

RESEARCH ARTICLE

Is the Change in the Nematological Biological Trait an Indicator of the Benthic Ecosystem Disturbance Following Sedimentary Enrichment in Micro-Sized Polyethylene (Pe-MPs): A Specific Focus on Free-Living Marine Nematofauna?

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, Institut of Biotechnology of Beja, Biology Department, BP: 382, Tunisia.

Received: 04 June 2024 Accepted: 19 June 2024 Published: 24 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

Free-living nematofauna are valuable biological indicators for different environmental disturbances. Their taxonomic structure and biological traits often respond to environmental changes. In our study, meiobenthic nematodes, collected from the Bizerte lagoon on the Tunisia northeastern coast, were examined in terms of their structure composition and functional traits after treatment with the utmost detected micro-sized polyethylene microplastics (PE-MPs) in marine biota. Three environmental PE-polymers levels: [PE1 (0.1 µg/g Dry weight (DW)), PE2 (1 µg/g DW), and PE3 (10 µg/g DW)], were progressively added and experiment was conducted for 30 days in the presence or absence of meiofauna assemblages. A lesser nematodes taxonomic structure was detected in all compartments tested. The non-parametric metric-multidimensional scaling (nMDS-2D plots) and the biological trait (relative abundances) of nematode species indicated that the change of all abundance. Nevertheless, all functional traits such as feeding diet, adult length, c-p score, tail shape, and amphid shape, exhibited a clear modification between the untreated and the treated compartments. The average dissimilarity between nematofauna species and biological traits groups augmented with PE-MPs-supplemented sediments. The nMDS second-stage ordination for matrices including nematodes species and functional traits displayed that the feeding diet was the closest to the taxonomic species distribution.

Keywords: PE-MPs Exposure, Nematodes Species, Population Response, Taxonomic Structure, Biological Traits.

1. Introduction

Coastal lagoons are intermediate milieus between continental terrestrial and marine ecosystems that represent about 13% of the ecosphere's coastlines (Pérez-Ruzafa et al., 2019). The coastal lagoons water

is commonly calm, and is changed by high and low tidal, river flows, wind action, rainfall, and evaporation (Kennish, 2016; Pérez-Ruzafa et al., 2019). Coastal lagoons present a great position via biodiversity preservation and for the coastal human populations safety (Newton et al., 2018; Pérez-Ruzafa et al., 2019).

Citation: Ahmed Nasri, Fouzi Bouleefah, Amel Hannachi, *et al.* Is the Change in the Nematological Biological Trait an Indicator of the Benthic Ecosystem Disturbance Following Sedimentary Enrichment in Micro-Sized Polyethylene (Pe-MPs): A Specific Focus on Free-Living Marine Nematofauna?. *Journal of Aquatic Science and Marine Biology*. 2024;5(1): 33-45.

©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.

These aquatic ecosystems are interconnected with the terrestrial environment; therefore, subject to changes brought about by the increase in rapid urbanization and industrialization over the past century. They are under menace produced by the pollutants accumulation, the interact of toxic elements with each other (Huang et al., 2021), causing disappearance various ecological and commercial importance species (Vázquez-Rowe et al., 2021).

Numerous studies have revealed that plastic waste is considered a new emerging pollutant reaching coastal ecosystems via direct discharges or arriving via rivers (Paduani, 2020). These synthetic compounds have specific characteristics such as low cost, durability, and resistance to degradation, therefore usable in many applications such as packing, construction, electrical and electronics industry. A subcategory of plastics in the environment are microplastics (MP), that are plastic particles of sizes <5 mm to 1 µm, which are considered a widely dispersed pollutant in many countries (Anagnosti et al., 2021). They have had global concern due to their degradation resistance and possible risk to littoral ecosystems.

The Bizerte littoral lagoon located in northern Tunisia is exposed to anthropogenic pressures such as the development of industrial activities, urban releases and various pressures (Aydi et al., 2022; Hannachi et al., 2022; Nasri et al., 2022b, 2022d, 2022a), touching biodiversity, ecological equilibrium (Allouche et al., 2021a; Nasri et al., 2021b, 2021a, 2020b, 2020a). This coastal lagoon has been considered as a sink for MPs, being a preferential shallow area for the accumulation of these polymers (Abidli et al., 2021). These compounds could come from discarded plastic that breaks down into smaller pieces (Julienne et al., 2019), while the presence of fibers could be associated with the discharged untreated wastewater (Haddout et al., 2021). Also, the dissemination of PMs could also be due to the movement of suspended matter in coastal waters and rivers (Haddout et al., 2021).

Sediments are defined as the final MPs destination in marine environments (Woodall et al., 2014). In Bizerte lagoon, Abidli et al. (2017) are identified the MPs presence in sediments with the number was at the range of 3000–18,000 items kg⁻¹ dry sediment. Due to their residence time in the environment estimated at 10 to 100 years (Zbyszewski and Corcoran, 2011), their location at 0-10 cm of sediments surface depth (Dong et al., 2020) with concentrations that can reach 1 to 10 g kg⁻¹ of dry sediment (Hurley et al., 2018), MPs consequently present a significant risk to

organisms living in this biotope, in particular benthic invertebrates given their food habits and their close contact with sediments (Scherer et al., 2020). In this context, meiobenthic fauna, in particular marine nematodes, have been used as bio-indicators of the environment quality enriched experimentally with increasing doses of PE-MPs, the most frequently plastic detected in aquatic ecosystems, thanks to the evaluation of namatofauna species taxonomic structure and biological traits.

2. Materials and Methods

2.1. Polyethylene-MPs

Marine nematodes have been subjected to the most studied PE-MPs of size (ø 40–48 µm) (Tosetto et al., 2016). The experimental environmental PE levels used were: 0.1 µg/g, 1 µg/g, and 10 µg/g with a control group was run in parallel with no PE-MPs.

2.2. Sampling Location and Experiment Onset

Sediment containing meiobenthic nematodes were collected from the Bizerte lagoon (Menzel Jemil), 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 60 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 PE-MPs 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 PE-MPs. After five 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 three environmental levels of PE-MPs: PE1 (0.1 µg.g⁻¹), PE2 (1 µg.g⁻¹) and PE3 (10 µg.g⁻¹) (Hurley et al., 2018).

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., 2016). 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., 2015). Then, the meiofauna was gathered on a 45 µm sieve and then sorted manually using a Nikon SMZ745 stereomicroscope. The first 100 marine nematodes were mounted on permanent slides and identified to

specific scale by following pictorial keys from (Platt and Warwick, 1983, 1988), (Warwick et al., 1998) and NeMys online identification keys (Nemys, 2022) using an Olympus BX51 compound microscope (Olympus, Tokyo, Japan).

Feeding diet and four biological traits (c-p score, tail shape, adult length, amphid shape of each nematodes species were investigated. Therefore, based on the buccal cavity type according to (Wieser, 1953), feeding diet were identified as: non-selective 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 tail shape was divided into four different 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 four groups (< 1 mm, 1–2 mm, 2–4 mm, and > 4 mm) (Schratzberger et al., 2007). The amphid shapes were grouped 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).

