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# Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran)

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#### ABSTRACT

Intertidal nematode assemblages along three beaches with different types of anthropogenic activity were collected in the northern part of the Persian Gulf, near Bandar Abbas (Iran). Forty-two genera of free-living nematodes belonging to 17 families were identified. Daptonema, Ptycholaimellus, and Promonhystera were the most abundant taxa in the area. The nematode assemblage structure was negatively affected by polychlorinated biphenyls (PCBs) and total organic matter (TOM), with an impact gradient from the polluted Power Plant (PP), over Khur Gursuzan (KG), to the cleaner 'Resalat' site (RE). The PP sites, characterized by industrial infrastructures, were mainly impacted by PCBs, while Khur Gursuzan (KG) is under the influence of domestic discharges, with high sedimentary TOM levels. However, PCBs and TOM seem closely associated and they likely lead to compounding effects on the fauna. Among the bioindicator genera found, Spirinia, Chromadorina, and Terschellingia may be recognized as resistant taxa to PCBs, while Daptonema, Sabateria, Promonhystera and Ptycholaimellus are opportunists able to exploit organic load, and Oncholaimus and Pomponema appeared sensitive genera being characteristic to unpolluted sediments. As expected, the spatial effects between the stations and concomitantly the different types of anthropogenic impacts played a much more important role than seasonal variability and related changes. This confirms that anthropogenic impact can be a major control factor at Persian Gulf beaches. Nematode female:male ratios were in favor of the females, especially in the sediments impacted by PCBs, corroborating the hypothesis that stressful conditions sustain the increasing female frequency in nematode populations. In terms of the ecological quality (EcoQ) status, genus percentages indicated lower EcoQ at PP and highest at RE. In contrast, Shannon-Wiener diversity (H') did not detect variations of EcoQ status at the three locations, while the Maturity Index did not indicate the presence of stressful conditions at PP, but it did at KG. It is possible that MI performs better when considering organic pollution compared to PCB contamination. However, we do not recommend its use in intertidal or transitional areas that are characterized by strong natural variations of the physical and chemical variables.

## 1. Introduction

Free-living nematodes are abundant and very species-rich in marine sediments (Giere, 2009; Heip et al., 1985). They play important ecological roles in the benthic ecosystem (Schratzberger and Ingels, 2018), including micro-bioturbation (Bonaglia et al., 2014; Murray et al., 2002; Nascimento et al., 2012), enhancing bio-mineralization and redistribution of organic matter, nutrient cycling, oxygenation of sediments, effects on microbial activity (De Mesel et al., 2003), and enhancement of micro-phytobenthic biofilm development (Hubas et al., 2010, Hubas et al., 2013). Variations in their assemblages are indicative of both natural and anthropogenic stressors and they are useful sentinels for ecological health in marine ecosystems (Alves et al., 2013; Boyd et al., 2000; Ferris and Bongers, 2006; Moreno et al., 2011;

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Fig. 1. Sampling locations on the coastline of Bandar Abbas.

Schratzberger and Jennings, 2002; Semprucci et al., 2013, 2015a, 2018). Accordingly, they can be used as indicators of different types of impact resulting from urbanization and human industrial activities (Astaraie-Imani et al., 2012; Limburg et al., 2005).

One of the more prominent industrial pollutants in marine ecosystems is the group of polychlorinated biphenyls (PCBs), which are endocrine disrupting compounds (EDCs) of significant concern because of their toxicity, persistence, bio-accumulative properties, and long-range atmospheric transport (Hotchkiss et al., 2008). EDCs are ubiquitous organic contaminants closely bound to the sediment particles, where they may significantly alter benthic assemblages that are associated with substratum or the water-sediment interface. In such environments, nematodes may take up leached compounds from interstitial water through their cuticle, and deposit feeding nematodes may ingest particles loaded with hydrophobic chemicals (Austen and McEvoy, 1997; Schratzberger et al., 2002). Despite their widespread occurrence in aquatic sediments, data available on the effects of PCBs are studied only in macrofauna (Mazurova et al., 2008), while data available on the phylum Nematoda are mainly focused on the model organism Caenorhabditis elegans and some parasitic nematodes (Höss and Weltje, 2007). Despite the lack of exhaustive knowledge on the endocrine pathways of nematodes, it seems that hormones are involved in the development, sex determination and molting process, which, if altered through PCB effects, could lead to unpredictable changes at all organizational levels, from molecule to assemblage levels (Höss and Weltje, 2007).

In recent years, coastal areas of Iran have been affected heavily by urbanization, industrialization and maritime activities. Bandar Abbas is a port city and the capital of the Hormuzgan province on the southern coast of Iran, which is located on the northern part of the Persian Gulf. The coasts of Bandar Abbas have been characterized by a substantial industrial development as well as rapid demographic growth with significant concerns for the health status of the coastal system, issues that have attracted attention only recently (Dibajnia et al., 2012; Nadim et al., 2008). One of the major pollutants in this area are PCBs along with sewage output (Mohebbi Nozar, 2013, 2014). Therefore, an assessment of the impact of these pollution sources on coastal nematode assemblages was carried out. To do this, three intertidal locations in Bandar Abbas, affected by different anthropogenic impacts (i.e. sewage and industrial contamination i.e. PCBs) were selected. The potential temporal variability of the nematode assemblages across seasons (August 2018, November 2018, January 2019, corresponding with summer, autumn, and winter, respectively) was also taken into consideration. In intertidal areas, variability of environmental factors can be very high on a daily basis, which causes distinct nematode assemblages that are generally adapted to the highly variable conditions (see Sahraean et al. 2017a,b for details) and hence seasonal variability may be less influential than in subtidal areas. It is therefore expected that nematode assemblage drivers between areas (and linked pollution types) may override temporal differences because of the general adaptable nature of the resident nematode assemblages. We tested the following hypotheses: 1) the spatial differences (which are associated to anthropogenic impacts in the form of PCBs and organic load) are more important than temporal dynamics in driving nematode assemblages in the intertidal area of Bandar Abbas; 2) the effects of PCBs are more severe on the nematode assemblages than sewage outputs in the study area and 3) nematode assemblage structure and different contributions of species can indicate different types of human impact in this coastal area.

# 2. Material and methods

### 2.1. Sampling sites and design

Our study sites were situated in the northern part of the Persian Gulf, along the coast near Bandar Abbas, the capital of the Hormuzgan province (Iran). The Persian Gulf represents a semi-enclosed area located in the subtropical and hyper-arid region of the north-western Indian Ocean. This Gulf is characterized by harsh environmental conditions related to the high evaporation and low freshwater inputs as well as by high salinity levels (Naderloo 2017). The Hormuzgan area (~500,000 inhabitants) is important because of its contributions to fisheries, industrial infrastructures, and international marine trade. In particular, oil-related activities (oil exploration and production, oil spills) have been developing rapidly in the area, with significant subsequent effects on marine ecosystems (Emtiazi et al., 2009; Hassanshahian, 2014, 2010; Mohebbi Nozar et al., 2014). Three intertidal locations varying in type of anthropogenic impact were selected along the coastal area of this city (Fig. 1). The most western site, 'Power plant' (PP), is characterized by industrial contamination and is highly impacted by PCBs (Mohebbi Nozar, 2013, 2014), while the middle site, 'Khur Gursuzan' (KG), suffers from major domestic sewage discharges. The most eastern site, 'Resalat' (RE), is considered a comparatively less disturbed site, with no heavy domestic or industrial pollution sources.

