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RESEARCH ARTICLE

Taxonomic Composition Change after Environmental Levels Addition of Polyethylene Microplastic (PE-MPs) to Mediterranean Sediment: Case Study of Bizerte Lagoon Nematodes Communities

Ahmed Nasri[1,2], Fouzi Bouleefah[1,3], Amel Hannachi[1], Ibrahem Bokharem[3], Mohamed Allouche[1,4], Hamouda Beyrem[1], Ezzeddine Mahmoudi[1]

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

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

[3]*University of Ajdabia, Faculty of Science, Zoology Department, Libya.*

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

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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

The current study examined the meiobenthic populations' response, to the influence of the most detected micro-sized microplastics polyethylene (PE-MPs) in aquatic system. Three concentrations [PE1 (0.1 µg/g Dry weight (DW)), PE2 (1 µg/g DW), and PE3 (10 µg/g DW)] were progressively applied and the experiment was run for a month in the existence or lack of meiofauna. Our results showed that PE-MPs declined meiofauna taxa abundance, as well as, marine nematodes univariate indices were significantly affected. A change in taxonomic structure has been recorded leading to an increase in the mean difference between nematode genera with increasing doses of PE-MPs. Differences in nematodes sensitivities to this polymer occurred. Cyatholaimus, Paracomesoma, Oncholaimus, Oncholaimellus, Ascolaimus, Synonchiella were present in all conditions and categorized as tolerant to PE-MPs. Nematodes detected only in control compartments, including Microlaimus, Desmodora, Paramonohystera, and Metoncholaimus were considered the most sensitive compared to Terschellingia, Calomicrolaimus, Paramonohystera, Daptonema, Metalinhomoeus, Odontophora.

Keywords: Pe-Mps Enriched Sediment, Meiobenthic Taxa, Nematodes Genus, Population Response, Taxonomic Structure.

1. Introduction

Currently, plastics have attracted public attention due to their ubiquity and high production, and more particularly because the poor waste management. These are synthetic solid particles with a size between 1 µm and 5 mm (Bajt, 2021). They are on the firstly of industrial, medical and cosmetic origin, and on secondly are generated thanks to processes of natural alteration to larger elements (Bajt, 2021). Due to their properties of persistence, lightness (Li et al., 2019), microplastics have the ability to accumulate in all matrices of the aquatic environment thanks to their resistance to degradation processes (Besseling et al., 2018). In this context, studies have clearly indicated the presence of microplastics in several living organisms such as copepods, amphipods, turtles and fish (Guzzetti et al., 2018).

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Coastal lagoons play a vital role in preserving biodiversity and providing ecosystem services (including food) on which human populations be contingent (Newton et al., 2018). These environments have been considered as a MPs sink, due to the fact that they are preferential shallow areas for accumulation (Quesadas-Rojas et al., 2021), which is the case of the most famous Mediterranean ecosystem, the Bizerte lagoon (Nasri et al., 2020a). The latter is subjected to anthropogenic stresses such as manufacturing activities, urban expansion, domestic discharges and various pollutions (Velez et al., 2020). These anthropogenic activities would therefore alter biodiversity, the ecological equilibrium and the ecosystem services quality (Espinosa-Díaz et al., 2021), due to the increase in the pollutant levels loads in water, sediments and living organisms (Nasri et al., 2022c, 2022d, 2021c).

Due to their final destination in marine sediments, polyethylene-MPs has been recognized as the most dominant plastic, with the highest abundance close industrial zones (Zbyszewski and Corcoran, 2011). It is considered one of the most abundant MPs in surface waters (Erni-Cassola et al., 2019). Polyethylene-MPs levels found in sediments are valued to be in the order of grams per kg of sediment (Hurley et al., 2018). Their residence time when released into the environment has been estimated to range from tens to hundreds of years (Zbyszewski and Corcoran, 2011). In the Bizerte lagoon, MPs were identified in sediments by (Abidli et al., 2017) and showed that the MPs number was in the range of 3 thousand to 18 thousand items kg^{-1} of dry sediments.