2.3. Data Analysis

Data analyzes followed standard methods described by (CLARKE, 1993; Clarke and Warwick (2001) using STATISTICA (v5.1) software (Nasri et al., 2013, 2022b, 2021a). 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; Clarke and Warwick, 2001). 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 PE-MPs via spatial distribution. SIMPER 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. Nematodes Structure

All nematodes species abundance was significantly changed compared to the controls (Table 1). Twenty-one species including 4 most abundant species were identified in the control conditions such as: *Terschellingia longicaudata* (15,33 ± 1,55 %), *Paramonohystera pilosa* (14,33 ± 1,11%), *Daptonema trabeculosum* (10,66 ± 1,77%), *Metalinhomoeus setosus* (16 ± 0,66%). In compartment PE1, eighteen species were recognized with five dominant named: *Terschellingia longicaudata* (13 ± 1,33 %), *Cyatholaimus prinzi* (6,66 ± 0,44%), *Paramonohystera pilosa* (5,33 ± 1,11%), *Daptonema trabeculosum* (8,33 ± 1,11%), and *Metalinhomoeus setosus* (11 ± 0,66%). In compartment PE2, seventeen were documented with five dominant named: *Terschellingia longicaudata* (11,66 ± 1,11%), *Cyatholaimus prinzi* (6,33 ± 0,44%), *Paramonohystera pilosa* (4,33 ± 1,55%), *Daptonema trabeculosum* (6,66 ± 1,55%), and *Metalinhomoeus setosus* (6 ± 0,66%). In the high treated PE3 compartment, sixteen species were found including four abundant named: *Terschellingia longicaudata* (6 ± 0,66%), *Paracomesoma dubium* (4 ± 1,33%), *Daptonema trabeculosum* (4,33 ± 1,11%), *Metalinhomoeus setosus* (5,33 ± 1,11%) (Table 1).

Table 1. List and biological traits of nematode genera identified in the control (C) and treated conditions with PE (PE1, PE2 and PE3). 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	Biologicals traits					Treatments	C	PE1	PE2	PE3
	FG	c-p	Am	T	AL					
<i>Terschellingia longicaudata</i>	1A	3	Cr	e/f	2–4	15,33 ± 1,55	13 ± 1,33	11,66 ± 1,11	6 ± 0,66	
<i>Terschellingia communis</i>	1A	3	Cr	e/f	2–4	1,66 ± 0,88	1,33 ± 1,11	0,66 ± 0,88	-	
<i>Anticoma acuminata</i>	1A	2	Pk	e/f	1–2	0,66 ± 0,44	3 ± 0,66	1,66 ± 1,55	1 ± 0,66	
<i>Prochromadorella longicaudata</i>	2A	2	Id	co	1–2	2,33 ± 1,11	1,33 ± 0,44	1 ± 0,66	1,66 ± 0,44	
<i>Cyatholaimus prinzi</i>	2A	3	Sp	co	2–4	4 ± 0,66	6,66 ± 0,44	6,33 ± 0,44	3,33 ± 0,44	

<i>Paracomerosoma dubium</i>	2A	2	Sp	cla	2–4	3 ± 0,66	1,33 ± 0,44	0,66 ± 0,88	4 ± 1,33
<i>Calomicrolaimus honestus</i>	2A	3	Sp	co	1–2	3,66 ± 1,77	1,33 ± 0,44	0,66 ± 0,88	0,66 ± 0,88
<i>Microlaimus cyatholaimoides</i>	2A	2	Cr	co	2–4	0,66 ± 0,44	-	-	-
<i>Desmodora de Man, 1889</i>	2A	2	REL	co	1–2	1,33 ± 0,44	-	-	-
<i>Paramonohystera pilosa</i>	1B	2	Cr	cla	1–2	14,33 ± 1,11	5,33 ± 1,11	4,33 ± 1,55	3,66 ± 1,11
<i>Promonohystera Wieser</i>	1B	2	Cr	cla	1–2	3,33 ± 1,11	-	-	-
<i>Daptonema trabeculosum</i>	1B	2	Cr	cla	1–2	10,66 ± 1,77	8,33 ± 1,11	6,66 ± 1,55	4,33 ± 1,11
<i>Metalinhomoeus setosus</i>	1B	2	Cr	e/f	2–4	16 ± 0,66	11 ± 0,66	6 ± 0,66	5,33 ± 1,11
<i>Odontophora villoti</i>	1B	2	Cr	co	2–4	6,33 ± 1,11	2 ± 0,66	1 ± 0,66	1 ± 0,66
<i>Steineria pilosa</i>	1B	2	Cr	cla	1–2	1,33 ± 0,44	3 ± 1,33	3 ± 0,66	0,33 ± 0,44
<i>Ascolaimus elongatus</i>	1B	2	Cr	co	2–4	1,33 ± 0,44	0,66 ± 0,88	1,33 ± 1,11	1 ± 0,66
<i>Synonchiella edax</i>	2B	4	Sp	e/f	2–4	1,33 ± 0,44	1,66 ± 0,88	1,66 ± 0,44	1 ± 0,66
<i>Viscosia cobbi</i>	2B	3	Pk	cla	1–2	1,66 ± 0,88	1,33 ± 0,44	0,66 ± 0,44	0,33 ± 0,44
<i>Metoncholaimus pristiurus</i>	2B	3	Pk	cla	2–4	0,66 ± 0,44	1,33 ± 0,44	-	-
<i>Oncholaimus campylocercoides</i>	2B	4	Pk	cla	2–4	1,33 ± 0,44	1,33 ± 0,44	1,33 ± 0,44	1,66 ± 0,88
<i>Oncholaimellus mediterraneus</i>	2B	4	Pk	cla	2–4	2,33 ± 0,44	1,66 ± 0,88	1,66 ± 0,88	1 ± 0,66

In the table 2, the simpser analysis showed that the average dissimilarities between control and treated compartments increased ((29,89 – 48,94%). Moreover, data indicated that the main modifications between the control and the PE-MPs

treatments were a result of the lessening of species identified *Paramonohystera pilosa*, *Metalinhomoeus setosus*, *Odontophora villoti*, *Paramonohystera wieser*, *Terschellingia longicaudata*, *Daptonema trabeculosum*, *Calomicrolaimus honestus*.

