitute the pollutants final sink, which can have dangers on marine life at all levels (Tangherlini et al., 2020), and specially on benthic organisms, which are directly exposed to chemicals in their biotope (Gambi et al., 2020), in addition to the lack of studies dealing with the mixtures effects of antibiotics and brominated flame retardants on benthic organisms. In our present study, meiobenthic organisms and in particular nematofauna were used in to assess the effects of these two emerging contaminants by monitoring taxa abundance, diversity indices, nematological community structure, trophic groups and the biological traits of nematodes which may reflect the quality of an environment enriched experientially with its two pollutants, after one month of incubation.

2. Materials and Methods

2.1 Emerging Contaminants (ECs) Exposure

Marine meiofauna have been subjected to the combined emerging contaminants (ECs) at low and highest concentrations of Ciprofloxacin and BDE-47, respectively, M1 = 50 μ g/g (Ciprofloxacin) + 25 ng/g (BDE-47) and M2 = 500 μ g/g (Ciprofloxacin) + 200 ng/g (BDE-47), in presence of control or non-treated compartment (Nasri et al., 2021b, 2020a).

2.2 Sampling Location and Experiment Onset

Sediment containing meiobenthic nematodes were collected from the Bizerte lagoon, Tunisia (37° 21'83.77" N, 9° 93' 58.83"E). Numerous cores were used to sample the top 10 cm of the sediment layer, at 70 cm water depth according to the method (Coull and Chandler, 1992). Then, once arriving at the laboratory, they were homogenized before being used for the ECs addition or the microcosms filling. Then, a portion of sediment was defaunaed by freezing (-20°C for 12 h) and thawing (ambient T° for 48 h, 3 times) (Nasri et al., 2020c), so that quantities of 100 g of dry weight (DW) will be used for the addition of ECs. After six days of acclimation, the water underlying the microcosms was removed and the 100 g (DW) of treated sediment was mixed with the 200 g of wet weight (WW) of sediment populated by meiofauna to obtain two ECs concentrations mixture: $M1 = 50 \mu g/g$ + 25 ng/g and M2 = 500 μ g/g + 200 ng/g.

At the end of experiment (30 days), sediment samples were fixed in a 4 % buffered formalin solution and marked with a rose Bengal solution (0.2 g. L-1) for >24 h (Nasri et al., 2014). The following day, the sediments were washed with filtered tap water and treated by flotation to extract the meiofauna by Ludox TM-50 centrifugation technique (Nasri et al., 2016). Then, the meiofauna was gathered on a 45 μ m sieve and then sorted manually using a Nikon SMZ745 stereomicroscope. The first 100 marine nematodes were mounted on slides and identified to specific scale by following pictorial keys from (Platt and Warwick, 1983, 1988), (Warwick et al., 1998) and NeMys online recognition keys (http://nemys.ugent.be) (Nasri et al., 2021a).

The morphological and functional attributes of each specimen (i.e., FG, life history c-p score, amphid shape, tail shape, and adult length) were investigated. Therefore, based on the buccal cavity type according to (Wieser, 1953), feeding diet were identified as: nonselective deposit feeders (1B), omnivores/predators (2B), selective deposit feeders (1A), and epigrowth feeders (2A). The life history (i.e. c-p score) was ranked from c-p = 1 (i.e., good colonizers, with short life cycle, high reproduction and tolerant to various types of stress) to c-p=5 (with long life cycle, few offspring and sensitive to stress), analogous to K/r strategists, following (Bongers et al., 1991). The amphid shapes were assembled into eight categories, of which four categories were used in our study: circular (Cr), spiral (Sp), pocket (Pk), and indistinct (Id) (Semprucci et al., 2018). The tail shape was distributed into 4 types: conical (co), clavate/conico-cylindrical (cla), short/ round (s/r), and elongated/filiform (e/f) (Thistle et al., 1995). The adult length was assigned to 4 groups (< 1 mm, 1-2 mm, 2-4 mm, and > 4 mm) (Schratzberger et al., 2007).

2.3 Data Analysis

Data analyzes followed standard methods described by (CLARKE, 1993) using STATISTICA (v5.1) software (Nasri et al., 2022a, 2022b, 2020b, 2013). Data were first tested for normality and homogeneity of variance. Then, one-way analysis of variance (1-ANOVA) was used to compare relative abundances of nematode functional traits, followed by Tukey's HSD post-hoc tests. Multivariate analyzes were then carried out using Primer v5.0 software via the standard methods described by (CLARKE, 1993). Non-metric multidimensional scaling (nMDS) ordination via the Bray-Curtis similarity matrix was performed to detect whether taxonomic structure or biological traits of marine nematodes responded to exposure to combined ECs via spatial distribution. Percentage similarity analysis was investigated to determine the contribution of each nematode species or functional trait (cumulative contribution of 70%) to the dissimilarity between treatments.