#### 2.2. Sample collection and analysis

Samples were collected in August (A18), November (N18) 2018, and January 2019 (J19) at intertidal beaches (Fig. 1). The times of sampling correspond with different seasons (i.e. summer, autumn and winter, respectively), to which we will refer in the results and discussion sections accordingly. At each location (PP, KG, RE), samples were taken in triplicate at mid-tide locations when the tide was at its lowest. Sediment samples for nematode assemblage analyses were taken using PVC hand corers with an inner diameter of 3.5 cm (9.62 cm<sup>2</sup> surface area), which were pushed into the sediment to a depth of 5 cm. Additional triplicate samples were taken for all the environmental variable analyses (i.e. grain size, total organic matter, polychlorinated biphenyls). Temperature, pH and salinity were measured directly in the overlying bottom water at the sampling locations with an Ocean Seven 316 CTD Probe.

Grain size analysis on triplicate samples was conducted with a Malvern Hydro 2000G Particle Size Analyzer and the sediment fractions were defined according to the Wentworth scale (Bale and Kenny, 2005).

#### Table 1

Sediment environmental variables at the three sampling locations (Khur Gursuzan (KG), Power Plant (PP), Resalat (Re)) for the three different months (August 2018 or A18, November 2018 or N18, January 2019 or J19). Data are means  $\pm$  SE (n = 3). TOM = total organic matter, PCB values are ng/g dry weight, temperatures are in °C.

|               |               | Temperature      | pH                | Salinity         | PCB               | TOM%            |
|---------------|---------------|------------------|-------------------|------------------|-------------------|-----------------|
|               | Khur Gursuzan | $35.72 \pm 0.67$ | 7.9 ± 0,04        | $36.50 \pm 0.25$ | $6.16 \pm 0.18$   | $3.71 \pm 0.06$ |
| August 2018   | Power Plant   | $35.30 \pm 0.65$ | $8.49 \pm 0.12$   | $33.97 \pm 0.18$ | $10.5 \pm 0.21$   | $2.22 \pm 0.10$ |
|               | Resalat       | $34.66 \pm 0.08$ | $8.08 \pm 0.10$   | $39.50 \pm 0.15$ | $0.98 \pm 0.11$   | $0.95 \pm 0.04$ |
|               | Khur Gursuzan | $29.87 \pm 0.19$ | $7.58 \pm 0.11$   | $29.10 \pm 0.06$ | $5.46 \pm 0.2$    | $4.07 \pm 0.07$ |
| November 2018 | Power Plant   | $28.60 \pm 0.31$ | $7.86 \pm 0.09$   | $25.63 \pm 0.09$ | $10.43 \pm 0.5$   | $2.76 \pm 0.03$ |
|               | Resalat       | $30.96 \pm 0.84$ | $7.92 \pm 0.03$   | $33.36 \pm 0.23$ | $0.66 \pm 0.09$   | $1.36 \pm 0.07$ |
|               | Khur Gursuzan | $24.83 \pm 0.36$ | $7.43 \pm 0.05$   | $28.20 \pm 0.12$ | $5.23 \pm 0.52$   | $4.44 \pm 0.05$ |
| January 2019  | Power Plant   | $24.70 \pm 0.29$ | $7.64 \pm 0.08$   | $24.30 \pm 0.12$ | $11.56 \pm 0.35$  | $2.85 \pm 0.03$ |
|               | Resalat       | $25.25~\pm~0.14$ | $7.73 ~\pm~ 0.06$ | $32.40~\pm~0.12$ | $0.63 ~\pm~ 0.22$ | $1.62 \pm 0.04$ |

Total Organic Matter (TOM) contents was analyzed in triplicate, using a FLASH 2000 CHN elemental analyzer on dried sediment samples after prior removal of inorganic carbon using a dilute HCl solution (Nieuwenhuize et al., 1994). Concentrations of Polychromatic biphenyls (PCB) were measured by the GCMS/MS method (Mohebbi Nozar et al., 2014). The three major PCB congeners, CB-138, CB-153 and CB-180, account for approximately 50% of the total PCB concentration (Nunes et al., 2011, 2014; Weber et al., 2018) and a simplified  $\Sigma$ PCB concentration was, therefore, calculated as the sum of congeners CB-138, CB-153 and CB-180.

Samples for nematode analysis were immediately preserved in 4% formaldehyde and transferred to the laboratory. In the laboratory, sediments were rinsed thoroughly with tap water and decanted 10 times over a 38-µm sieve. The nematodes from the fraction retained on that sieve were extracted by triple centrifugation using Ludox© HS40 diluted to specific density 1.18 (Vincx, 1996) and collected on a 38  $\mu$ m mesh. The supernatant fractions following each centrifugation were pooled per sample in 4% buffered formaldehyde. From each replicate sample, 100 nematodes were randomly picked out and transferred through a graded series of ethanol-glycerol solutions Seinhorst (1959), and mounted on glass slides. All selected nematodes were identified down to genus level, by using the pictorial keys of Platt and Warwick (1983) and Warwick et al. (1998) and the Nemys database (Bezerra et al., 2019, http://nemys.ugent.be). The number of male and female individuals were recorded and the sex ratio calculated (female:male). Maturity index (MI), based on a range of life strategists of nematodes (classes of colonizers-persisters or c-p), was calculated according to Bongers (1990) and Bongers et al. (1991, 1995). Furthermore, Shannon-diversity index (log2), MI as well as genus percentages were utilized as biological quality elements allowing the classification of the Ecological Quality (EcoQ) status of the study area in five classes: "bad," "poor," "moderate," "good," and "high" (for details see e.g. Moreno et al. 2011; Semprucci et al. 2015a,b).

#### 2.3. Data analysis

Different measures of nematode diversity were calculated: genera richness, Shannon-Wiener diversity (H', log 2), Simpson's diversity index (Si), and Pielou's evenness (J), using the DIVERSE routine in PRIMER 7 (Clarke and Gorley, 2015).

Differences in individual environmental variables (temperature, TOM, pH, PCB, salinity), total nematode abundances, diversity/evenness indices were tested across different locations and months in twoway crossed PERMANOVAs on univariate data (Anderson et al., 2008) with 999 permutations (Euclidean distance was used as similarity measure for univariate tests). For nematode assemblage structure, we tested for spatial and temporal differences using the same two-way PERMANOVA test but with multivariate nematode genus data (Bray Curtis resemblance on standardized and square root transformed data). The Estimated Components of Variation (ECV) were used to assess the variance explained by the test factors. The homogeneity of multivariate dispersions was tested using the PERMDISP routine to assess whether significant PERMANOVA differences were only factor differences or whether there was a contribution from dispersion heterogeneity to the group differences. Pairwise tests were used to assess the differences between levels of factors within significant main test effects under the full two-way model. The two-way PERMANOVA pairwise tests allowed us to assess the importance of the location versus the sampling month in determining differences of abundance, diversity/evenness, maturity index and assemblage structure. In analyses with < 100 available permutations, Monte Carlo estimates were applied.