Table 2. Dissimilarity percentages (bold values) between control (C) and treated microcosms with PE (PE1, PE2 and PE3) 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. P1	C vs. P2	C vs. P3
Species	29,89%	38,69%	48,94%
	<i>Paramonohystera pilosa</i> (-)	<i>Paramonohystera pilosa</i> (-)	<i>Metalinhomoeus setosus</i> (-)
	<i>Metalinhomoeus setosus</i> (-)	<i>Metalinhomoeus setosus</i> (-)	<i>Paramonohystera pilosa</i> (-)
	<i>Odontophora villoti</i> (-)	<i>Odontophora villoti</i> (-)	<i>Terschellingia longicaudata</i> (-)
	<i>Paramonohystera Wieser</i> (-)	<i>Daptonema trabeculosum</i> (-)	<i>Daptonema trabeculosum</i> (-)
	<i>Terschellingia longicaudata</i> (-)	<i>Terschellingia longicaudata</i> (-)	<i>Odontophora villoti</i> (-)
	<i>Daptonema trabeculosum</i> (-)	<i>Paramonohystera Wieser</i> (-)	<i>Paramonohystera Wieser</i> (-)
	<i>Cyatholaimus prinzi</i> (-)	<i>Cyatholaimus prinzi</i> (+)	<i>Calomicrolaimus honestus</i> (-)
FG	21,33%	29,13%	43,97%
	1B (-)	1B (-)	1B (-)
	2A (-)	2A (-)	2A (-)
	1A (-)	1A (-)	1A (-)
Adult length	17,68%	28,53%	43,97%
	1–2 (-)	1–2 (-)	1–2 (-)
	2–4 (-)	2–4 (-)	2–4 (-)
C-p score	19,57%	29,47%	43,97%
	2 (-)	2 (-)	2 (-)
	3 (-)	3(-)	3(-)
Tail shape	18,22%	28,53%	43,97%
	cla (-)	cla (-)	cla (-)
	co (-)	co (-)	co (-)
	e/f (-)	e/f (-)	e/f (-)
Amphid shape	21,32%	30,05%	44,66%
	Cr (-)	Cr (-)	Cr (-)
	Pk (+)	Pk (+)	SP (-)
	SP (-)	SP (-)	

3.2. Nematodes Biological Traits

The 2D-nMDS plots in Figure (1 – 6) shows that the functional traits indicating significant stress values fluctuating between 0.01 and 0.03, and a clear difference between control and treated compartments with PE-MPs, forming a cluster separated from the one comprising the uncontaminated communities.

Feeding diet were dominated by types 1B and 1A,

representing $57.07 \pm 1.8\%$ and $19.02 \pm 2.68\%$ of the control nematode community, respectively. Contamination by PE-MPs induced significant decrease of trophic group 1B in PE2 and PE3. Conversely, an increase in trophic group 2A was reported only in the PE3 compartment. However, no effect was noticed for trophic groups 1A and 2B compared to controls (Fig. 1).

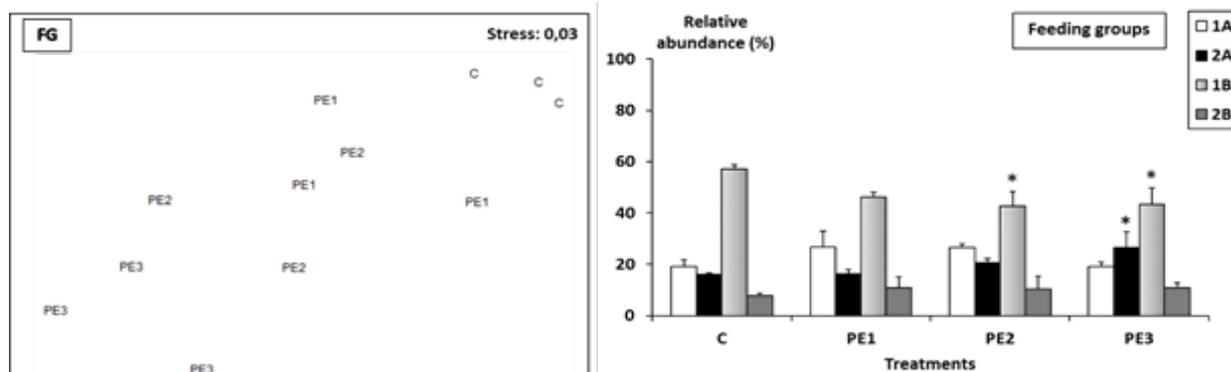


Figure 1. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Selective deposit feeders (1A); non-selective deposit feeders (1B); epigrowth feeders (2A); omnivores-carnivores (2B). Asterisks indicate significant differences from the control ($\sqrt{\text{transformed data}}$: * = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$; **** = $0.0001 \leq p$).

Life history indices or c-p score showed that nematode communities were dominated by c-p2 ($65.7 \pm 2.25\%$) and c-p3 ($28.96 \pm 2.85\%$) species in the

control compartment. Only c-p2 showed a significant decrease in only the PE1-treated weak compartment (Fig. 2).

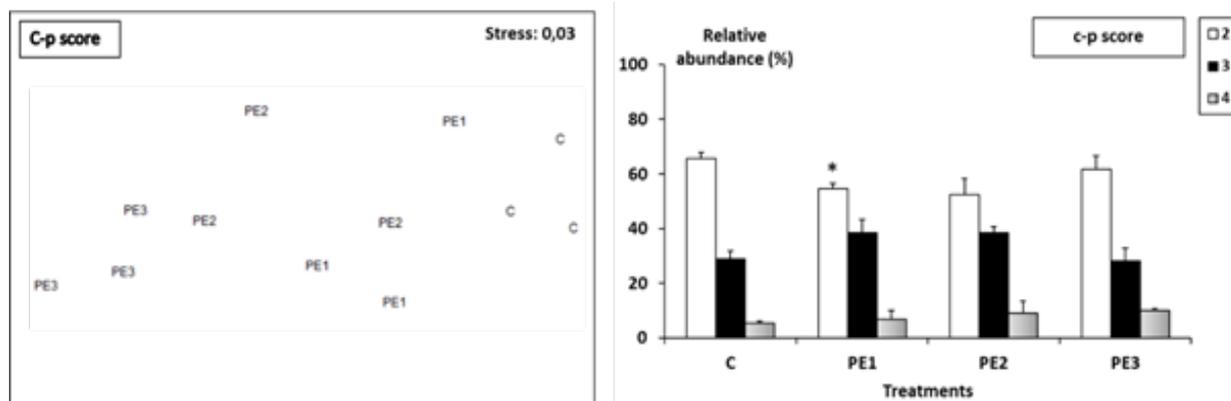


Figure 2. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Asterisks indicate significant differences from the control ($\sqrt{\text{transformed data}}$: * = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$; **** = $0.0001 \leq p$).

The amphid shapes were dominated by the form Cr and to a lesser extent by the form Sp, representing $76.1 \pm 1.01\%$ and 12.77 ± 2.18 , respectively, of the nematodes community in control compartments. Sediment enrichment in MPs induced a significant decrease in the type of REL form in all treated compartments, and Cr only in PE3. However, the Sp form showed a significant increase in PE2 and PE3 treatments compared to the control. The Id and Pk forms did not show any significant variation (Fig. 3).

The tail shapes were dominated by the e/f type ($37.65 \pm 4.21\%$) and to a lesser extent by cla ($41.23 \pm 5.02\%$), from the control nematode community. No significant change was noticed for these two forms e/f and cla, as well as for the co form, after addition of the PE-MPs (Fig. 4).