Because the presence of microplastics in surface sediments at a depth of 0-10 cm (Dong et al., 2020) with levels reaching 1-10 g kg^{-1} of dry sediment (Hurley et al., 2018), they therefore pose a significant threat to biota, especially benthic invertebrates since they can ingest MPs due to their feeding behavior and close contact with sediments (Scherer et al., 2020). In this context, meiofauna, in particular free-living marine nematodes have been used in the present study as bio-indicators to qualitatively indicate the quality of their environment experimentally enriched with increasing doses of polyethylene-MPs, via changes in the species abundances, the specific structure and variation of ecological indices.

2. Materials and Methods

2.1. Polyethylene-MPs

In our study, méiofauna were subjected to PE-MPs $(C2H4)n$ (ø 40–48 µm), the most manufactured plastic polymer and common sizes used in the studies reviewed (Tosetto et al., 2016). PE particles were purchased from Sigma Aldrich, USA. The experimental treatments used: 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 Meiofauna Community Study

Sediment samples were taken from the Menzel Jemil station located in the Bizerte lagoon, Tunisia (37° 21′83.77″ N, 9° 93′ 58.83"E). A number of hand-cores (surface of 10 cm^2) were used to sample the first 10 cm of the sediment layer, at 60 cm below water surface following (Coull and Chandler, 1992) method. On coming back to the laboratory, sediments were homogenized with spatula before they were used for addition PE-MPs or filling microcosms.

Before beginning the contamination process, a sediments portion was defaunated by repeating a defaunation process, freezing the sediments at -20°C for 12 h and thawing them at room temperature for 48 h, 3 times (Nasri et al., 2015), in order to use several 100 g dry weight (DW) quantities for the addition of PE-MPs at the end. After five days of acclimation, the overlying water was eliminated and 100 g (DW) of treated sediment was gently mixed using a large spatula with the 200 g wet weight (WW) of sediment populated with meiofauna to obtain three doses of PE: PE1 (0.1 $µg.g^{-1}$), PE2 (1 $µg.g^{-1}$) and PE3 (10 $µg.g^{-1}$). The doses were chosen based on environmental concentrations found in aquatic sediments (Hurley et al., 2018).

After one month of experiment, sediment samples were conserved with 4% formalin and marked with a rose Bengal solution (0.2 $g.L^{-1}$) for >24 h (Nasri et al., 2021b). The following day, the sediments were washed with filtered tap water and treated by flotation to extract the meiofauna from the Ludox TM-50 centrifugation technique (Aldrich Chemical Company, gravity of 1.13 g/cm^3) (Allouche et al., 2021; Hannachi et al., 2022). Then, the meiofauna was collected on a 45 µm sieve and then manually sorted using a Nikon SMZ745 stereomicroscope. The first 100 nematodes were mounted on permanent slides and recognized to genus level following the pictorial keys of (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). Species number (S); Margalef's species richness (d); Shannon diversity index (H'); Pielou's evenness (J') were selected as environmental quality signs in this study.

2.3. Statistical Data Processing

The statistical analyses followed the standard methods defined by (CLARKE, 1993) and Clarke and Warwick (2001) and were accomplished with STATISTICA (v5.1) software (Nasri et al., 2021a, 2020c, 2020b). Data were first verified for normality and variance homogeneity, via Bartlett test and Kolmogorov–Smirnov test, respectively. Afterwards, one-way analysis of variance (1-ANOVA) was used to compare univariate indices between conditions: abundance, species number (S), Margalef's species richness (d), diversity (Shannon-Weaner index, H'), and evenness (Pielou) (J'), followed by Tukey's HSD post-hoc tests (log-transformed data). Moreover, multivariate analyzes were realized with the PRIMER v.5 software (Clarke and Gorley, 2001). Thus, the Similarity Percentages procedure (CLARKE, 1993) was used to determine each genera participation to the average Bray-Curtis difference between the control and treatments conditions.

3. Results

3.1. Meiofauna Abundance

The control microcosms 'C' had significantly the maximum average abundances (nematodes = 168.66 ± 13.55; copepods = 37 ± 3.33; oligochaetes = 9 ± 0.88; polychaetes = 7 ± 0.66). Those related to all conditions applied indicated significantly diminished abundances according to the taxon studied (Fig. 1, 1-ANOVA, $p < 0.001$, Tukey's HSD test: $p < 0.00001$).