The nematode assemblage structure was also analyzed using nonmetric multidimensional scaling (nMDS) with superimposed vectors representing genera correlations, on Bray-Curtis similarity values after square root-transformation of the data. Genera contributing most to the dissimilarities between different locations and months were identified by a two-way crossed SIMPER analysis. A DISTLM analysis was performed to assess to what extent the environmental variables (including PCBs) influence the nematode assemblages. This was accompanied by a dbRDA to visualize the relatedness of environmental variables with the assemblage data. Principal component analysis (PCA) on normalized environmental variables in distinguishing the different sampling locations and sampling months (including vector correlations). Furthermore, a chi-squared test ( $\chi$ 2) was carried out to test significant differences in sex ratio between the spatial and temporal levels.

# 3. Results

#### 3.1. Environmental data analysis

Table 1 shows physical and chemical environmental data for each season and location, and Table 2 shows the granulometric parameters of the sediments at the three different locations.

Sediments at KG and PP were much finer than at RE, where over 55% was characterized as coarse sand, compared to 1.56% and 2.03% at KG and PP, respectively (supported by PERMANOVA, Pseudo-F:43.411, p = 0.001).

Temperatures did not differ between locations but did differ

# Table 2

Sediment granulometry at the three locations (mean  $\pm$  standard deviation (n = 3)).

|  | Khur Gursuzan   | Power Plant  | Resalat   |
|--|---|--|---|
| Median grain size<br>Silt<br>%very fine sand<br>%Fine sand<br>%Medium sand<br>%coarse sand | $\begin{array}{rrrrr} 129.95 \ \pm \ 5.07 \\ 2.32 \ \pm \ 0.56 \\ 20.55 \ \pm \ 1.09 \\ 49.16 \ \pm \ 2.13 \\ 24.31 \ \pm \ 0.8 \\ 1.56 \ \pm \ 0.11 \end{array}$ | $\begin{array}{rrrrr} 113.74 \ \pm \ 6.77 \\ 3.46 \ \pm \ 0.63 \\ 26.44 \ \pm \ 2.35 \\ 57.57 \ \pm \ 0.87 \\ 10.08 \ \pm \ 1.72 \\ 2.03 \ \pm \ 0.42 \end{array}$ | $\begin{array}{rrrr} 166.26 \ \pm \ 4.19 \\ 0.03 \ \pm \ 0.03 \\ 3.37 \ \pm \ 1.18 \\ 15.98 \ \pm \ 0.87 \\ 24.49 \ \pm \ 2.01 \\ 55.46 \ \pm \ 1.86 \end{array}$ |

#### Table 3

PERMANOVA main test results (two-way crossed design with Location and Month as main factors, univariate data) for environmental variables. Df = degrees of freedom, SS/SM = sums and means of squares, P(perm) = p-value obtained through permutations, perms = number of unique permutations,  $\forall$ ECV = square root of estimated component of variation, PERMDISP = p-value for PERMDISP homogeneity of dispersion test. P(perm) values < 0.05 are in bold. LO = Location, MO = Month, LO × MO = Location × month interaction factor, Res = Residual.

| Temperature          | df | SS       | MS       | Pseudo-F | P(perm) | perms | √ECV         | PERMDISP |
|----------------------|----|----------|----------|----------|---------|-------|--------------|----------|
| LO                   | 2  | 2.9196   | 1.4598   | 2.232    | 0.15    | 999   | 0.29922      | 0.963    |
| MO                   | 2  | 478.15   | 239.07   | 365.53   | 0.001   | 998   | 5.1469       | 0.212    |
| LOxMO                | 4  | 7.6966   | 1.9241   | 2.9419   | 0.051   | 999   | 0.65067      | 0.318    |
| Res                  | 18 | 11.773   | 0.65404  |          |         |       | 0.80873      |          |
| Total                | 26 | 500.54   |          |          |         |       |              |          |
| pH                   | df | SS       | MS       | Pseudo-F | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                   | 2  | 0.63722  | 0.31861  | 16.769   | 0.001   | 998   | 0.18246      | 0.039    |
| MO                   | 2  | 1.456    | 0.72801  | 38.316   | 0.001   | 999   | 0.28068      | 0.073    |
| LOxMO                | 4  | 0.25402  | 0.063506 | 3.3424   | 0.024   | 999   | 0.1218       | 0.801    |
| Res                  | 18 | 0.342    | 0.019    |          |         |       | 0.13784      |          |
| Total                | 26 | 2.6893   |          |          |         |       |              |          |
| Salinity             | df | SS       | MS       | Pseudo-F | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                   | 2  | 228.53   | 114.27   | 1531.6   | 0.001   | 999   | 3.562        | 0.614    |
| MO                   | 2  | 372.33   | 186.16   | 2495.2   | 0.001   | 997   | 4.5471       | 0.563    |
| LOxMO                | 4  | 5.8319   | 1.458    | 19.542   | 0.001   | 999   | 0.67906      | 0.505    |
| Res                  | 18 | 1.3429   | 0.074607 |          |         |       | 0.27314      |          |
| Total                | 26 | 608.03   |          |          |         |       |              |          |
| Total organic matter | df | SS       | MS       | Pseudo-F | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                   | 2  | 34.322   | 17.161   | 1769.9   | 0.001   | 999   | 1.3805       | 0.93     |
| MO                   | 2  | 2.1085   | 1.0542   | 108.73   | 0.001   | 998   | 0.34068      | 0.955    |
| LOxMO                | 4  | 0.063459 | 0.015865 | 1.6362   | 0.203   | 999   | 0.045345     | 0.753    |
| Res                  | 18 | 0.17453  | 0.009696 |          |         |       | 0.09847      |          |
| Total                | 26 | 36.668   |          |          |         |       |              |          |
| PCB                  | df | SS       | MS       | Pseudo-F | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                   | 2  | 456.71   | 228.35   | 828.98   | 0.001   | 998   | 5.0341       | 0.111    |
| MO                   | 2  | 0.65722  | 0.32861  | 1.1929   | 0.325   | 999   | 0.076846     | 0.864    |
| LOxMO                | 4  | 3.4089   | 0.85222  | 3.0938   | 0.046   | 998   | 0.43847      | 0.17     |
| Res                  | 18 | 4.9583   | 0.27546  |          |         |       | 0.52485      |          |
| Total                | 26 | 465.73   |          |          |         |       |              |          |

significantly between seasons (p = 0.001, Table 3), with the highest values recorded in summer (A18) and lowest in winter (J19) at all locations (Table 1).

Salinity was significantly higher in summer compared to autumn, and higher in autumn compared to winter, overall as well as at each location separately. Salinity also differed significantly between locations, overall and in each separate season (Table 3, Table S1). It was clear that salinity was highest at RE (average of 39.50 in summer), followed by KG, and lowest at PP, with values as low as 24.30 in winter at PP. Salinity differed more between seasons than between locations (see  $\sqrt{ECV} = 4.55$  for season, vs 3.56 for location and only 0.68 for the interaction, suggesting little interaction effect) (Tables 3, S1).

pH was highest in summer, lower in autumn and lowest in winter for each location (Table 1), but significant differences were only expressed for certain pairwise comparisons (Table S1). There was a significant heterogeneity in dispersion of pH values between sites (p = 0.039, Table 3), indicating pH variability may be greater at some stations compared to others (Table S1). The significant interaction term was mainly caused by seasonal differences as confirmed by a higher estimated component of variation ( $\sqrt{ECV}$ ) for the latter (see Tables 3 and S1).

TOM was highest at KG and lowest at RE, while it was highest in winter, followed by autumn and summer, and consistently so across locations (Tables 1 and 3). However, TOM differences between locations were much greater than between seasons ( $\sqrt{ECV} = 1.38$  for location, 0.34 for season, Table 3).