Finally, the adults were dominated by species with a body-size of 2–4 mm and 1–2 mm, comprising $58.03 \pm 4.49\%$ and $41.96 \pm 4.49\%$ of the control nematode population. However, these species did not show significant changes among treatments (Fig. 5).

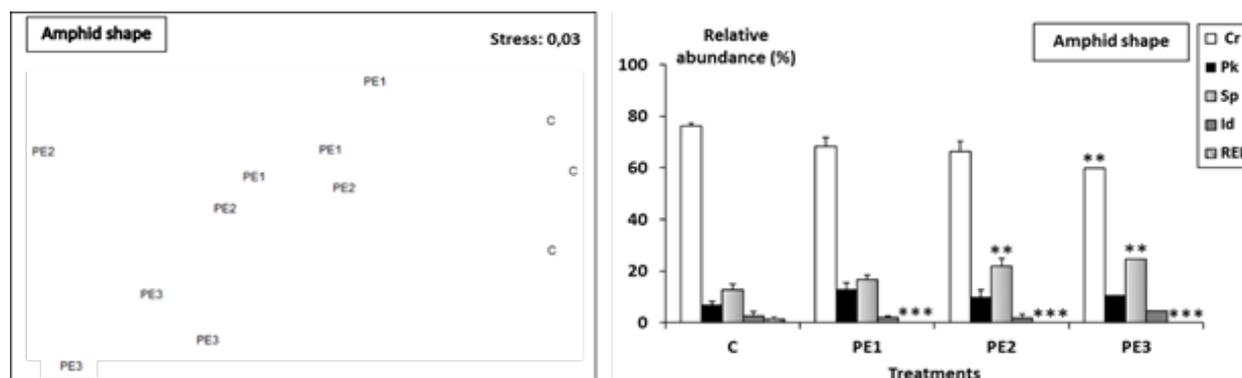


Figure 3. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Circular (Cr) ; indistinct (Id) ; pocket-like (Pk); spiral (SP); REL. Asterisks indicate significantly differences from the control (\sqrt{n} -transformed data : * = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$; **** = $0.0001 \leq p$).

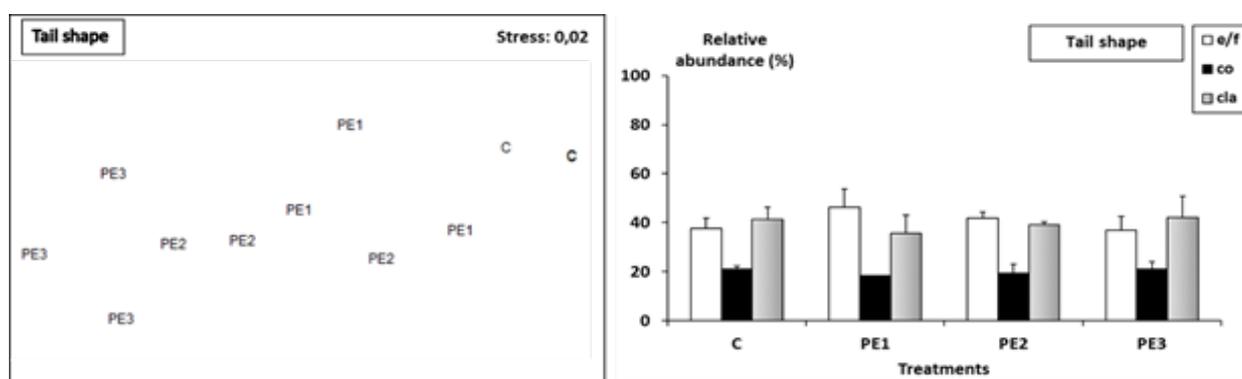


Figure 4. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Elongated/filiform (e/f); conical; (co) ; clavate/conical-cylindrical (cla). Asterisks indicate significantly differences from the control (\sqrt{n} -transformed data : * = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$; **** = $0.0001 \leq p$).

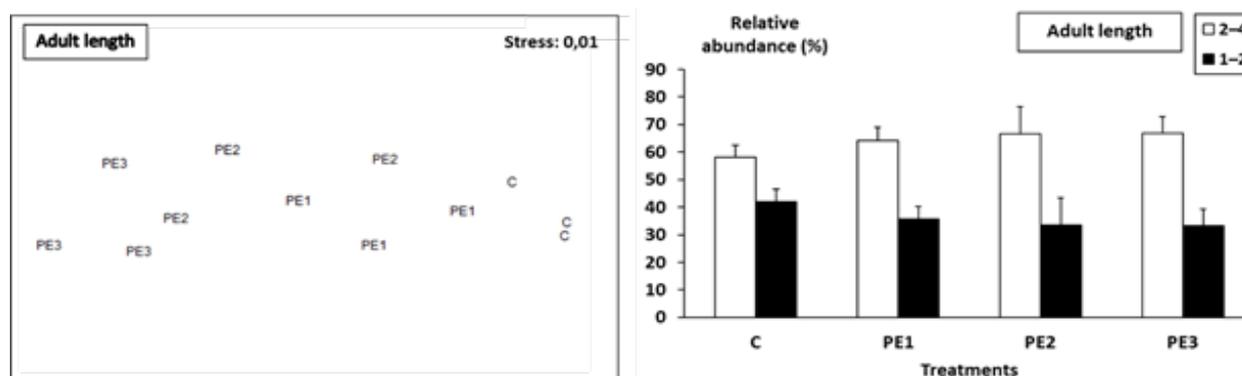


Figure 5. Non-parametric metric-multidimensional scaling (nMDS) 2D plots (left) and relative abundances of functional groups of nematode assemblages (right) from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Asterisks indicate significantly differences from the control (\sqrt{n} -transformed data : * = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$; **** = $0.0001 \leq p$).

Dissimilarity values for all functional traits were recorded in the high PE-MPs-treated compartments and were in the range of 43.97%, 43.97%, 43.97%, 43.97%, and 44.66%, respectively for Feeding diet, Adult length, c-p score, tail shape, and amphid shape. The table 2 indicated that the main change between the control and the treated compartments were a result of the all functional traits lessening such as FG: [1B↓, 2A↓, 1A↓] ; adult length: [1-2↓, 2-4↓] ;

c-p score: [2↓, 3↓] ; tail shape: [cla ↓, co ↓, e/f ↓] ; amphid shape: [Cr↓, SP↓]. The nMDS second-step ordination revealed that nematode response to sediment PE-MPs enrichment mainly depended on trophic groups (92.09%), tail forms (90.85%), and d life history (90.12%). Adult body size (88.88%) and amphid shapes (88.71%) were at least affected and located farther from the species in the graphical representation (Fig. 6)