3. Results

3.1 Meiobenthic Taxa Abundances

In Figure 1, significant changes between the compartment (C) and those enriched with mixed treatments (M1, and M2) (p < 0.05 for all) were registered following Tukey's HSD test. All taxa (nematodes, copepods, oligochaetes, and polychaetes) abundance was reduced for two compartment and importantly with the highest M2.



Figure 1. Abundances of meiobenthic taxa from control (C) and treated microcosms with M1 and M2. Asterisks indicate significantly differences from the control (* = p < 0.05; ** = $0.05 \le p < 0.001$; *** = $0.001 \le p < 0.001$).

3.2. Ecological Indices and Taxonomic Composition of Nematodes

In the present study, only the number of species (S) and the Species richness of Margalef (d) diminished significantly in the tested mixture compartments (M1

and M2) compared to the untreated compartment (C) (Fig. 2). However, the Shannon-Wiener index (H') and the Pielou's evenness (J') showed no significant variation in pairwise comparisons.



Figure 2. Univariate indices values for each microcosm treatment. Species number (S); Species richness (d); Shannon diversity (H'); Equitability or Pielou's evenness (J'). Asterisks indicate significantly differences from the control (* = p < 0.05; ** = $0.05 \le p < 0.001$; *** = $0.001 \le p < 0.0001$).

Twenty-seven meiobenthic nematodes were recognized in the untreated compartments including seven most abundant species were Terchellengia longicaudata ($21,33 \pm 2,22\%$), Paracosoma dubium

were $(19,66 \pm 1,11\%)$, Odontophora villoti $(25 \pm 2,66\%)$, uding Paramonohysteria pilosa $(7,66 \pm 5,55\%)$, and engia Metoncholaimus pristiurus $(16,66 \pm 2,44\%)$. (Table 1).

Table 1. *List and biological traits of nematode genera identified in the control (C) and treated conditions with M1 and M2. Feeding groups according to Wieser (1953) (FG): selective deposit-feeders (1A); epistratum-feeders (2A); non-selective deposit-feeders (1B); omnivores-carnivores (2B); Colonizers-Persisters scores (c-p); Amphid shape (Am): circular (Cr); pocket-like (Pk); spiral (Sp); indistinct (Id); Tail shape (T): conical (co); elongated/filiform (e/f); clavate (cla); Adult length (AL); species absent (-).*

Species	Biolog	icals tr	aits			Treatments		
	FG	c-p	Am	Т	AL	С	M1	M2
Terchellengia longicaudata	1A	3	Cr	e/f	2-4	$21,33 \pm 2,22$	18,66 ± 1,55	6,66 ± 1,11
Terchellengia communis	1A	3	Cr	e/f	2-4	$1,66 \pm 1,11$	$0,33 \pm 0,44$	-
Antichoma acuminata	1A	2	Pk	e/f	1-2	$0,\!33 \pm 0,\!44$	$3,33 \pm 0,44$	$1,33 \pm 1,77$
Marylinia stekhoveni	2A	3	Sp	e/f	1-2	$1,\!66 \pm 0,\!44$	-	$0,\!66\pm0,\!44$
comesoma sp	2A	3	Sp	со	>1	$0,33 \pm 0,44$	-	-
Prochromadoremlla longicaudata	2A	2	Id	со	1-2	$1,33 \pm 0,44$	3 ± 0,66	$0,\!33 \pm 0,\!44$
Cyatholaimus prinzi	2A	3	Sp	со	2-4	$0,\!33 \pm 0,\!44$	-	-
Paracosoma dubium	2A	2	Sp	cla	2-4	19,66 ± 1,11	9 ± 1,33	$7,\!33\pm3,\!77$
Calomicrolaimus honestus	2A	3	Sp	со	1-2	$0,33 \pm 0,44$	28,66 ± 11,11	$14,\!33\pm8,\!22$
Cobbia truncata	2A	3	Cr	e/f	>1	$0,33 \pm 0,44$	-	-
Desmodora de Man, 1889	2A	2	REL	со	1-2	1	$0,\!33 \pm 0,\!44$	-
Spirinia parasitifera (Bastian, 1865)	2A	3	REL	со	2-4	$0,\!33 \pm 0,\!44$	-	-
Odontophora villoti	1B	2	Cr	co	2-4	$25 \pm 2,66$	4,33 ± 2,22	$2,33 \pm 1,77$
Steineria pilosa	1B	2	Cr	cla	1-2	$0,\!66\pm0,\!88$	3 ± 1,33	$0,\!33 \pm 0,\!44$
Ascolaimus elongatus (Butschli, 1874)	1B	2	Cr	co	2-4	1	$1,66 \pm 0,44$	$0,\!66\pm0,\!44$
Theristus sp	1B	2	Cr	со	>1	$0,\!66 \pm 0,\!44$	1	-
Paramonohysteria pilosa	1B	2	Cr	cla	1-2	$7,66 \pm 5,55$	3 ± 1,33	$3,33 \pm 1,77$
Sabatieria granifer	1B	2	Sp	cla	1-2	$0,\!66 \pm 0,\!44$	1	$0,\!66\pm0,\!88$
Daptonema trabeclosum	1B	2	Cr	cla	1-2	3,66 ± 1,55	4 ± 1,33	$3,33 \pm 1,55$