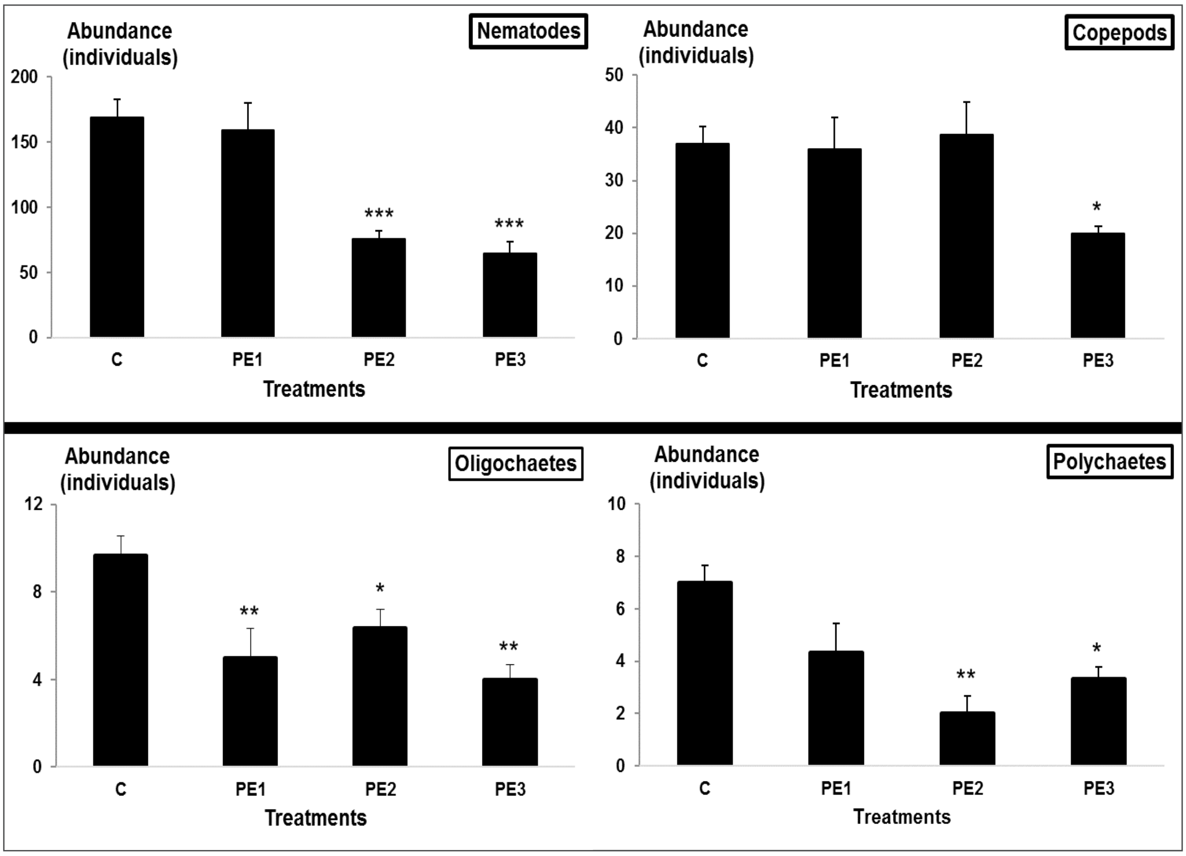

Fig 1. *Abundances of meiobenthic taxa from control (C) and treated microcosms with PE (PE1, PE2 and PE3). Asterisks indicate significantly differences from the control (* = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$).*

3.2. Univariates Indices

At the end of the experimentation, the nematofauna showed significant reduction in abundance in the PE2 and PE3 compartments. The analysis of univariate indices showed that the number of species (S) and species richness (d) decreased significantly in highest contaminated compartments (PE2 and PE3) compared to control "C". However, no significant effect was observed for Shannon diversity index (H'). In contrast, Equitability or Pielou's evenness (J') showed significant change in only compartments PE3 (Fig. 2).