The PCBs were significantly different between locations (for pooled data across all seasons, as well as for location comparisons within each season), with consistent values of more than 10 ng/g dry weight at PP, followed by 5.23–6.16 ng/g dry weight at KG and lower than 1 ng/g dry weight at RE (Table 1, Table 3). The PERMANOVA location  $\times$  season interaction effect was also significant caused by a summer vs. autumn difference at KG (Table 3, Table S1). The location differences in PCBs,

however, was over five times greater ( $\sqrt{ECV}$ , Table 3), with concentrations at KG and PP five to ten times higher than at RE (Table 1).

PCA results show a clear separation between the two impacted locations (PP, KG) and RE, the latter characterized by high salinity and low TOM% and PCB concentrations (Fig. 2). The seasonal effect is less pronounced at RE compared to the other two localities in which there was more evident distinction of summer period (A18). However, a temperature and pH influence is visible in the study area, with a gradient from high to low temperatures from summer to winter seasons (i.e. A18, over N18, to J19; left to right along PC1 in Fig. 2).



**Fig. 2.** Principal Component Analysis (PCA) based on the environmental parameters: temperature, salinity, pH, total organic matter (TOM %) and polychlorinated biphenyls (PCB). Vectors indicate correlations with the PCA axes, indicating strength of contribution of the environmental variables to the ordination patterns. Symbols indicate location: KG = Khur Gursuzan, PP = Power Plant, RE = Resalat. Letters indicate month/season (A = August 2018/summer, N = November 2018/autumn, J = January 2019/winter).



Fig. 3. Total nematode abundance (ind. 10 cm<sup>-2</sup>) per location for each month (mean values  $\pm$  SE, n = 3).



Fig. 4. Genus richness (number of genera) per location for each month (mean values  $\pm$  SE, n = 3).

November

### 3.2. Nematode data analyses

Nematode abundance ranged from 189 to 1320 ind. 10 cm<sup>-2</sup> in individual cores. The lowest abundance was found at PP (site characterized by industrial pollution) and highest abundance was observed at the RE site (i.e. location far from domestic or industrial pollution sources). Nematode abundances declined over time, with highest values in August 2018/summer and lowest in January 2019/winter for each location (Fig. 3). PERMANOVA showed these differences to be significant (including the interaction term (Table 4), but  $\sqrt{ECV}$  values indicated the greatest differences were caused by the location effect

(20.73 for location vs 12.16 for season), which can also be seen in the pairwise comparison results (Table S2) and plainly visible in Fig. 3.

Forty-two genera belonging to 17 families were identified in the present study (Fig. 4). Genus richness differed significantly between locations and seasons (PERMANOVA Table 4, location: p = 0.008; season: p = 0.021; interaction effects: p = 0.018). Highest genus richness was observed at RE and lowest at PP, and a gradual genus richness decline was observed over time (34 genera in summer, 30 in autumn and only 25 in winter) (Fig. 4, Tables 4 and S2). Pielou's evenness and Simpson's diversity index did not differ significantly between months or locations (Table 4), while H' showed a significant

### Table 4

PERMANOVA main test results (two-way crossed design with Location and Month as main factors, univariate data) for nematode abundance and diversity/evenness indices. Df = degrees of freedom, SS/SM = sums and means of squares, P(perm) = p-value obtained through permutations, perms = number of unique permutations,  $\forall$ ECV = square root of estimated component of variation, PERMDISP = p-value for PERMDISP homogeneity of dispersion test. P(perm) values < 0.05 are in bold. LO = Location, MO = Month, LO × MO = Location × month interaction factor, Res = Residual.

Number of genera

0

August

| ,                         |    |           |           | , |         |       |              |          |
|---------------------------|----|-----------|-----------|---|---------|-------|--------------|----------|
| Abundance                 | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 7903.4    | 3951.7    | 46.738                                  | 0.001   | 999   | 20.729       | 0.576    |
| MO                        | 2  | 2831.5    | 1415.8    | 16.745                                  | 0.001   | 999   | 12.162       | 0.991    |
| LOxMO                     | 4  | 995.29    | 248.82    | 2.9429                                  | 0.01    | 998   | 7.3998       | 0.75     |
| Res                       | 18 | 1521.9    | 84.55     |   |         |       | 9.1951       |          |
| Total                     | 26 | 13,252    |           |   |         |       |              |          |
| Genus richness            | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 66.889    | 33.444    | 7.0547                                  | 0.008   | 998   | 1.7859       | 0.07     |
| MO                        | 2  | 48.667    | 24.333    | 5.1328                                  | 0.021   | 996   | 1.4755       | 0.18     |
| LOxMO                     | 4  | 81.778    | 20.444    | 4.3125                                  | 0.018   | 999   | 2.2879       | 0.476    |
| Res                       | 18 | 85.333    | 4.7407    |   |         |       |              |          |
| Total                     | 26 | 282.67    |           |   |         |       |              |          |
| Pielou's evenness         | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 0.008636  | 0.004318  | 2.3446                                  | 0.12    | 998   | 0.0166       | 0.973    |
| MO                        | 2  | 0.00242   | 0.00121   | 0.65704                                 | 0.522   | 998   | -            | 0.811    |
| LOxMO                     | 4  | 0.002551  | 0.000638  | 0.34624                                 | 0.848   | 999   | -            | 0.485    |
| Res                       | 18 | 0.033151  | 0.001842  |   |         |       | 0.0429       |          |
| Total                     | 26 | 0.046758  |           |   |         |       |              |          |
| Simpson's diversity index | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 0.005907  | 0.002954  | 2.3677                                  | 0.127   | 999   | 0.0138       | 0.007    |
| MO                        | 2  | 0.006164  | 0.003082  | 2.4708                                  | 0.112   | 998   | 0.0153       | 0.155    |
| LOxMO                     | 4  | 0.003112  | 0.000778  | 0.62373                                 | 0.652   | 999   | -            | 0.43     |
| Res                       | 18 | 0.022453  | 0.001247  |   |         |       | 0.0353       |          |
| Total                     | 26 | 0.037637  |           |   |         |       |              |          |
| Shannon-Wiener diversity  | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 0.36963   | 0.18481   | 2.9039                                  | 0.082   | 998   | 0.116        | 0.007    |
| MO                        | 2  | 0.55614   | 0.27807   | 4.3691                                  | 0.027   | 998   | 0.154        | 0.159    |
| LOxMO                     | 4  | 0.44219   | 0.11055   | 1.737                                   | 0.196   | 999   | 0.125        | 0.485    |
| Res                       | 18 | 1.1456    | 0.063644  |   |         |       | 0.252        |          |
| Total                     | 26 | 2.5136    |           |   |         |       |              |          |
| Maturity Index            | df | SS        | MS        | Pseudo-F                                | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
| LO                        | 2  | 3.32      | 1.66      | 136.44                                  | 0.001   | 999   | 0.4279       | 0.054    |
| MO                        | 2  | 0.0088889 | 0.004444  | 0.3653                                  | 0.699   | 999   | -            | 0.853    |
| LOxMO                     | 4  | 0.016556  | 0.0041389 | 0.34018                                 | 0.846   | 999   | -            | 0.132    |
| Res                       | 18 | 0.219     | 0.012167  |   |         |       | 0.1103       |          |
| Total                     | 26 | 3.5645    |           |   |         |       |              |          |
|                           |    |           |           |   |         |       |              |          |

January



Fig. 5. Nematode indices for each location and each month. Maturity Index, Shannon-Wiener's diversity index, Simpson's dominance index, and Pielou's Evenness index (means ± SE, n = 3).

variation only between months (p = 0.027) with higher values in winter (J19) than in the other two seasons. Fig. 5 shows clearly the differences in MI between locations (p = 0.001, Table 4), consistent across the seasons (season and interaction factor non-significant, Table 4). The MI increases from KG to PP and RE (the lowest, intermediate and highest MI values, respectively).