	,							
Methalinhomeus setosus	1B	2	Cr	e/f	2–4	$2,66 \pm 1,11$	$9\pm0,\!66$	$5,66 \pm 2,22$
Promonohysteria pilosa	1B	2	Cr	e/f	1-2	$1,33 \pm 1,11$	-	-
Desmolaimus de Man, 1880	1B	2	Cr	cla	1-2	$1,33 \pm 0,44$	-	-
Synonchella edax	2B	4	Sp	e/f	2–4	$4 \pm 1,33$	$2,66 \pm 1,11$	$1,33 \pm 0,44$
Viscosia glabra	2B	3	Pk	cla	1-2	$0,\!66\pm0,\!88$	-	-
Metoncholaimus pristiurus	2B	3	Pk	cla	2–4	$16,66 \pm 2,44$	$22,33 \pm 6,22$	20 ± 4
Oncholaimus campylocercoides	2B	4	Pk	cla	2–4	$0,\!66\pm0,\!88$	$4 \pm 2,\!66$	-
Oncholaimellus mediterraneus	2B	4	Pk	cla	2–4	$0,66 \pm 0,44$	10,66 ± 3,11	$6,33 \pm 1,77$

Nineteen species were identified in the compartment mixture M1 with four dominant nematodes included Terchellengia longicaudata (21.33 \pm 2.22%). Calomicrolaimus honestus (28,66 ± 11,11%), Metoncholaimus pristiurus $(22,33 \pm 6,22\%)$, and Oncholaimellus mediterraneus $(10,66 \pm 3,11\%)$. Finally, sixteen species were registered in the highest compartment M2 including four principal nematodes comprised (i.e; Terchellengia longicaudata (6,66 \pm 1,11%), Calomicrolaimus honestus (14,33 \pm 8,22%), Metoncholaimus pristiurus ($20 \pm 4\%$), and Oncholaimellus mediterraneus $(6,33 \pm 1,77\%)$ (Table 1).

In Table 2, the dissimilarity percentages between untreated and treated compartments (C vs. M1; and C vs. M2) was augmented respectively (47,88%; and 53,38%). The difference between control and all mixtures conditions was due mainly to the increasing percentage of four species including Calomicrolaimus honestus, Oncholaimellus mediterraneus, Metoncholaimus pristiurus, Metalinhomoeus setosus, and in parallel with the decrease in species named Terchellengia longicaudata, Odontophora villoti, Paracosoma dubium, Paramonhystera pilosa.

Table 2. Dissimilarity percentages (bold values) between control (C) and treated microcosms with M1 and M2 and results of Similarity Percentage analysis (SIMPER) based on square-root transformed data. Species and functional groups accounting for ~ 70% of overall dissimilarity are ranked in order of importance of their contribution. More abundant (+); less abundant (-).

	C vs. M1	C vs. M2		
Species	47,88%	53,38%		
	Calomicrolaimus honestus (+)	Odontophora villoti (-)		
	Odontophora villoti (-)	Terchellengia longicaudata (-)		
	Paracosoma dubium (-)	Calomicrolaimus honestus (+)		
	Oncholaimellus mediterraneus (+)	Paracosoma dubium (-)		
	Metoncholaimus pristiurus (+)	Oncholaimellus mediterraneus (+)		
	Metalinhomoeus setosus (+)	Paramonhystera pilosa (-)		
FG	22,03%	28,31%		
	1B (-)	1B (-)		
	2B (+)	1A(-)		
	2A (+)	2B (+)		
Adult length	15,72%	29,70%		
	1-2 (+)	1-2 (+)		
	2-4 (-)	2-4 (-)		
C-p score	25,29%	25,26%		
	3 (+)	2 (-)		
	2 (-)	3(-)		
	4 (+)			
Tail shape	13,15%	22,51%		
	co (+)	co (-)		
	cla (+)	cla (-)		
	e/f (-)	e/f (-)		
Amphid shape	25,44%	32,18%		
	Cr (-)	Cr (-)		
	Pk (+)	Pk (+)		
	SP (+)	SP (-)		