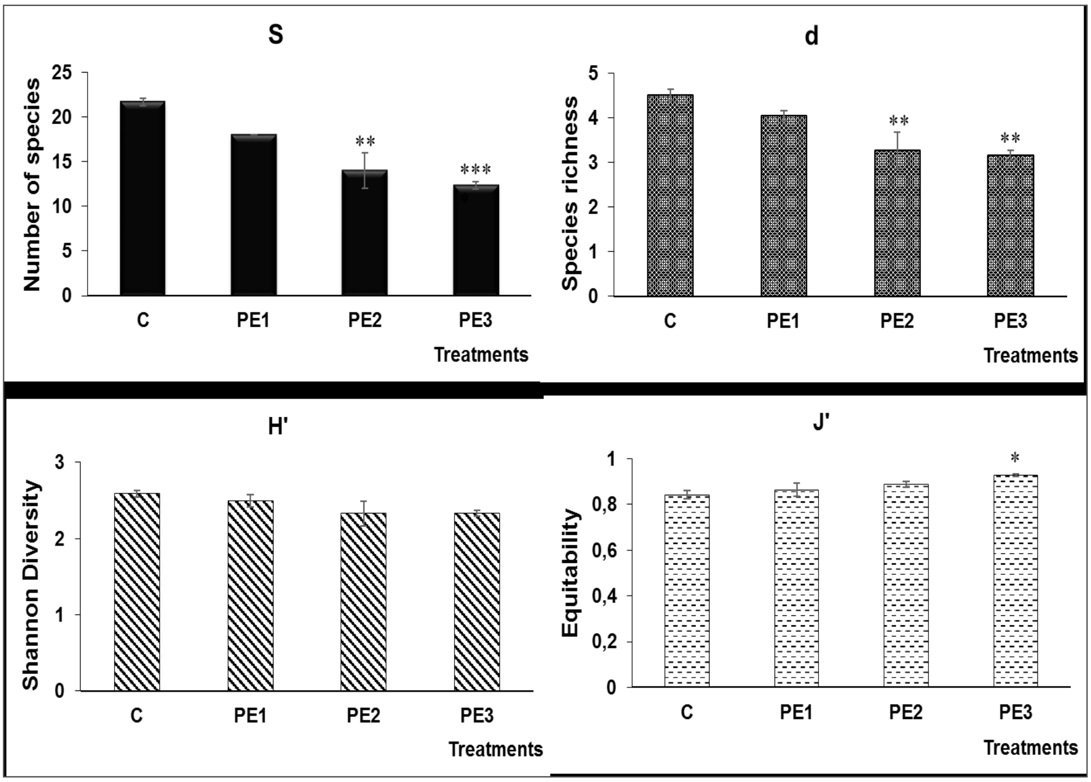

Fig 2. *Univariate indices values for each microcosm treatment. Species number (S); Margalef's species richness (d); Shannon diversity index (H'); Pielou's evenness (J'). Asterisks indicate significantly differences from the control (* = $p < 0.05$; ** = $0.05 \leq p < 0.001$; *** = $0.001 \leq p < 0.0001$).*

3.2. Taxonomic Composition

Twenty genera of meiobenthic nematodes were recognized in the control compartments with four most abundant genera were *Terschellingia* (17 ± 0,66%), *Metalinhomoeus* (16 ± 0,66%), *Paramonohystera* (14,33 ± 1,11%), and *Daptonema* (10,66 ± 1,77%) (Table 1). Seventeen genera were identified in the treatment PE1 with six nematodes were dominant, and included *Terschellingia* (14,33 ± 2,44%), *Metalinhomoeus* (11 ± 0,66%), *Daptonema* (8,33 ± 1,11%), *Cyatholaimus* (6,66 ± 0,44%), *Paramonohystera* (5,33 ± 1,11%), and *Anticoma* (3 ± 0,66%). 16 in treatments PE2, with six taxa were remarkably present, namely, *Terschellingia* (12,33 ± 1,55%), *Daptonema* (6,66 ± 1,55%), *Cyatholaimus* (6,33 ± 0,44%), *Metalinhomoeus* (6 ± 0,66%), *Paramonohystera* (4,33 ± 1,55%), and *Steineria* (3 ± 0,66%). Regarding replicates highly treated with PE3, 16 genera were identified with 6 taxa were regularly inventoried, namely, *Terschellingia* (6 ± 0,66%), *Metalinhomoeus* (5,33 ± 1,11%), *Daptonema* (4,33 ± 1,11%), *Paracomesoma* (4 ± 1,33%), *Paramonohystera* (3,66 ± 1,11%), and *Cyatholaimus* (3,33 ± 0,44%) (Table 1).