The nMDS showed clear differences of the assemblage structure between locations, but no clear distinction between months (Fig. 6). This is supported by PERMANOVA showing a significant location effect (p = 0.001), with an effect size six times larger ( $\sqrt{ECV}$  42.7 vs 7.1) than the significant differences between seasons/months (p = 0.004; Table 5). The significant interaction effect was likely caused by significant differences between all pairs of locations in each month (as seen in pairwise comparisons, Table S3). Upon closer inspection, we can also see a tighter grouping of the PP samples in the nMDS, compared to KG and RE, which could explain the significant PERMDISP for location (Table 5). These results in accordance with the nMDS patterns (Fig. 6)



**Fig. 6.** Non-metric multidimensional scaling (nMDS) of nematode assemblages. Nematode genera data were standardized and square-root transformed prior to calculating Bray-Curtis resemblance. Symbols represent location (KG = Khur Gursuzan, PP = Power Plant, RE = Resalat). Letters indicate month/season (A = August 2018/summer, N = November 2018/autumn, J = January 2019/winter). Vectors overlain on plot show correlations of genera (greater than0.7) with nMDS axes, indicating important genera that differentiate the assemblages between locations.

suggest that the location effect causing the extreme grouping is of greater magnitude compared to the relatively small, but significant, dispersion effect for location groups. The relative abundance of observed nematode genera is reported in Table 6. The SIMPER analysis revealed the highest dissimilarities between Power Plant (PP) vs. Resalat (RE) (84.42%), followed by Khur Gursuzan (KG) vs. Power Plant (PP) (82.53%), while 67.43% of dissimilarity resulted between Khur Gursuzan (KG) vs. Resalat (RE) (Table S4). In particular, Daptonema, Sabateria, Promonhystera and Ptycholaimellus were the discriminating genera in Khur Gursuzan (KG); which is confirmed in the vector plot on the nMDS (genera correlations with nMDS axes greater than 0.7, Fig. 6). The genera that distinguished Power Plant (PP) from the others were Spirinia, Chromadorina and Terschellingia, while Oncholaimus, Pomponema and Viscosia mainly characterized Resalat (RE) (Table 6, S4). The level of dissimilarity found between months was lower than between locations (from 59.58 to 61.81%) (Table S5). The highest values were found between A18 vs. J19 (61.81%), followed by N18 vs. J19 (60.99%) and A18 vs. N18 (59.58%). Genera were relatively evenly distributed across seasons, with only minor differences between sampling months compared to between locations (Tables S4 and S5).

Furthermore, the relation between nematode assemblage distribution and environmental variables was analyzed using DISTLM and dbRDA (Fig. 7). The DISTLM analysis indicated that all 5 environmental variables explained over 86% of total variation, and over 96% of the variability of the fitted model. Taken separately, PCB, TOM% and salinity were the variables that explained most of the assemblage structure. The dbRDA (Fig. 7) shows clearly the correlation with PCB and the PP assemblages (PCB-polluted site), and the association of TOM with the KG nematode assemblages (domestic pollution).

The effect of different amounts of PCB on the sex-ratio of nematodes is shown in Fig. 8. There were significant differences in the female to male ratio at the Power Plant compared to the other two locations, in August ( $\chi 2 = 21.5$ , df = 1,  $\alpha = 0.05$ ), November ( $\chi 2 = 28.8$ , df = 1,  $\alpha = 0.05$ ) and January ( $\chi 2 = 18.7$ , df = 1,  $\alpha = 0.05$ ). There is a striking gradient of female to male ratios, with maximum values at the PCB-polluted PP location, medium values at the unpolluted RE site.

The genera selected as indicators by Moreno et al. (2011) and found in the study area were reported in the Table 7. In particular, the genera that reached more than 10% of the total nematode assemblage (in bold) allowed us the assign correspondent EcoQ classes for the ecological assessment of the Bandar Abbas beaches. When more than one EcoQ class was designated for each site, we assigned the intermediate EcoQ

#### Table 5

Nematode assemblages PERMANOVA main test results (two-way crossed design with Location and Month as main factors, multivariate data). Df = degrees of freedom, SS/SM = sums and means of squares, P(perm) = p-value obtained through permutations, perms = number of unique permutations,  $\forall$ ECV = square root of estimated component of variation, PERMDISP = p-value for PERMDISP homogeneity of dispersion test. P(perm) values < 0.05 are in bold. LO = Location, MO = Month, LO × MO = Location × month interaction factor, Res = Residual.

| Assemblages | df | SS     | MS     | Pseudo-F | P(perm) | perms | $\sqrt{ECV}$ | PERMDISP |
|-------------|----|--------|--------|----------|---------|-------|--------------|----------|
| LO          | 2  | 33,389 | 16,695 | 57,488   | 0.001   | 997   | 42.693       | 0.026    |
| MO          | 2  | 1477.8 | 738.9  | 25,444   | 0.004   | 998   | 7.0592       | 0.762    |
| LOxMO       | 4  | 2641.8 | 660.45 | 22,743   | 0.008   | 994   | 11.106       | 0.799    |
| Res         | 18 | 5227.3 | 290.4  |          |         |       | 17.041       |          |
| Total       | 26 | 42,736 |        |          |         |       |              |          |

#### Table 6

Genus composition and relative abundances (%) of nematodes at the three sampling locations and averaged over all sampling locations. The genera *Hypodontolaimus, Doliolaimus,* and *Polysigma* were only represented by 1 nematode each in the entire dataset. All data are means of three different months (August 2018 or A18, November 2018 or N18 and January 2019 or J19), with 3 replicates per location.