3.3 Nematodes Functional Traits

In Figure 3, a significant stress values (0.01 - 0.03), and a clear difference between untreated and treated compartments with combined emerging contaminants forming a cluster separated from the one comprising the non-treated communities, were reported in the 2D-nMDS plots. In control compartment, feeding type were represented by 1A, 2A, 1B, and 2B groups, respectively, $20,11 \pm 2,7$; $21,93 \pm 3,65$; $38,05 \pm 6,94$; and $19,89 \pm 5,9$. In the M1 compartment 2A and 2B were augmented while 1B decreased significantly. In the highly contaminated compartment, there was a decrease in addition to group 1A compared to control compartment. Life history indices of nematode control communities were dominated by c-p 2 and c-p 3, respectively, $57.6 \pm 3.72\%$ and $37.81 \pm 4.04\%$. In the both mixture compartment M1 and M2, abundance

of c-p 2 were reduced, while, c-p 3 augmented. Amphid shapes were dominated by three form in control compartment (i.e; $Cr = 57,63 \pm 9,19\%$; Pk = $16,77 \pm 5,93\%$; Sp = 23,22 ± 2,83%). Compartment enriched M1, characterized by the diminished of two form Cr and REL, respectively, $34,77 \pm 1,87\%$; $0,22 \pm$ 0,38%. A further increase in REL form was recorded at the highest contaminated value compartment, $37,74 \pm 6,51\%$. The tail shapes were represented by three form type in the control nematode community, $e/f = 28,72 \pm 4,59\%$; co = 26,05 ± 2,9%; and cla = 45,21± 7,01%. No significant modification was reported in mixture compartment M1 and M2. Also, the control compartment was dominated by two abundant adult length represented by body-size of 2-4 mm and 1-2 mm, comprising $81,98 \pm 5,71\%$; and $16,84\pm 5,94\%$. However, these species did not show significant variation in mixture treated compartment.



Figure 3. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with M1 and M2. Selective deposit feeders (1A); non-selective deposit feeders (1B); epigrowth feeders (2A); omnivores-carnivores (2B); elongated/filiform (e/f); conical; (co); clavate/conical-cylindrical (cla); circular (Cr); indistinct (Id); pocket-like (Pk); spiral (SP). Asterisks indicate significantly differences from the control ($\sqrt{-transformed data: * = p < 0.05; ** = 0.05 \le p < 0.001; *** = 0.001 \le p < 0.0001; *** = 0.0001 \le p$).

Dissimilarity values for all biological traits were exhibited in the high mixture-treated compartments M2 with a value 28,31%; 29,70%; 25,26%; 22,51%; and 32,18%, respectively for feeding diet, adult length, c-p score, tail shape, and amphid shape. The main transformation between the untreated and the treated compartments were a consequence of the modification of all biological traits abundance such as, FG: $[1B\downarrow, 1A\downarrow, 2B\uparrow]$; adult length: $[1-2\uparrow, 2-4\downarrow]$; c-p score: $[2\downarrow, 3\downarrow]$; tail shape: $[co \downarrow, cla \downarrow, e/f \downarrow]$; amphid

shape: $[Cr\downarrow, SP\downarrow, Pk\uparrow]$ table 2. The nMDS secondstep ordination revealed that nematode response to sediment emerging contaminants mixture enrichment essentially depended on amphid shapes (81.17%), adult body size (78.86%) and in second degree on life history (75.56%), feeding diet (73.05%). Tail shape (59.53%) was at least affected and located farther from the taxonomic composition in the graphical representation (Fig. 4).



Figure 4. Non-parametric metric-multidimensional scaling (nMDS) second-stage ordination of inter-matrix rank correlations. Values indicate average similarity percentages between nMDS related to species and those related to functional traits.

4. Discussion

Currently, our knowledge of the combined toxic effects of antibiotics and flame retardants and their interactions on meiobenthic species is very limited and even rare. The main objective of this work was to fill the lack of knowledge on the effects of chronic exposures of two compounds, Ciprofloxacin and Tetrabromodiphenyl ether "BDE-47", on meiobenthic taxa in particular on marine nematofauna. To understand the extent of specific changes within the community, this study focused on the fluctuations degree in abundance, diversity, biological traits within the nematode population. The results of our present study showed that the density of meiobenthic communities decreased significantly in the M1 and M2 mixture compartments compared to the control (Figure 1). In addition, the diversity indices (species number and species richness), and the generic taxonomic list of free-living marine nematodes assessed also showed a lessening (Figure 2, Table 1). The control nematological assemblages were mainly

represented by species with specific biological characteristics such as Terchellengia longicaudata [1A, 3, Cr, e/f, 2–4], Paracosoma dubium [2A, 2, Sp, cla, 2–4], Odontophora villoti [1B, 2, Cr, co, 2–4], Paramonohysteria pilosa [1B, 2, Cr, cla, 1–2], and Metoncholaimus pristiurus [2B, 3, Pk, cla, 2–4]. Exposure to mixtures of emerging contaminants at low and high concentrations, respectively, M1 and M2, led to a proliferation of epistrate-eating species 2A and omnivores 2B and a decrease in species bacteriophages 1A and 1B.