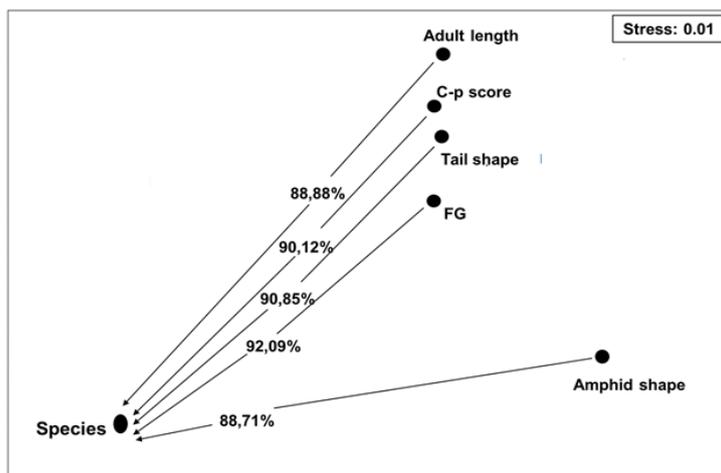


Figure 6. Non-parametric metric-multidimensional scaling (nMDS) second-stage ordination of inter-matrix rank correlations. Values indicate average similarity percentages between nMDS related to species and those related to functional traits.

4. Discussion

Negative effects were recorded after one month of sediment enrichment experiment with PE-MPs using a microcosm approach. In detail, nematode community structure and biological traits were modified as found in the results of other papers (Hannachi et al., 2022; Nasri et al., 2021b, 2020b). Given their usefulness as ecological indicators and as signs of environmental quality for several ecosystems (Moreno et al., 2011), the nematodes used in our present study have been highlighted as good tools for monitoring anthropogenic disturbances in many studies (Allouche et al., 2021b; Hannachi et al., 2022; Nasri et al., 2022c, 2020c).

Several researchers have investigated in laboratories the effects of different MPs types and sizes on multiple parameters in several living beings. In fish, many effects were registered after plastic exposure. The swimming behavior was disturbed (Rist et al., 2016); the predation capacity and the larvae efficiency were reduced (de Sá et al., 2015) as well as a decreasing of acetylcholinesterase (AChE) activity in the nervous system, have been reported in the goby *Pomatoschistus microps* (Oliveira et al., 2013). A decreased microvilli number, and mitochondria number increased as the intestinal epithelial cell ultrastructure alteration consequence, as well as a metabolomics profiles changes and genes expression associated with a diverse set of functions, were recorded in zebrafish *Danio rerio* (Lei et al., 2018). In addition of the vitellogenin (vtg 1) up-regulation in the liver, and his weak expression in intestine and gills, followed by slight cyp 1a up-regulation in the liver tissue (Wang et al., 2005). In European seabass *Dicentrarchus labrax*, microplastics induced a genes modification related to

response to stress, immunity, reparation of DNA and signaling pathways of lipid metabolism (Brandts et al., 2018). All of these effects were associated with locomotor behavior velocity disruption (Barboza et al., 2018). In sole *Solea senegalensis*, ingestion of PE-MPs resulted in physical damage associated with adverse physiological effects depending on their size (Lei et al., 2018) and particle density (Silva et al., 2020).

In mollusks, exposure to MPs induced: diminishes byssus production, food clearance, and respiration by the green mussel *Perna viridis*; oxidative damage, inflammatory responses, and neurotoxicity in *Mytilus edulis* (Brandts et al., 2018; Jeong et al., 2018); oxidative stress, immune responses and cell apoptosis (Ogonowski et al., 2016), DNA damage and micronuclei production in hemocytes in *Mytilus galloprovincialis* (Avio et al., 2015; Ribeiro et al., 2017); an histopathological modifications including degenerated cilia, hemocytic infiltration, as well as changed of oxidative stress biomarkers alike SOD, CAT and GST in the tissue in the mussel *Perna viridis*; neurotoxicity, increased activities of superoxide dismutase and catalase, reduction in lipid oxidative damage, inflammation, and destabilization of lysosomal membranes in *Scrobicularia plana* (Ribeiro et al., 2017). In addition, MPs caused a larval development retardation (Sussarellu et al., 2016), feeding rate lessening (Xu et al., 2020), and energy reserve reduction (Wright et al., 2013) in bivalves.

In crustacean, a filtration process alteration causing food intake reduction and mortality was signaled globally (Jemec et al., 2016). An inhibition of both feeding activity and energy reserves (Besseling et al., 2013), as well as an increased of reactive oxygen

content (Suman et al., 2020) were recorded in *Artemia salina*; a decreased reproductive output reported *Ceriodaphnia dubia* (Ziajahromi et al., 2017); a feeding capacity alteration as well as the decline of reproductive output the result of diminished of egg size and success of hatching linked to decreased energy intake due to MP ingestion were recorded in the pelagic copepod *Calanus helgolandicus* and *Parvocalanus crassirostris* (Cole et al., 2015; Heindler et al., 2017); growth and reproduction change in the amphipods *Hyaella azteca* (Syakti et al., 2019); an oxidative stress generation and energy metabolism alteration led a locomotor behavior disruption (Han et al., 2022; Mehennaoui et al., 2021) were observed both in gammarids and hepatopancreas.

In worm's species, for example *Arenicola marina*, a lethality was reported after repression of energy reserves and feeding activity following MPs ingestion (Wright et al., 2013); for *Lumbriculus variegatus*, the increased levels of biochemical markers such as glutathione, glutathione S-transferase known to play a greater role in antioxidant defense (Kristoff et al., 2008), appear to be triggered to protect against oxidative damage caused by MPs (Trestrail et al., 2020). In meiofaunal taxa, the number of MPs ingested was high in nematodes who are considered the most consuming organisms, as *Caenorhabditis elegans* (Fueser et al., 2020), causing; (1) alteration of survival rates, body length, and reproduction (Lei et al., 2018); (2) free radicals formation leading to oxidative stress and glutathione S-transferase augmentation (Lei et al., 2018).

Microplastics effects on benthic taxonomic groups were universally negative (Wright et al., 2013). The taxonomic diversity in our study was changed and we have registered a gradual nematodes populations restructuring. As consequence, of the certain species abundance lessening after PE-MPs sediment enrichment, noting, *Paramonohystera pilosa*, *Metalinhomoeus setosus*, *Odontophora villoti*, *Paramonohystera wieser*, *Terschellingia longicaudata*, *Daptonema trabeculosum*, *Calomicrolaimus honestus*, respectively representative by functional groups [1B, 2, Cr, cla, 1–2], [1B, 2, Cr, e/f, 2–4], [1B, 2, Cr, co, 2–4], [1B, 2, Cr, cla, 1–2], [1A, 3, Cr, e/f, 2–4], [1B, 2, Cr, cla, 1–2], [2A, 3, Sp, co, 1–2]. In addition to the increased abundance of the species named *Cyatholaimus prinzi* [2A, 3, Sp, co, 2–4]. The second-stage nMDS ordination results exhibited that the response of nematodes to PE-MPs