Table 1. *List of nematode genera identified in the control (C) and treated conditions with PE (PE1, PE2 and PE3).*

Genera	Treatments			
	C	PE1	PE2	PE3
Terschellingia	17 ± 0,66	14,33 ± 2,44	**12,33 ± 1,55**	**6 ± 0,66**
Anticoma	0,66 ± 0,44	3 ± 0,66	1,66 ± 1,55	1 ± 0,66
Prochromadorella	2,33 ± 1,11	1,33 ± 0,44	1 ± 0,66	1,66 ± 0,44
Cyatholaimus	4 ± 0,66	6,66 ± 0,44	**6,33 ± 0,44**	**3,33 ± 0,44**
Paracomesoma	3 ± 0,66	1,33 ± 0,44	0,66 ± 0,88	**4 ± 1,33**
Calomicrolaimus	3,66 ± 1,77	1,33 ± 0,44	0,66 ± 0,88	0,66 ± 0,88
Microlaimus	0,66 ± 0,44	-	-	-
Desmodora	1,33 ± 0,44	-	-	-
Paramonohystera	14,33 ± 1,11	5,33 ± 1,11	**4,33 ± 1,55**	**3,66 ± 1,11**

Promonohystera	3,33 ± 1,11	-	-	-
Daptonema	10,66 ± 1,77	8,33 ± 1,11	**6,66 ± 1,55**	**4,33 ± 1,11**
Metalinhomoeus	16 ± 0,66	11 ± 0,66	**6 ± 0,66**	**5,33 ± 1,11**
Odontophora	6,33 ± 1,11	2 ± 0,66	1 ± 0,66	1 ± 0,66
Steineria	1,33 ± 0,44	3 ± 1,33	**3 ± 0,66**	0,33 ± 0,44
Ascolaimus	1,33 ± 0,44	0,66 ± 0,88	1,33 ± 1,11	1 ± 0,66
Synonchiella	1,33 ± 0,44	1,66 ± 0,88	1,66 ± 0,44	1 ± 0,66
Viscosia	1,66 ± 0,88	1,33 ± 0,44	0,66 ± 0,44	0,33 ± 0,44
Metoncholaimus	0,66 ± 0,44	1,33 ± 0,44	-	-
Oncholaimus	1,33 ± 0,44	1,33 ± 0,44	1,33 ± 0,44	1,66 ± 0,88
Oncholaimellus	2,33 ± 0,44	1,66 ± 0,88	1,66 ± 0,88	1 ± 0,66

The SIMPER data are presented in Table 2. The Dissimilarity percentages between control and treated microcosms (C vs. PE1; C vs. PE2; and C vs. PE3) was augmented respectively (29,89%; 38,69%; and 48,94%). The main difference between the untreated control and all treated conditions was due mainly to a decline in the percentage of six genera, namely *Paramonohystera*, *Metalinhomoeu*s, *Odontophora*, *Paramonohystera*, *Terschellingia*, and *Daptonema* except in the PE2 compartment, marked by an increase in the genus *Cyatholaimus*, which induced a restructuring of the nematode populations in the treated microcosms compared to the control (Table 2).

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. More abundant (+); less abundant (–).*

	C vs. P1	**C vs. P2**	**C vs. P3**
Genera	**29,89%**	**38,69%**	**48,94%**
	Paramonohystera (-)	*Paramonohystera* (-)	*Metalinhomoeus* (-)
	Metalinhomoeus (-)	*Metalinhomoeus* (-)	*Paramonohystera* (-)
	Odontophora (-)	*Odontophora* (-)	*Terschellingia* (-)
	Paramonohystera (-)	*Daptonema* (-)	*Daptonema* (-)
	Terschellingia (-)	*Terschellingia* (-)	*Odontophora* (-)
	Daptonema (-)	*Paramonohystera* (-)	*Paramonohystera* (-)
	Cyatholaimus (-)	*Cyatholaimus* (+)	*Calomicrolaimus* (-)