|                    | Khur Gursuzan | Power Plant | Resalat | Average |
|--------------------|---------------|-------------|---------|---------|
| Actinonema         | 0.00          | 0.00        | 0.31    | 0.10    |
| Camacolaimus       | 0.11          | 0.51        | 0.00    | 0.20    |
| Chromadora         | 0.20          | 0.00        | 0.10    | 0.10    |
| Chromadorina       | 0.22          | 25.05       | 0.09    | 8.45    |
| Cyatholaimus       | 0.11          | 0.00        | 0.00    | 0.04    |
| Daptonema          | 27.62         | 2.32        | 8.70    | 12.88   |
| Desmodora          | 0.28          | 0.00        | 0.00    | 0.09    |
| Dichromadora       | 0.19          | 0.56        | 0.00    | 0.25    |
| Doliolaimus        | 0.00          | 0.00        | 0.00    | 0.00    |
| Eleutherolaimus    | 5.63          | 8.47        | 7.37    | 7.16    |
| Enoploides         | 0.00          | 0.11        | 0.22    | 0.11    |
| Enoplolaimus       | 0.11          | 0.11        | 0.22    | 0.15    |
| Eumorpholaimus     | 1.00          | 0.00        | 2.00    | 1.00    |
| Graphonema         | 0.11          | 0.11        | 0.10    | 0.11    |
| Haliplectus        | 0.10          | 0.21        | 0.00    | 0.10    |
| Hypodontolaimys    | 0.11          | 0.00        | 0.00    | 0.04    |
| Metachromadora     | 0.55          | 0.00        | 0.21    | 0.25    |
| Metalinhomous      | 0.00          | 0.00        | 1.80    | 0.60    |
| Metoncholaimus     | 2.28          | 0.42        | 0.09    | 0.93    |
| Nygmatonchus       | 0.44          | 0.09        | 0.00    | 0.18    |
| Odontophora        | 0.11          | 0.00        | 0.00    | 0.04    |
| Oncholaimus        | 7.35          | 3.88        | 25.63   | 12.29   |
| Onyx               | 0.54          | 0.00        | 0.33    | 0.29    |
| Paracanthonchus    | 0.65          | 0.00        | 0.00    | 0.22    |
| Paraethmolaimus    | 0.84          | 0.11        | 0.10    | 0.35    |
| Paramonhystera     | 0.74          | 7.01        | 1.57    | 3.10    |
| Phanoderma         | 0.11          | 0.00        | 0.11    | 0.07    |
| Polysigma          | 0.00          | 0.00        | 0.00    | 0.00    |
| Pomponema          | 0.32          | 0.00        | 20.91   | 7.08    |
| Prochromadora      | 0.33          | 0.00        | 0.47    | 0.27    |
| Promonhystera      | 15.88         | 5.52        | 1.29    | 7.56    |
| Ptycholaimellus    | 11.20         | 0.20        | 11.81   | 7.74    |
| Rhynchonema        | 0.43          | 0.00        | 0.00    | 0.14    |
| Sabateria          | 20.90         | 0.00        | 0.18    | 7.03    |
| Spilophorella      | 0.09          | 0.00        | 0.29    | 0.13    |
| Spirinia           | 0.10          | 31.71       | 0.09    | 10.63   |
| Thalassomonhystera | 0.00          | 0.00        | 0.26    | 0.09    |
| Theristus          | 0.57          | 0.60        | 0.94    | 0.70    |
| Terschellingia     | 0.79          | 12.93       | 0.82    | 4.84    |
| Tripyloides        | 0.00          | 0.00        | 0.19    | 0.06    |
| Viscosia           | 0.00          | 0.11        | 13.59   | 4.57    |
| Xenolaimus         | 0.00          | 0.00        | 0.20    | 0.07    |

class. Accordingly, the high contribution of *Terschellingia* (from 7 to 17%) and *Spirinia* (23–44%) at PP resulted in a poor EcoQ. Instead, the occurrence of representatives of both the bad-poor classes (i.e. mainly *Sabatieria* and *Daptonema* for an overall contribution of 29–69%) as well as good-high classes (i.e. *Ptycholaimellus* and *Oncholaimus*, 3–38%) led to the assignment of a moderate EcoQ status at KG. RE was the site with the highest EcoQ (good), with mainly genera that indicate high EcoQ as dominant taxa (i.e. *Ptycholaimellus*, *Oncholaimus* and *Pomponema*),



Fig. 7. Db-RDA plot showing nematode assemblage structure differences between samples. The three clusters are entirely separated based on study sites with almost no influence of the month of sampling. Environmental variable fitting into the DISTLM model showed an important role of TOM and PCB content in structuring the nematode assemblages; which is confirmed by the correlation vector size (blue circle represents a correlation value of 1). Symbols indicate location: KG = Khur Gursuzan, PP = Power Plant, RE = Resalat. Letters indicate month/season (A = August 2018/summer, N = November 2018/autumn, J = January 2019/winter).



Fig. 8. Ratio of female to male (F/M) of three sampling locations of three different months. Data shown are means  $\pm 1$  SE of three replicate samples, per location.

while much less abundant taxa such as *Daptonema* indicated poor EcoQ (i.e. *Daptonema*). Regarding the univariate measures, H' suggested mainly a moderate EcoQ (it narrowly exceeded the lowest thresholds of the poor EcoQ), while MI showed the best EcoQ at RE, followed by PP and KG in a clear gradient (Table 7).

| Stations | Faunal parameters | Bad  | Poor   | Moderate                                 | Good                                  | High  |
|----------|-------------------|--|--|--|---------------------------------------|---|
| ЬЬ       | Genera<br>MI      | Terschellingia (7.0–17.4%)                           | Theristus (0.0–1.7%), Daptonema (0.0–5.9%)                       | Spirinia (22.6–43.7%)                    | Ptycholaimellus (0–0.9%)<br>2.79–2.80 | Oncholaimus (0.0–6.4%)                                |
|          | H'                |  | 2.49–2.73  |  |                                       |   |
| KG       | Genera            | Sabateria (10.9–30.6%), Terschellingia<br>(0.0–2.5%) | Daptonema (17.8–38.4%), Odontophora (0–1.0%), Theristus (0–2.5%) | Desmodora (0–1.6%), Spirinia<br>(0–0.9%) | Ptycholaimellus (2.0–20.8%)           | <b>Oncholaimus</b> (1.0–16.7%), Pomponema<br>(0–1.0%) |
|          | MI                |  | 2.30–2.39  |  |                                       |   |
|          | H                 |  |  | 2.52-3.22                                |                                       |   |
| RE       | Genera            | Sabateria (0.0–0.8%), Terschellingia<br>(0.0–2.9%)   | Daptonema (1.7–13.3%), Theristus (0.0–4.9%)                      | Spirinia (0–0.8%)                        | Ptycholaimellus (8.1–15.9%)           | Oncholaimus (15.9–32.8%), Pomponema<br>(11.2–34.1%)   |
|          | IM                |  |  |  |                                       | 3.18-3.25   |
|          | H'                |  |  | 2.78–2.92                                |                                       |   |

**Fable** 7

#### 4. Discussion

# 4.1. Marine nematodes in Persian Gulf are mainly driven by spatial differences owing to different types of environmental disturbances

The study presented here is the first to document both the spatial and temporal trends of free-living nematodes in the Iranian part of the Persian Gulf, an area previously covered in only two investigations (Sahraean et al., 2017a, b). The faunal distribution was analyzed in relation to different types of anthropogenic pollution (i.e. domestic sewage input and industrial contamination), providing evidence for anthropogenic impact on certain nematode assemblage characteristics.

Spatial patterns (i.e. difference between locations) and associated pollution types (PP and KG) or lack of pollution (RE) were the most significant factors affecting nematode abundance, richness and lifestrategies in the intertidal area of Bandar Abbas. The PP site, the beach close to the industrial power plant, showed about twice the PCB concentration compared to the site characterized mainly by domestic sewages (KG) and concentrations ten times higher than at RE, which had very low levels of PCB (and TOM) confirming RE as a relatively undisturbed site. Despite KG not being in the immediate vicinity of the industrial area, sediments there contained the highest levels of organic matter enrichment (TOM), in addition to the increased levels of PCBs. It has been shown before for other geographical areas that the accumulation of TOM and fine sediment grain size can play an important role in the retention of PCBs in the marine sediments (Kampire et al., 2017). This could explain the concomitant high levels of PCB and TOM in the study area, particularly at KG. Moreover, the statistical results indicate clearly how the sedimentary PCB and TOM concentrations were markedly different between locations, while seasonal influence on PCB and TOM concentrations was shown to be less important, although a TOM increase was observed from summer, over autumn and into winter.