sediment enrichment depended on functional traits trophic group (FG = 92.09%), followed by tail shape (T = 90.85%) and life history indices (c-p score = 90.12%). These results show that the species which share in their biological traits a 1A or 1B diet, a tail type (cla, co, e/f) and a life history of type c-p 2, are the species responsible for this structural restructuring. population, due to their sensitivity to the enrichment of PE-MPs in the sediments. Moreover, our current data are in agreement with the results showing that the feeding strategy is the key explanatory of toxic effects in benthic organisms (Bertoli et al., 2022). It is the deposit-feeders feeding on particles incorporated into the sediments, and easily ingesting the MPs retained in the substrate (Bertoli et al., 2022), which accumulate more MPs and are the most affected compared to the other trophic groups, this is the case of selective deposit-feeders (1A) and non-selective deposit-feeders (1B) in our study (Table 1, 2) where a false diet of PE-MPs is the consequence of damage or blockages of the digestive tract leading to the progressive death of the species (de Sá et al., 2015). These deposit feeders, which nurse on detritus and microbes in the sediment (Levin, 2013), showed an enormous capacity to ingest plastic particles than those predators which were more selective in feeding on living organisms (Gérino et al., 2003).

5. Conclusion

Several studies have shown that the presence of MPs in biota is the apparent consequence of toxic effects at all trophic levels in many organisms; leading to impaired survival, respiration, growth, development, reproduction and other vital functions of organisms, resulting in large-scale degradation of biodiversity and ecosystem services (Silva et al., 2022). Our present study has just validated its effects but on the benthic nematofauna where a taxonomic restructuring as well as biological traits have been recorded following the enrichment of the sediments in PE-MPs, showing that the type of diet is the central explanatory key of these impacts and that the deposit-eating organisms are the most sensitive. These results support the use of these organisms as in vivo and in situ biological models to detect environmental variations following anthropogenic pressures (Nasri et al., 2022b, 2022a).

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.

References

1. Abidli, S., Akkari, N., Lahbib, Y., Trigui El Menif, N., 2021. First evaluation of microplastics in two commercial fish species from the lagoons of Bizerte and Ghar El Melh (Northern Tunisia). *Reg. Stud. Mar. Sci.* 41, 101581. <https://doi.org/10.1016/J.RSMA.2020.101581>
2. Abidli, S., Toumi, H., Lahbib, Y., Trigui El Menif, N., 2017. The First Evaluation of Microplastics in Sediments from the Complex Lagoon-Channel of Bizerte (Northern Tunisia). *Water. Air. Soil Pollut.* 228, 1–10. <https://doi.org/10.1007/S11270-017-3439-9/FIGURES/7>
3. Allouche, M., Nasri, A., Harrath, A.H., Mansour, L., Alwasel, S., Beyrem, H., Plăvan, G., Rohal-Lupher, M., Boufahja, F., 2021a. Meiobenthic nematode *Oncholaimus campyloceroides* as a model in laboratory studies: selection, culture, and fluorescence microscopy after exposure to phenanthrene and chrysene. *Environ. Sci. Pollut. Res.* 28, 29484–29497. <https://doi.org/10.1007/S11356-021-12688-1/TABLES/4>
4. Allouche, M., Nasri, A., Harrath, A.H., Mansour, L., Alwasel, S., Beyrem, H., Plăvan, G., Rohal-Lupher, M., Boufahja, F., 2021b. Meiobenthic nematode *Oncholaimus campyloceroides* as a model in laboratory studies: selection, culture, and fluorescence microscopy after exposure to phenanthrene and chrysene. *Environ. Sci. Pollut. Res.* 28, 29484–29497. <https://doi.org/10.1007/S11356-021-12688-1/TABLES/4>
5. Anagnosti, L., Varvaresou, A., Pavlou, P., Protopapa, E., Carayanni, V., 2021. Worldwide actions against plastic pollution from microbeads and microplastics in cosmetics focusing on European policies. Has the issue been handled effectively? *Mar. Pollut. Bull.* 162, 111883. <https://doi.org/10.1016/J.MARPOLBUL.2020.111883>
6. Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., D’Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ. Pollut.* 198, 211–222. <https://doi.org/10.1016/J.ENVPOL.2014.12.021>
7. Aydi, A., Ghannem, S., Nasri, A., Hessine, R., Mezni, A., 2022. Evaluation of heavy metals contamination and pollution indices levels in surface sediments of the Bizerte coastal line, Tunisia. *Mar. Pollut. Bull.* 184, 114171. <https://doi.org/10.1016/J.MARPOLBUL.2022.114171>
8. Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018. Single and combined effects of microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*): Changes in behavioural responses and reduction of swimming velocity and resistance time. *Environ. Pollut.* 236, 1014–1019. <https://doi.org/10.1016/J.ENVPOL.2017.12.082>
9. Bertoli, M., Pastorino, P., Lesa, D., Renzi, M., Anselmi, S., Prearo, M., Pizzul, E., 2022. Microplastics accumulation in functional feeding guilds and functional habit groups of freshwater macrobenthic invertebrates: Novel insights in a riverine ecosystem. *Sci. Total Environ.* 804, 150207. <https://doi.org/10.1016/J.SCITOTENV.2021.150207>
10. Besseling, E., Wegner, A., Foekema, E.M., Van Den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environ. Sci. Technol.* 47, 593–600. https://doi.org/10.1021/ES302763X/SUPPL_FILE/ES302763X_SI_001.PDF
11. 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.* 76, 135–142. <https://doi.org/10.3354/meps076135>
12. Brandts, I., Teles, M., Tvarijonaviciute, A., Pereira, M.L., Martins, M.A., Tort, L., Oliveira, M., 2018. Effects of polymethylmethacrylate nanoplastics on *Dicentrarchus labrax*. *Genomics* 110, 435–441. <https://doi.org/10.1016/J.YGENO.2018.10.006>
13. 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>
14. Clarke, K.R., Warwick, R.M., 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd ed.; PRIMER-E., Ltd., Plymouth Marine Laboratory: London. PRIMER v5: User manual/tutorial. PRIMER-E: Plymouth, UK. 91.
15. Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environ. Sci. Technol.* 49, 1130–1137. https://doi.org/10.1021/ES504525U/ASSET/IMAGES/LARGE/ES-2014-04525U_0005.JPEG
16. Coull, B.C., Chandler, G., 1992. Pollution and meiofauna - field, laboratory, and mesocosm studies. *Oceanogr. Mar. Biol.*
17. de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental

- conditions. *Environ. Pollut.* 196, 359–362. <https://doi.org/10.1016/J.ENVPOL.2014.10.026>
18. Dong, M., Luo, Z., Jiang, Q., Xing, X., Zhang, Q., Sun, Y., 2020. The rapid increases in microplastics in urban lake sediments. *Sci. Reports* 2020 101 10, 1–10. <https://doi.org/10.1038/s41598-020-57933-8>
19. Fueser, H., Mueller, M.T., Traunspurger, W., 2020. Ingestion of microplastics by meiobenthic communities in small-scale microcosm experiments. *Sci. Total Environ.* 746, 141276. <https://doi.org/10.1016/J.SCITOTENV.2020.141276>
20. Gérino, M., Stora, G., François-Carcaillet, F., Gilbert, F., Poggiale, J.C., Mermillod-Blondin, F., et al., 2003. Macro-invertebrate functional groups in freshwater and marine sediments: a common mechanistic classification. *Middle Life* 53 (4), 221 - 231.
21. Haddout, S., Gimiliani, G.T., Priya, K.L., Hogueane, A.M., Casila, J.C.C., Ljubenkov, I., 2021. Microplastics in Surface Waters and Sediments in the Sebou Estuary and Atlantic Coast, Morocco. <https://doi.org/10.1080/00032719.2021.1924767> 55, 256–268. <https://doi.org/10.1080/00032719.2021.1924767>
22. Han, Y., Zhang, X., Liu, P., Xu, S., Chen, D., Liu, J.N., Xie, W., 2022. Microplastics exposure causes oxidative stress and microbiota dysbiosis in planarian *Dugesia japonica*. *Environ. Sci. Pollut. Res.* 29, 28973–28983. <https://doi.org/10.1007/S11356-022-18547-X/FIGURES/5>
23. Hannachi, A., Nasri, A., Allouche, M., Aydi, A., Mezni, A., D'Agostino, F., Avellone, G., Gambi, C., Beyrem, H., Mahmoudi, E., 2022. Diuron environmental levels effects on marine nematodes: Assessment of ecological indices, taxonomic diversity, and functional traits. *Chemosphere* 287, 132262. <https://doi.org/10.1016/J.CHEMOSPHERE.2021.132262>
24. Heindler, F.M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S.J., Vamvounis, G., van Herwerden, L., 2017. Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl) phthalate on the calanoid copepod, *Parvocalanus crassirostris*. *Ecotoxicol. Environ. Saf.* 141, 298–305. <https://doi.org/10.1016/J.ECOENV.2017.03.029>
25. Huang, W., Song, B., Liang, J., Niu, Q., Zeng, G., Shen, M., Deng, J., Luo, Y., Wen, X., Zhang, Y., 2021. Microplastics and associated contaminants in the aquatic environment: A review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. *J. Hazard. Mater.* 405, 124187. <https://doi.org/10.1016/J.JHAZMAT.2020.124187>
26. Hurley, R., Woodward, J., Rothwell, J.J., 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci.* 2018 114 11, 251–257. <https://doi.org/10.1038/s41561-018-0080-1>
27. Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. *Environ. Pollut.* 219, 201–209. <https://doi.org/10.1016/J.ENVPOL.2016.10.037>
28. Jeong, C.B., Kang, H.M., Lee, Y.H., Kim, M.S., Lee, Jin Sol, Seo, J.S., Wang, M., Lee, Jae Seong, 2018. Nanoplastic Ingestion Enhances Toxicity of Persistent Organic Pollutants (POPs) in the Monogonont Rotifer *Brachionus koreanus* via Multixenobiotic Resistance (MXR) Disruption. *Environ. Sci. Technol.* 52, 11411–11418. https://doi.org/10.1021/ACS.EST.8B03211/ASSET/IMAGES/LARGE/ES-2018-032118_0007.JPG
29. Julienne, F., Delorme, N., Lagarde, F., 2019. From macroplastics to microplastics: Role of water in the fragmentation of polyethylene. *Chemosphere* 236, 124409. <https://doi.org/10.1016/J.CHEMOSPHERE.2019.124409>
30. Kennish, M.J., 2016. Coastal Lagoons 140–143. https://doi.org/10.1007/978-94-017-8801-4_47
31. Kristoff, G., Verrengia Guerrero, N.R., Cochón, A.C., 2008. Effects of azinphos-methyl exposure on enzymatic and non-enzymatic antioxidant defenses in *Biomphalaria glabrata* and *Lumbriculus variegatus*. *Chemosphere* 72, 1333–1339. <https://doi.org/10.1016/J.CHEMOSPHERE.2008.04.026>
32. Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Sci. Total Environ.* 619–620, 1–8. <https://doi.org/10.1016/J.SCITOTENV.2017.11.103>
33. Levin, S.A., 2013. *Encyclopedia of Biodiversity*. (2nd ed.).
34. Mehennaoui, K., Cambier, S., Minguez, L., Serchi, T., Guérol, F., Gutleb, A.C., Giamberini, L., 2021. Sub-chronic effects of AgNPs and AuNPs on *Gammarus fossarum* (Crustacea Amphipoda): From molecular to behavioural responses. *Ecotoxicol. Environ. Saf.* 210, 111775. <https://doi.org/10.1016/J.ECOENV.2020.111775>
35. Moreno, M., Semprucci, F., Vezzulli, L., Balsamo, M., Fabiano, M., Albertelli, G., 2011. The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. *Ecol. Indic.* 11, 328–336. <https://doi.org/10.1016/J.ECOLIND.2010.05.011>
36. 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>
37. 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>
38. 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>
39. 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>
40. 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>
41. 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., 2022c. 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>
42. 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>
43. 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>
44. Nasri, A., Jouili, S., Boufahja, F., Hedfi, A., Mahmoudi, E., Aïssa, P., Essid, N., Beyrem, H., 2015. Effects of increasing levels of pharmaceutical penicillin G contamination on structure of free living nematode communities in experimental microcosms. *Environ. Toxicol. Pharmacol.* 40, 215–219. <https://doi.org/10.1016/J.ETAP.2015.06.007>
45. Nasri, A., Jouili, S., Boufahja, F., Hedfi, A., Saidi, I., Mahmoudi, E., Aïssa, P., Essid, N., Hamouda, B., 2016. Trophic restructuring (Wieser 1953) of free-living 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/TABLES/4>
46. Nasri, A., Mhadhbi, T., Allouche, M., Hannachi, A., Demey, H., Aïssa, P., Beyrem, H., Mahmoudi, E., Nasri, A., Mhadhbi, T., Allouche, M., Hannachi, A., Demey, H., Aïssa, P., Beyrem, H., Mahmoudi, E., 2022d. Persistent Organic Pollutants in the Bizerte Lagoon Ecosystem: Occurrence, Distribution, and Ecotoxicological Assessment Using Marine Organism. <https://doi.org/10.5772/INTECHOPEN.100549>
47. Nasri, A., Boufahja, F., Hedfi, A., Mahmoudi, E., Aïssa, P., Essid, N., 2013. Réponse des différents taxons méiofaunistiques à la contamination par un antibiotique (la Penicilline G) : étude microcosmique.
48. Nemys eds., 2022. Nemys: World Database of Nematodes. <https://nemys.ugent.be> on 2022-12-27. doi:10.14284/366.
49. Newton, A., Brito, A.C., Icely, J.D., Derolez, V., Clara, I., Angus, S., Schernewski, G., Inácio, M., Lillebø, A.I., Sousa, A.I., Béjaoui, B., Solidoro, C., Tosic, M., Cañedo-Argüelles, M., Yamamuro, M., Reizopoulou, S., Tseng, H.C., Canu, D., Roselli, L., Maanan, M., Cristina, S., Ruiz-Fernández, A.C., Lima, R.F. d., Kjerfve, B., Rubio-Cisneros, N., Pérez-Ruzafa, A., Marcos, C., Pastres, R., Pranovi, F., Snoussi, M., Turpie, J., Tuchkovenko, Y., Dyack, B., Brookes, J., Povilanskas, R., Khokhlov, V., 2018. Assessing, quantifying and valuing the ecosystem services of coastal lagoons. *J. Nat. Conserv.* 44, 50–65. <https://doi.org/10.1016/J.JNC.2018.02.009>

50. Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The Effects of Natural and Anthropogenic Microparticles on Individual Fitness in *Daphnia magna*. PLoS One 11, e0155063. <https://doi.org/10.1371/JOURNAL.PONE.0155063>
51. Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647. <https://doi.org/10.1016/J.ECOLIND.2013.06.019>
52. Paduani, M., 2020. Microplastics as novel sedimentary particles in coastal wetlands: A review. Mar. Pollut. Bull. 161, 111739. <https://doi.org/10.1016/J.MARPOLBUL.2020.111739>
53. Pérez-Ruzafa, A., Pérez-Ruzafa, I.M., Newton, A., Marcos, C., 2019. Coastal Lagoons: Environmental Variability, Ecosystem Complexity, and Goods and Services Uniformity. Coasts Estuaries Futur. 253–276. <https://doi.org/10.1016/B978-0-12-814003-1.00015-0>
54. Platt, H.M., Warwick, R.M. (Richard M., 1988. Free-living marine nematodes. Pt. 2, British chromadorids. 502.
55. Platt, H.M., Warwick, R.M., 1983. Free-living marine nematodes. Part 1. British Enoplids. Synopses of the British Fauna No. 28.
56. Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122, 379–391. <https://doi.org/10.1016/J.MARPOLBUL.2017.06.078>
57. Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Porschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. Mar. Pollut. Bull. 111, 213–220. <https://doi.org/10.1016/J.MARPOLBUL.2016.07.006>
58. Scherer, C., Wolf, R., Völker, J., Stock, F., Brennhold, N., Reifferscheid, G., Wagner, M., 2020. Toxicity of microplastics and natural particles in the freshwater dipteran *Chironomus riparius*: Same same but different? Sci. Total Environ. 711, 134604. <https://doi.org/10.1016/J.SCITOTENV.2019.134604>
59. Schratzberger, M., Warr, K., Rogers, S.I., 2007. Functional diversity of nematode communities in the southwestern North Sea. Mar. Environ. Res. 63, 368–389. <https://doi.org/10.1016/j.marenvres.2006.10.006>
60. 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>
61. Silva, C.J.M., Machado, A.L., Campos, D., Rodrigues, A.C.M., Patrício Silva, A.L., Soares, A.M.V.M., Pestana, J.L.T., 2022. Microplastics in freshwater sediments: Effects on benthic invertebrate communities and ecosystem functioning assessed in artificial streams. Sci. Total Environ. 804, 150118. <https://doi.org/10.1016/J.SCITOTENV.2021.150118>
62. Silva, M.S.S., Oliveira, M., Valente, P., Figueira, E., Martins, M., Pires, A., 2020. Behavior and biochemical responses of the polychaeta *Hediste diversicolor* to polystyrene nanoplastics. Sci. Total Environ. 707, 134434. <https://doi.org/10.1016/J.SCITOTENV.2019.134434>
63. Suman, T.Y., Jia, P.P., Li, W.G., Junaid, M., Xin, G.Y., Wang, Y., Pei, D.S., 2020. Acute and chronic effects of polystyrene microplastics on brine shrimp: First evidence highlighting the molecular mechanism through transcriptome analysis. J. Hazard. Mater. 400, 123220. <https://doi.org/10.1016/J.JHAZMAT.2020.123220>
64. Syakti, A.D., Jaya, J.V., Rahman, A., Hidayati, N.V., Raza'i, T.S., Idris, F., Trenggono, M., Doumenq, P., Chou, L.M., 2019. Bleaching and necrosis of staghorn coral (*Acropora formosa*) in laboratory assays: Immediate impact of LDPE microplastics. Chemosphere 228, 528–535. <https://doi.org/10.1016/J.CHEMOSPHERE.2019.04.156>
65. Toso, L., Brown, C., Williamson, J.E., 2016. Microplastics on beaches: ingestion and behavioural consequences for beach hoppers. Mar. Biol. 163, 1–13. <https://doi.org/10.1007/S00227-016-2973-0/FIGURES/5>
66. Trestrail, C., Nuggeoda, D., Shimeta, J., 2020. Invertebrate responses to microplastic ingestion: Reviewing the role of the antioxidant system. Sci. Total Environ. 734, 138559. <https://doi.org/10.1016/J.SCITOTENV.2020.138559>
67. Vázquez-Rowe, I., Ita-Nagy, D., Kahhat, R., 2021. Microplastics in fisheries and aquaculture: implications to food sustainability and safety. Curr. Opin. Green Sustain. Chem. 29, 100464. <https://doi.org/10.1016/J.COGSC.2021.100464>
68. Wang, H., Tan, J.T.T., Emelyanov, A., Korzh, V., Gong, Z., 2005. Hepatic and extrahepatic expression of vitellogenin genes in the zebrafish, *Danio rerio*. Gene 356, 91–100. <https://doi.org/10.1016/J.GENE.2005.03.041>
69. Warwick, R., Platt, H.M., Somerfield, P., 1998. Free-living marine nematodes: Part III. Monhysterida. Synopses of the British Fauna No. 53.
70. Wieser, W., 1953. Die Beziehung zwischen Mundhohlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Ark. für Zoologie.

71. Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* 1. <https://doi.org/10.1098/RSOS.140317>
72. Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.* 178, 483–492. <https://doi.org/10.1016/J.ENVPOL.2013.02.031>
73. Xu, S., Ma, J., Ji, R., Pan, K., Miao, A.J., 2020. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. *Sci. Total Environ.* 703, 134699. <https://doi.org/10.1016/J.SCITOTENV.2019.134699>
74. Zbyszewski, M., Corcoran, P.L., 2011. Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. *Water. Air. Soil Pollut.* 220, 365–372. <https://doi.org/10.1007/S11270-011-0760-6/FIGURES/3>
75. Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D.L., 2017. Impact of Microplastic Beads and Fibers on Waterflea (*Ceriodaphnia dubia*) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures. *Environ. Sci. Technol.* 51, 13397–13406. https://doi.org/10.1021/ACS.EST.7B03574/ASSET/IMAGES/LARGE/ES-2017-03574C_0004.JPEG