4. Discussion

Meiobenthos (40 µm - 1 mm, (Vitiello et al., 1979) includes many phyla that have shown the characteristics of being effective bioindicators in biomonitoring and ecotoxicology experiments. Between the numerous meiobenthic assemblages, the greatest abundant (up to 23 million m2) and diversified (more than 7000 known species) is that of free-living marine nematodes (Warwick and Price, 1979). Their usefulness as appropriate ecological indicators were emphasized by numerous studies (Nasri et al., 2021b, 2021a, 2020a, 2020b, 2020c, 2016). They have been widely recognized as effective signs of environmental quality for several ecosystems (Moreno et al., 2011). They are very sensitive to different categories of stressors such as physical and chemical and anthropogenic disturbances, leading to environmental change followed by restructuring of living communities' composition (Schratzberger and Ingels, 2018).

Polyethylene (PE), is one of the most popular microplastic polymers, found significantly in marine environments (Wang et al., 2022). Due to their small size, these microplastics can be ingested/inhaled and bio-accumulated by organisms mistakenly and inadvertently, leading to potential health hazards (Wright et al., 2013). In our present study, using a microcosm approach, we detected a negative effect of sediments enriched in PE-MPs on the abundance of meiofauna and the nematode communities structure. Nevertheless, the impact of these polymers presence in the sediments seems to be caused following their absorption on the organism cuticles and also by their direct ingestion at the level of the substrate particles (Di Lorenzo et al., 2023). Exposure to PE-MPs resulted in a reduction in the various meiobenthic taxa abundance, nematode species richness (d) and Shannon diversity (H'), resulting in subsequent elimination of the most sensitive species, especially with the highest doses PE2 and PE3 (Figure 1, 2). In this context, the MPs ingestion has been approved in several invertebrates' species (Scherer et al., 2017). These microplastics have been detected in aquatic biota at different trophic levels, such as planktons, crustaceans, bivalves and fish (Cole et al., 2019). Hurley et al. (2017) showed that the presence of these compounds in sediments plays a major role in contamination throughout the food chain.

Numerous studies have described the ecotoxicological effects of PE-MPs on aquatic organisms at the individual, cellular and molecular levels (Cole and Galloway, 2015). At the individual level, typically microplastics when ingested can have adverse physiological effects that affect diet, behavior, growth, development, and reproduction (Harmon, 2018). In *Arenicola marina*, it has been shown that the feeding process as well as the energy reserves are inhibited, which leads to a decrease in the survival of arenaceans (Besseling et al., 2013). In the three species of copepod *Centropages typicus*, the oyster *Pinctada margaritifera* and the crab *Carcinus maenas*, a reduction in ingestion rate, assimilation efficiency, energy balance and swimming speed has been demonstrated (Gardon et al., 2018). A decreased byssal production, food clearance, and respiration in the green mussel *Perna viridis* (Rist et al., 2016). A reduction in body size, number of hatchlings, as well as the appearance of various malformations in the bristles of the antennae, the vertebral column and the vacuoles around the ovary have been reported in Daphnia magna (Rehse et al., 2016). In bivalves, microplastics caused reduced feeding activity, leading to morphological alterations as well as decreased fecundity and growth of offspring (Sussarellu et al., 2016).

At the cellular level, adverse effects induced following exposure to MPs have been demonstrated. In *Paracentrotus lividus* sea urchin embryos, the development of a thick ectodermal membrane, an abnormal proliferation as well as an alteration of the skeletal rods and a reduction in the length of the arms, have been demonstrated (Della Torre et al., 2014). Changes in the ultrastructure of intestinal epithelial cells marked by a decrease in the number of microvilli and an increase in the number of mitochondria have been reported in *Artemia parthenogenetica* and the zebrafish *Danio rerio*, respectively (Lei et al., 2018). In rotifers, crabs, mussels, fish and corals, the occurrence of neurotoxic effects and inflammatory responses in addition to developmental defects have been established (Ding et al., 2018; Jeong et al., 2018; Tang et al., 2020). Additionally, in zebrafish larvae, alterations in metabolomic profiles and homeostasis have also been reported (Wan et al., 2019).