It can be assumed that a certain synergy of effect between the two contaminants tested, as reported in other studies on other emerging pollutants (Billionnet et al., 2012; González-Naranjo et al., 2015). Thus, research into the characteristics of each molecule shows that BDE-47 is weakly degradable, hydrophobic, persistent with a lifespan of the order of five months (Tomy et al., 2004), and intensely adsorbed on particles sediments (Li et al., 2012).

Ciprofloxacin, a compound with strong adsorption affinity to sediment particles (Halling-Sørensen et al., 2003), and weakly biodegradable (Kümmerer et al., 2000). These two pollutants have shown toxic effects when exposed separately in past works. For example, all meiofauna abundances as well as ecological diversity indices of nematodes were considerably modified following the sedimentary enrichment in BDE-47 (Nasri et al., 2021a, 2021b). In addition, a restructuring of the nematode assemblages marked by a decrease in the abundance of the genera Terschellingia [1A, e/f, cr, 2-4], Paracomesoma [2A, cla, sp, 2-4], Paramonohystera [1B, Cr, cla, 1-2] and Odontophora [1B, Cr, co, 2-4], and an increase in the genera of Oncholaimellus [2B, Pk, cla, > 4] and Metoncholaimus [2B, cla, pk, 2-4], were responsible for these community edits (Nasri et al., 2021b). Also, these are the three biological groups, feeding group, adult length and amphid shape were the most modified, and that the genera with selective deposit-diet (1A), of circular amphid type and of length between 2 and 4 mm, such as Terschellingia; and omnivorouscarnivorous (2B) with functional-type genera [Pk, cla, 2-4], such as Metoncholaimus; are both responsible for modifying the generic list. For ciprofloxacin, it altered the abundance of all meiobenthic taxa, as well as altering nematode community structure (Nasri et al., 2020c). This restructuring is the consequence on the one hand, of a lessening in the abundance of the genera Terschellingia [1A, e/f, cr, 3], Anticoma [1A, e/f, Pk, 2], Prochromadorella [2A, co, Id, 2], Paracomesoma [2A, cla, sp, 2]; and on the other hand, an increase in the genera Synonchiella [2B, e/f, Sp, 4], and Metoncholaimus [2B, cla, pk, 3] (Nasri et al., 2020b). Moreover, they are both functional traits, i.e., trophic-diet and tail shape, were the most touched by ciprofloxacin and responsible to these taxonomic modifications (Nasri et al., 2020b).

The sediments constitute a biotope characterized by the presence of the food sources necessary for the life of the various meiobenthic taxa, thus, the presence of toxic compounds risks causing major problems for these organisms (Ravera, 2001). Sediment enrichment with weak or strong mixtures of emerging pollutants M1 and M2 has created an imbalance in these experimental ecosystems. The explanation of the present results suggests that these two contaminants present similar mechanisms of toxicity targeting the composition of bacterial assemblages, causing a decrease in their abundance and community diversity within the biotope (Girardi et al., 2011; Li et al., 2018). An alteration in the abundance of bacteria which present the main food source for the bacteriophage population located in the sediments. This is the case observed in our study where the addition of ciprofloxacin and PDE-47 in a mixture induced a reduction in the abundances of 1A and 1B eaters of microbes, and the increase in those of opportunists, 2B and 2A, classified respectively as omnivorous predators and epistrate-eaters (2A), leading to a generic restructuring of nematodes as already recorded in other works (Nasri et al., 2021c, 2020b).

5. Conclusion

Due to their hydrophobic properties, persistence and strong adsorption on sedimentary particles, the chronic exposure of ciprofloxacin/BDE-47 mixture causes negative effects on meiobenthic taxa from Bizerte lagoon, especially, ecological indices, taxonomic structure, and functional trait of marine nematodes. Our data support the use of diet biological trait as an indicator to detect compounds targeting bacterial-like nutrient sources.

Acknowledgments

Faculty of Science of Bizerte, University of Carthage, was supported this work. We thank Professor Patricia Aïssa, a specialist of meiobenthic nematodes for nematode identification and paper revision.

6. References

- Barbier, E.B., 2017. Marine ecosystem services. Curr. Biol. 27, R507–R510. https://doi.org/10.1016/J. CUB.2017.03.020
- Ben Ameur, W., El Megdiche, Y., Eljarrat, E., Ben Hassine, S., Badreddine, B., Souad, T., Bèchir, H., Barceló, D., Ridha Driss, M., 2013. Organochlorine and organobromine compounds in a benthic fish (Solea solea) from Bizerte Lagoon (northern Tunisia): Implications for human exposure. Ecotoxicol. Environ. Saf. 88, 55–64. https://doi.org/10.1016/J. ECOENV.2012.10.021
- Billionnet, C., Sherrill, D., Annesi-Maesano, I., 2012. Estimating the Health Effects of Exposure to Multi-Pollutant Mixture. Ann. Epidemiol. 22, 126–141. https://doi.org/10.1016/J.ANNEPIDEM.2011.11.004
- 4. Bongers, T., Alkemade, R., Yeates, G.W., 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar. Ecol. Prog. Ser. https:// doi.org/10.3354/meps076135
- 5. Bradley, P.M., Battaglin, W.A., Clark, J.M., Henning, F.P., Hladik, M.L., Iwanowicz, L.R., Journey, C.A.,