The total mean abundances recorded in the present study are of the same order of magnitude as values previously documented in the Persian Gulf (Sahraean et al. 2017a) as well as from other beaches in the Mediterranean (e.g. Gheskiere et al., 2005; Moreno et al. 2006; Kotwicki et al. 2014) and the North Sea (e.g. Maria et al. 2018). However, these abundances are lower compared to those reported for tropical beaches, for instance in Indian and Atlantic Oceans (e.g. Semprucci et al., 2010b, 2011; Maria et al. 2013a; Santos et al. 2019). In intertidal areas, low abundances of meiofauna or nematodes may be related to numerous natural environmental variables including hydrodynamic conditions, daily variations of temperature and salinity (Heip et al., 1985; Maria et al. 2013b; Sahraean et al., 2017a). Nematode abundance differed significantly between locations (Table 4) with lowest values at PP, followed by KG, and highest at RE. This is concomitant with a likely negative effect of firstly PCBs, and secondly of TOM, on abundances. Furthermore, given that the sewage outfalls alone generally lead to an increase of the total meiofaunal abundance (see Maria et al. 2016 for review), there may be compounding effects of contaminants and organic load on nematode abundances at PP and KG. Aside spatial differences, there were some relatively minor seasonal differences in abundance as well (Table 4). The lower abundances in winter may be a result of low temperatures, or an increased disturbance frequency caused by flooding and water circulation in the winter period (which could also explain increased TOM levels in winter). Several authors have documented that nematode abundances are positively related to temperature increases because of 1) the more rapid generation times of nematode species at higher temperatures; 2) the decrease in nutrient absorption at lower temperatures and 3) the availability of food (Heip et al., 1985; Moens and Vincx, 2000; Semprucci et al., 2010a; Vranken et al., 1986). Moreover, temperature can affect nematode abundances directly, via changing osmotic pressures, dehydration and/or effects on reproduction, as well as indirectly, by controlling the growth of food items such as bacteria and diatoms (Harris,

1972; Semprucci et al., 2018). It is worth noting that temperature regimes can also have a substantial effect on the interspecific interactions and life-history characteristics of marine nematodes (De Meester et al., 2015a,b).

Nematode genus richness was low compared to other geographical regions (e.g. Baldrighi et al. 2019; Gheskiere et al., 2004, 2005; Maria et al. 2013a,b, 2018; Santos et al., 2019), and likely caused by the harsh conditions found in the intertidal area of the Persian Gulf as well as the recent increase in human pressures in the general area. Similar to what we found for nematode abundance, genus richness differences were greater between locations compared to differences between seasons (cf. PERMANOVA, Table 4). Lowest nematode genus richness and H' diversity, and highest PCB concentration at PP (i.e. industrial site), suggest a PCB impact on the number of nematode taxa and diversity. However, there were no significant H' diversity differences between locations.

With regard to nematode assemblage structure, the nMDS showed much greater assemblage differences between locations than between months/seasons. However, the primary effects of PCB and TOM as environmental variables explaining most of the assemblage structure was corroborated by the DISTLM and dbRDA results. A temporal sampling effect was present, but was much smaller than the location or spatial effect. Nematode assemblage differences between seasons were mainly caused by the contrast between the two extreme seasons, summer vs. winter, and is likely related to the different biological cycles of the various species as well as to the different food availability in those periods.

In summary, we show that the observed nematode trends are not mainly driven by environmental differences between seasons as has been documented in previous studies (Sahraean et al., 2017a), but rather by spatial differences owing to different types and amounts of contaminants. This may also be attributed to the unique environmental conditions of the Persian Gulf, which harbors comparatively low local and habitat-specific biodiversity (Ejlali Khanaghah et al., 2010; Naderloo and Tuerkay, 2012).

# **4.2.** Ecological quality status and severity of polychlorinated biphenyls effects and sewage outputs on the nematode assemblage

When we evaluated ecological quality status according to the thresholds defined by Moreno et al. (2011), lowest EcoQ was observed at PP caused by low percentages of sensitive taxa in the sediments near the industrial site (Table 7). The best ecological conditions were found in RE sediments, where genus indicators of high sediment quality were prevalent. Our EcoQ results indicate a gradient of increasing impact on the nematode assemblages and ecological quality, similar to the gradient observed in PCB levels (despite these levels being lower than the NOAA's threshold values). The univariate measures so far utilized for the definition of the EcoQ classes (namely H' and MI) did not appear suitable for the discrimination of the different EcoQ status in the study area: H' practically detected the same EcoQ status in all three stations (there was a minor crossover of EcoQ from predominantly moderate to poor based on H' at PP), while MI did not indicate poor conditions at PP, but it did at KG. This finding corroborates the observations that the discriminating genera (see section 4.3) may be regarded as bioindicators of specific ecological conditions. A few considerations regarding MI have to be made. MI distinguishes clearly between locations, but it may be that the MI performs better when considering organic pollution at KG (cf. TOM%) compared to the specific PCB contamination observed at PP. The fact that MI, in the context of EcoQ, does not grade the locations as expected by the environmental variables and in line with the expected toxicity of PCB contamination, is not that surprising given the highly variable intertidal habitat in the Persian Gulf. Indeed, there are several studies that have demonstrated that this index based on lifestrategies is not able to detect the real ecological status in areas characterized by notable variations of the physical-chemical features

(Semprucci et al., 2014, 2019; Jouili et al. 2017). Furthermore, the list of the c-p classes for the calculation of MI in marine habitats is too limited in terms of the number of genera for which this information has been published; it should be increased in accordance with existing literature. Eleven genera recorded in the present study were absent in the Bongers' list (i.e. Doliolaimus, Graphonema, Eumorpholaimus, Nygmatonchus, Paraethmolaimus, Phanoderma, Polysigma, Prochromadora, Promonhystera, Thalassomonhystera, Xenolaimus) and so assignment of the c-p scores on the family level was necessary (as also suggested by Bongers et al. (1991) for homologous families). This does increase the risk of an incorrect ecological status assignment as genera and species within a family can vary in terms of colonizer vs. persistence behaviors and life cycles. An additional problem with MI is related to the lack of empirical evidence on the life-strategies of most nematode taxa. This would require a revision of the categories assigned by Bongers, and differentiation on the base of the disturbance type should also be considered (Semprucci et al., 2015b). In this context, the genus Chromadorina, originally considered c-p 3, being more abundant in the industrial contaminated sediments (PP site), could be regarded as c-p 2, especially in areas affected by PCB disturbance, while Promonhystera, absent in the Bongers' list, could be regarded as c-p 3, being more abundant in the area characterized by domestic sewage inputs (KG site).

PCB concentration may affect hormones involved in the development and sex determination. In our study the sex ratio appeared influenced by the concentrations of this chemical, with highest ratio values, and hence dominance of females, at the PP site. The greater relative number of females is often interpreted as a way to sustain the populations in unstable environments (Tahseen, 2012). This suggests that there is a possible influence of PCB levels on the sex ratio. Our findings did not support a positive relation between higher temperature and increasing male presence as suggested by dos Santos et al. (2008).

# 4.3. Nematode assemblage structure and the occurrence of certain species can indicate different types of human impacts in this coastal area.