At the molecular level, the effects concern changes in gene expression and changes in physiological functions. In the gilthead seabream *Sparus aurata*, nematode *Caenorhabditis elegans* and the juvenile crab *Eriocheir sinensis*, modifications in gene expression related to the stress response have been demonstrated (Espinosa et al., 2017; Lei et al., 2018). In the mussel *Mytilus galloprovincialis*, the fish *Dicentrarchus labrax* and *Danio rerio*, they reported alterations in the genes expression related to biotransformation, DNA repair, defense against stress, the immune system and signaling pathways linked to metabolisms (LeMoine et al., 2018). Seoane et al. (2019) found also a significant diminution in esterase activity and lipid content in *Chaetoceros neogracile* after MPs treatments. A significant increase in superoxide dismutase and catalase activities, showing oxidative stress (Lu et al., 2016) and a decrease in glutathione peroxidase (GPx) and alkaline phosphatase (Tang et al., 2018).

In marine invertebrate communities, MPs have been found to reduce the biodiversity and abundance of organisms (Green, 2016) and decrease the benthic primary producer's biomass (Green et al., 2016). In our current study, MP-enriched sediments induced a restructuring of nematofauna populations. Thus, this modification is due to a decrease in the genera abundance, namely *Paramonohystera*, *Metalinhomoeus*, *Odontophora*, *Promonhystera*, *Terschellingia*, and *Daptonema*, and an increase in the genus *Cyatholaimus*. The similarity in size between MPs and food located at the sediment could be confused and taken as prey by various benthic marine organisms (Lee et al., 2013; Thompson et al., 2004). Once ingested, microplastics cannot be digested or absorbed, since these benthic organisms do not have specific enzymatic pathways to degrade synthetic polymers and could therefore be considered bio-inert compounds (Andrady, 2011). A decrease in energy reserves as a result not only due to an inflammatory response in the tissues, but also to a decrease in nutrition due to an accumulation of microplastics in the digestive cavities causing a proportional increase in mortality (Wright et al., 2013).

The benthic microplastics are found in the upper cm of sediment (Martin et al., 2017), where grazing species (and some omnivores) feed mainly (Duchêne and Rosenberg, 2001). Feeding strategy acting a key role in understanding the impact of the presence of microplastics on these organisms. Studies have shown that omnivores and deposit feeders are the most affected (Naji et al., 2018) than filter feeders (Bour et al., 2018). In our study, the majority of species seriously affected in their abundances are classified as selective deposit-feeders represented by the genus "*Terschellingia*" and non-selective deposit-feeders grouping the genera "*Paramonohystera*, *Promonhystera*, *Metalinhomoeus*, *Odontophora*". In addition to direct effects on nematode composition, Lei et al. (2018), demonstrated that exposure of the *Caenorhabditis elegans* nematode to PE-MPs microplastics reduced their survival rates, body length and reproduction. The microplastics effects on benthic composition groups were universally negative. Reduced growth has been documented, likely a consequence of reduced feeding due to damage or blockage of the digestive area or misperception between microplastics and prey (de Sá et al., 2015). In this context, Hodgson (2018) showed that 58.8% of nematodes exhibited energy loss due to the consumption of microplastic particles. Also, Qu et al. (2018) found that MPs particles deregulated the genes expression involved in the oxidative stress control and the activated of Nrf signaling pathway expression in *Caenorhabditis elegans*. Lei et al. (2018) recommended that MPs cause oxidative stress by activating glutathione S-transferase expression and ROS production resulting in DNA damage.

5. Conclusion

Our study supports the fact that the presence of polyethylene MPs in the benthic environment leads to negative effects on natural populations, in particular, on the abundance, the diversity of meiobenthic taxa and specifically the taxonomic structure of free-living marine nematodes. These invertebrates clearly showed a significant response depending on the enrichment levels of these polymers in the sediment. Our results support the use of nematodes as bio-indicative models for anthropogenic pressures caused by plastic pollution in aquatic ecosystem biomonitoring programs, given their characteristics of small body size, short life cycle, high abundances and the omnipresent dissemination in many marine environments (Nasri et al., 2022b, 2022a).

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