Riley, J.W., Romanok, K.M., 2017. Widespread occurrence and potential for biodegradation of bioactive contaminants in Congaree National Park, USA. Environ. Toxicol. Chem. 36, 3045–3056. https://doi.org/10.1002/ETC.3873

- Bramwell, L., Fernandes, A., Rose, M., Harrad, S., Pless-Mulloli, T., 2014. PBDEs and PBBs in human serum and breast milk from cohabiting UK couples. Chemosphere 116, 67–74. https://doi.org/10.1016/J. CHEMOSPHERE.2014.03.060
- CLARKE, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x
- Costa, L.G., Giordano, G., Tagliaferri, S., Caglieri, A., Mutti, A., 2008. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potential adverse health effects. Acta Biomed. 79, 172–183.
- Coull, B.C., Chandler, G.T., 1992. Pollution and meiofauna: field, laboratory, and mesocosm studies, in: Oceanography and Marine Biology: An Annual Review. Vol. 30. University College London Press, pp. 191–271.
- De Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583– 624. https://doi.org/10.1016/S0045-6535(01)00225-9
- El Megdiche, Y., Ameur, W. Ben, Bèchir, H., Hassine, S. Ben, Badreddine, B., Touil, S., Driss, M.R., Eljarrat, E., Barceló, D., 2017. Anthropogenic (PBDE) and naturally-produced (MeO-PBDE) brominated compound levels in Bizerte Lagoon clams (Ruditapes decussatus): Levels and human health risk assessment. Mar. Pollut. Bull. 125, 176–185. https:// doi.org/10.1016/J.MARPOLBUL.2017.08.005
- Gambi, C., Dell'Anno, A., Corinaldesi, C., Lo Martire, M., Musco, L., Da Ros, Z., Armiento, G., Danovaro, R., 2020. Impact of historical contamination on meiofaunal assemblages: The case study of the Bagnoli-Coroglio Bay (southern Tyrrhenian Sea). Mar. Environ. Res. 156, 104907. https://doi.org/10.1016/J. MARENVRES.2020.104907
- Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. J. Hazard. Mater. 198, 22–30. https://doi.org/10.1016/J.JHAZMAT.2011.10.004
- González-Naranjo, V., Boltes, K., de Bustamante, I., Palacios-Diaz, P., 2015. Environmental risk of combined emerging pollutants in terrestrial environments: Chlorophyll a fluorescence analysis. Environ. Sci. Pollut. Res. 22, 6920–6931. https://doi. org/10.1007/S11356-014-3899-Z/FIGURES/3

- 15. Haddad, T., Baginska, E., Kümmerer, K., 2015. Transformation products of antibiotic and cytostatic drugs in the aquatic cycle that result from effluent treatment and abiotic/biotic reactions in the environment: An increasing challenge calling for higher emphasis on measures at the beginning of the pipe. Water Res. 72, 75–126. https://doi.org/10.1016/J. WATRES.2014.12.042
- Halling-Sørensen, B., Sengeløv, G., Ingerslev, F., Jensen, L.B., 2003. Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquindox due to environmental processes. Arch. Environ. Contam. Toxicol. 44, 7–16. https://doi.org/10.1007/S00244-002-1234-Z/ METRICS
- 17. Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Indic. 8, 1–13. https://doi.org/10.1016/J.ECOLIND.2007.06.002
- Kümmerer, K., Al-Ahmad, A., Mersch-Sundermann, V., 2000. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere 40, 701–710. https://doi.org/10.1016/S0045-6535(99)00439-7
- Li, C.Y., Dempsey, J.L., Wang, D., Lee, S.W., Weigel, K.M., Fei, Q., Bhatt, D.K., Prasad, B., Raftery, D., Gu, H., Cui, J.Y., 2018. PBDEs altered gut microbiome and bile acid homeostasis in male C57BL/6 mice. Drug Metab. Dispos. 46, 1226–1240. https://doi. org/10.1124/DMD.118.081547/-/DC1
- Li, Y., Lin, T., Chen, Y., Hu, L., Guo, Z., Zhang, G., 2012. Polybrominated diphenyl ethers (PBDEs) in sediments of the coastal East China Sea: Occurrence, distribution and mass inventory. Environ. Pollut. 171, 155–161. https://doi.org/10.1016/J. ENVPOL.2012.07.039
- Nasri, A., Aïssa, P., Beyrem, H., Mahmoudi, E., Nasri, A., Aïssa, P., Beyrem, H., Mahmoudi, E., 2022a. New Approach for the Evaluation of Ecological Quality in the Mediterranean Coastal Ecosystems, Case Study of Bizerte Lagoon: Marine Nematodes Functional Traits Assessment. Nematodes - Recent Adv. Manag. New Perspect. https://doi.org/10.5772/ INTECHOPEN.98815
- Nasri, A., Allouche, M., Hannachi, A., Barhoumi, B., Wahbi, A., Harrath, A.H., Mahmoudi, E., Beyrem, H., Boufahja, F., 2021a. Ecotoxicity of polybrominated diphenyl ether (BDE-47) on a meiobenthic community with special emphasis on nematodes: Taxonomic and trophic diversity assessment. Environ. Pollut. 277, 116727. https://doi.org/10.1016/j. envpol.2021.116727
- 23. Nasri, A., Allouche, M., Hannachi, A., Barkaoui, T., Barhoumi, B., Saidi, I., D'Agostino, F., Mahmoudi, E.,