Marine nematodes are influenced by interacting physical and biological processes, leading to variations in their distribution at different spatial and temporal scales. In addition, pollution and disturbance are known to cause significant differences of nematodes at different organization levels (Semprucci and Balsamo, 2012). The levels of PCB and organic enrichment observed in this intertidal beach were lower than the thresholds reported by the National Oceanographic and Atmospheric Administration's (NOAA's) sediment quality guidelines (Long et al., 1995), but they appeared enough to influence nematode assemblages and, above all, genus richness, abundance and MI (discussed in context of EcoQ). Even in the presence of temporal differences, spatial effects between locations dominated and were related to the differences in human impact.

As reported in studies worldwide, the assemblage structure and the occurrence of specific sentinel species are important tools that allow detection of anthropogenic impact (Losi et al., 2013; Moreno et al., 2011). Although, the structure of the nematode assemblages showed a certain heterogeneity among the replicates of the same sampling site (see PERMDISP results, Table 4) a distinctive nematode assemblage for each environmental condition was observed (Fig. 6). Nematode assemblages at Resalat, which is unaffected from sources of human disturbance and/or pollution, were mainly dominated by genera that generally live in pristine environments like the Oncholaimidae Oncholaimus and the Cyatholaimidae Pomponema, which are reported by numerous authors as bioindicators of healthy sea floors (Hedfi et al., 2013; Moreno et al., 2011; Semprucci and Balsamo, 2012). The Oncholaimidae family and especially Oncholaimus life strategy is rather controversial (Semprucci et al., 2015b), because there are Oncholaimus species that are typical k-strategists (large, slow development, long generation time, cf. Bongers et al. (1991)), but other species belonging

to this genus are recognized as r-strategist species. For example, *O. campylocercoides* is able to detoxify metal sulphides (McMullin et al., 2000; Thiermann et al., 1994, 2000) and survive high concentrations of trace elements (e.g. Boufahja and Semprucci, 2015) and the more recently described, *O. dyvae* has shown affinity for extreme conditions at a deep-sea hydrothermal vent where it likely lives in association with bacterial symbionts (Bellec et al., 2018; Zeppilli et al., 2019).

Khur Gursuzan (KG) was discriminated by Daptonema and Promonhystera (Xyalidae), Sabateria (Comesomatidae) and to some extent Ptycholaimellus (Cyatholaimidae) from the other two sites. This is confirmed in the SIMPER results (Table S4) and the genus correlations with KG in the nMDS (Fig. 6). These taxa are considered sentinels of a poor ecological quality status because of their well-known tolerance to pollution (Austen and Somerfield, 1997; Gambi et al., 2003; Schratzberger et al., 2006; Soetaert et al., 1995; Steyaert et al., 2007; Warwick et al., 1997). Furthermore, representatives of Sabatieria and Daptonema (both non-selective deposit feeders, 1B trophic group Moens and Vincx, 1997) have been reported as bioindicators of organic enrichment (Gyedu-Ababio et al., 1999; Moens et al., 2013; Montagna and Harper, 1996) which indeed occurs at KG. Numerous species belonging to both the genera Sabatieria and Daptonema are adapted to live in permanently hypoxic or anoxic sediments and high sulfide concentrations and often thrive under conditions that are unsuitable for other nematodes (Guilini et al., 2012; Liu et al., 2007; Steyaert et al., 1999; Tietjen, 1980; Vanreusel et al., 2010). Ptycholaimellus species are epigrowth feeders (2A trophic group, Moens and Vincx, 1997) that generally occur in the surface layer (upper 1 cm) of intertidal sediments characterized by microphytobenthic biofilms (Commito and Tita, 2002; Steyaert et al., 2003; Van Colen et al., 2009). They are able to respond rapidly to microphytobenthos blooms (Van Colen et al., 2009) and are abundant in sediments under fish-farm cages (Danovaro et al., 2009). Accordingly, the domestic sewage inputs at KG, leading to a nutrient increase, could have a stimulatory effect on both 2A and 1B nematode feeding types.

The Power Plant area of Bandar Abbas did not show levels of PCBs high enough to exceed official standards during the sampling period, but yet are still suspected to modify the nematode assemblages in the sediments. Mohebbi Nozar et al. (2014) documented high PCB levels as a result of the zinc production smelting and electrical power generation, primary contributors to this chemical in Iran. Chemical releases may have exerted selective pressure, resulting in a specific nematode fauna composition favouring genera like Spirinia (Desmodoridae), Chromadorina (Chromadoridae) and Terschellingia (Linhomoeidae) (SIMPER results Table S4, nMDS correlations with PP in Fig. 6). The r-strategy of the genus Spirinia (i.e., S. parasitifera) has been documented in microcosom experiments to indicate presence of Polycyclic Aromatic Hydrocarbons (PAHs) and its effect on nematode assemblages (Louati et al., 2014). A dominance of the genus Spirinia has also been observed in sediments enriched with organic pollutants (Beyrem et al., 2010) or trace elements (Mahmoudi et al., 2007; Losi et al., 2013). In the same way, Chromadorina increased in relation to the enhancement of concentration of the antifouling biocide (Hannachi et al., 2016). Terschellingia has been linked to organic enrichment as well as contaminants and is recognized as an indicator of poor ecological status because of its well-known tolerance to pollution (Moreno et al., 2011; Balsamo et al., 2012 and references therein). In the present study, Terschellingia is the third-most abundant genus at PP, with 12.93% relative abundance; much higher than at KG and RE (0.79 and 0.82%). This presents more evidence of the contaminated nature of the sediments at PP and its influence on nematode assemblage structure. Thus, given the gradients observed, we can recognize Spirinia, Chromadorina and Terschellingia as resistant taxa and sentinels of PCB presence in the sediments, while Daptonema, Promonhystera, Sabateria and Ptycholaimellus as opportunistic genera, and mainly indicators of organic enrichment.

#### 5. Conclusions

Our study shows that local anthropogenic pressure (i.e. organic enrichment and sediment contamination) has marked effects on meiobenthic nematodes, even in intertidal habitats that generally experience notable physical-chemical fluctuations. The spatial differences, which are associated to anthropogenic impacts in the form of PCBs and organic load, are indeed more important than temporal dynamics in driving nematode assemblages in the intertidal beaches of Bandar Abbas (Hypothesis 1). We found that the effects of PCBs have a greater effect on the nematode assemblages compared to sewage outputs in the study area, mainly in terms of abundance, richness and female/male ratio and in assemblage structure, therefore confirming our second hypothesis. With regard to third hypothesis; nematode assemblage structure and especially the different contributions of species can indicate different types of human impact in this coastal area. Sets of indicator genera were identified to assess the ecological quality of the different environmental conditions at the three beaches. The overall low level of efficiency of the use of the maturity index in assessing ecological quality of sediments in Bandar Abbas area suggests that the colonizers-persister (c-p) scoring system may require updating, particularly in the context of variable intertidal areas as is the case here.

# CRediT authorship contribution statement

Narjes Sahraeian: Conceptualization, Data curation, Investigation, Methodology, Resources, Writing - original draft. Homayoun Hosseinzadeh Sahafi: Funding acquisition, Project administration, Supervision. Hadi Mosallanejad: Writing - original draft, Methodology. Jeroen Ingels: Formal analysis, Writing - review & editing. Federica Semprucci: Formal analysis, Writing - review & editing.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106697.

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