Beyrem, H., Boufahja, F., 2020a. Nematodes trophic groups changing via reducing of bacterial population density after sediment enrichment to ciprofloxacin antibiotic: Case study of Marine Mediterranean community. Aquat. Toxicol. 228, 105632. https://doi. org/10.1016/J.AQUATOX.2020.105632

- Nasri, A., Allouche, M., Hannachi, A., Harrath, A.H., Aldahmash, W., Alwasel, S., Mahmoudi, E., Beyrem, H., Boufahja, F., 2020b. Restructuring of a meiobenthic assemblage after sediment contamination with an antibacterial compound: Case study of ciprofloxacin. Ecotoxicol. Environ. Saf. 205, 111084. https://doi. org/10.1016/J.ECOENV.2020.111084
- 25. Nasri, A., Boufahja, F., Hedfi, A., Mahmoudi, E., Aïssa, P., Essid, N., 2014. Impact of penicillin G on the trophic diversity (Moens & Vincx, 1997) of marine nematode community: Results from microcosm experiments. Cah. Biol. Mar. 56, 65–72.
- 26. Nasri, A., Boufahja, F., Hedfi, A., Mahmoudi, E., Aïssa, P., Essid, N., 2013. REPONSE DES DIFFERENTS TAXONS MEIOFAUNISTIQUES A LA CONTAMINATION PAR UN ANTIBIOTIQUE (LA PENICILLINE G) : ETUDE MICROCOSMIQUE, Bull. Inst. Natn. Scien. Tech. Mer de Salammbô.
- 27. Nasri, A., Hannachi, A., Allouche, M., Aydi, A., Aïssa, P., Beyrem, H., Mahmoudi, E., Nasri, A., Hannachi, A., Allouche, M., Aydi, A., Aïssa, P., Beyrem, H., Mahmoudi, E., 2022b. Marine Free-Living Nematodes as Tools for Environmental Pollution Assessment: A Special Focus on Emerging Contaminants Impact in the Tunisian Lagoon Ecosystems. Toxic. Environ. Pollut. https://doi.org/10.5772/INTECHOPEN.104721
- 28. Nasri, A., Hannachi, A., Allouche, M., Barhoumi, B., Barkaoui, T., Wahbi, A., D'Agostino, F., Mahmoudi, E., Beyrem, H., Boufahja, F., 2021b. Using meiobenthic taxa, nematofauna biological traits, and bacterial abundance to assess the effects of the polybrominated diphenyl ethers compound: Case study of tetrabromo diphenyl ether BDE-47. Sci. Total Environ. 770, 145251. https://doi.org/10.1016/J. SCITOTENV.2021.145251
- 29. Nasri, A., Hannachi, A., Allouche, M., Barhoumi, B., Barkaoui, T., Wahbi, A., D'Agostino, F., Mahmoudi, E., Beyrem, H., Boufahja, F., 2021c. Using meiobenthic taxa, nematofauna biological traits, and bacterial abundance to assess the effects of the polybrominated diphenyl ethers compound: Case study of tetrabromo diphenyl ether BDE-47. Sci. Total Environ. 770, 145251. https://doi.org/10.1016/j. scitotenv.2021.145251
- Nasri, A., Hannachi, A., Allouche, M., Barhoumi, B., Saidi, I., Dallali, M., Halim Harrath, A., Mansour, L., Mahmoudi, E., Beyrem, H., Boufahja, F., 2020c.

Chronic ecotoxicity of ciprofloxacin exposure on taxonomic diversity of a meiobenthic nematode community in microcosm experiments. J. King Saud Univ. - Sci. 32, 1470–1475. https://doi.org/10.1016/j. jksus.2019.11.044

- 31. Nasri, A., Jouili, S., Boufahja, F., Hedfi, A., Saidi, I., Mahmoudi, E., Aïssa, P., Essid, N., Hamouda, B., 2016. Trophic restructuring (Wieser 1953) of freeliving nematode in marine sediment experimentally enriched to increasing doses of pharmaceutical penicillin G. Ecotoxicology 25, 1160–1169. https:// doi.org/10.1007/s10646-016-1670-6
- Noyes, P.D., Kelly, S.M., Mitchelmore, C.L., Stapleton, H.M., 2010. Characterizing the in vitro hepatic biotransformation of the flame retardant BDE 99 by common carp. Aquat. Toxicol. 97, 142–150. https://doi.org/10.1016/J.AQUATOX.2009.12.013
- Parolini, M., Pedriali, A., Binelli, A., 2012. Variation of antioxidant activity in dreissena polymorpha specimens exposed to 2,2',4,4',5,6'-hexa BDE (BDE-154). Water. Air. Soil Pollut. 223, 3067–3076. https:// doi.org/10.1007/S11270-012-1089-5/TABLES/1
- 34. Platt, H.M., Warwick, R.M., 1983. Freeliving marine nematodes. Part 1. British Enoplids. Synopses of the British Fauna No. 28.
- 35. Platt, H.M., Warwick, R.M. (Richard M., 1988. Freeliving marine nematodes. Pt. 2, British chromadorids. 502.
- Ravera, O., 2001. Monitoring of the aquatic environment by species accumulator of pollutants: a review. J. Limnol. 60, 63–78. https://doi.org/10.4081/ JLIMNOL.2001.S1.63
- Schratzberger, M., Warr, K., Rogers, S.I., 2007. Functional diversity of nematode communities in the southwestern North Sea. Mar. Environ. Res. https:// doi.org/10.1016/j.marenvres.2006.10.006
- Semprucci, F., Cesaroni, L., Guidi, L., Balsamo, M., 2018. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? Mar. Environ. Res. 135, 114–122. https:// doi.org/10.1016/j.marenvres.2018.02.001
- Soclo, H.H., Garrigues, P., Ewald, M., 2000. Origin of Polycyclic Aromatic Hydrocarbons (PAHs) in Coastal Marine Sediments: Case Studies in Cotonou (Benin) and Aquitaine (France) Areas. Mar. Pollut. Bull. 40, 387–396. https://doi.org/10.1016/S0025-326X(99)00200-3
- 40. Tang, J., Shi, T., Wu, X., Cao, H., Li, X., Hua, R., Tang, F., Yue, Y., 2015. The occurrence and distribution of antibiotics in Lake Chaohu, China: Seasonal variation, potential source and risk assessment. Chemosphere 122, 154–161. https://doi. org/10.1016/J.CHEMOSPHERE.2014.11.032

- Tangherlini, M., Corinaldesi, C., Rastelli, E., Musco, L., Armiento, G., Danovaro, R., Dell'Anno, A., 2020. Chemicalcontaminationcanpromoteturnoverdiversity of benthic prokaryotic assemblages: The case study of the Bagnoli-Coroglio bay (southern Tyrrhenian Sea). Mar. Environ. Res. 160, 105040. https://doi. org/10.1016/J.MARENVRES.2020.105040
- 42. Thistle, D., Lambshead, P.J.D., Sherman, K.M., 1995. Nematode tail-shape groups respond to environmental differences in the deep sea. Vie Milieu.
- Todd, P.A., Heery, E.C., Loke, L.H.L., Thurstan, R.H., Kotze, D.J., Swan, C., 2019. Towards an urban marine ecology: characterizing the drivers, patterns and processes of marine ecosystems in coastal cities. Oikos 128, 1215–1242. https://doi.org/10.1111/ OIK.05946
- 44. Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: A review. Environ. Sci. Technol. 35, 3397– 3406. https://doi.org/10.1021/ES0003021/ASSET/ IMAGES/MEDIUM/ES0003021E00004.GIF

- 45. Tomy, G.T., Palace, V.P., Halldorson, T., Braekevelt, E., Danell, R., Wautier, K., Evans, B., Brinkworth, L., Fisk, A.T., 2004. Bioaccumulation, Biotransformation, and Biochemical Effects of Brominated Diphenyl Ethers in Juvenile Lake Trout (Salvelinus namaycush). Environ. Sci. Technol. 38, 1496–1504. https:// doi.org/10.1021/ES035070V/ASSET/IMAGES/ LARGE/ES035070VF00005.JPEG
- Warwick, R., Platt, H.M., Somerfield, P., 1998. Freeliving marine nematodes: Part III. Monhysterida. Synopses of the British Fauna No. 53.
- 47. Wieser, W., 1953. Die Beziehung zwischen Mundh o"hlengestalt,Erna"hrungsweise und Vorkommen bei freilebenden marinen Nematoden. Ark. fu"r Zoolgie.
- Zuccato, E., Castiglioni, S., Bagnati, R., Melis, M., Fanelli, R., 2010. Source, occurrence and fate of antibiotics in the Italian aquatic environment. J. Hazard. Mater. 179, 1042–1048. https://doi. org/10.1016/J.JHAZMAT.2010.03.110