

Are free-living nematodes effective environmental quality indicators? Insights from Bohai Bay, China

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ABSTRACT

Free-living marine nematodes are sensitive biological indicators of environmental quality in marine sediments. This study investigated nematode communities in coastal waters in Bohai Bay, China. Nematode community taxonomic structure, colonizer–persister classes, maturity index (MI), index of trophic diversity (ITD), Shannon–Wiener diversity index (H'), together with the Hakanson's Ecological Risk Index, were applied to assess the environmental quality of the investigated stations. The taxonomic structure, MI, ITD, and H' varied among the study stations. In addition to the stochastic causes, variability in environmental variables together with metal concentrations explained, to a certain extent, the observed variations in nematode features. The differentiation in nematode species' composition was attributed to changes in water depth, temperature, salinity, dissolved oxygen concentration, sediment medium diameter, organic matter, chlorophyll-*a* and pheophytin-*a* concentrations, and concentrations of manganese (Mn) and cobalt (Co). Mn was the most influential metal in explaining nematode diversity. MI appeared to be affected more by metals than by other environmental variables. According to the nematode features, the environmental quality ranged from bad to good at the majority of stations, although the rankings varied among different indices. Taxonomic structure, life strategy traits, ITD, and H' were all effective in highlighting the areas subjected to severe metal contamination in the bay, and could provide more comprehensive assessments when used in combination. These indices suggested a more severe pollution status than the results obtained from the Hakanson's Ecological Risk Index method, and may be more sensitive to ecological quality deterioration. However, their effectiveness requires further testing over larger spatial ranges and with more data.

1. Introduction

Bohai Bay is a semi-enclosed bay located in the western part of the Bohai Sea (37°07'–41°00' N, 117°35'–121°10' E), China. In addition to the Hebei province, Bohai Bay (west of 118.6° E) is surrounded by the megacities of Beijing and Tianjin, i.e., some of the most commercially important regions in China. Rapid economic development of the surrounding areas has led to increasing pollution, especially due to heavy metals, in Bohai Bay, which is considered to be one of the most contaminated coastal areas in China (Xu et al., 2016). It was reported that from 1997 to 2007, the mean Cd, Cu, Hg, Pb, and Zn concentrations in the surface sediments of the Bay increased compared with their background values in sediments, which were largely attributed to human activities (Gao et al., 2014). Rivers are considered to be the most

important sources of heavy metal inputs into Bohai Bay. The Haihe River is the largest river flowing into the bay, and delivers large amounts of fresh water, sediments, and pollutants (Song and Duan, 2019). The heavy metal concentrations in surface sediments decrease with increasing distance from the Haihe River Estuary and then further decrease to the open sea (Xu et al., 2012).

Heavy metals are toxic pollutants that may cause serious biological harm to organisms (Tam and Wong, 1997). Among the heavy metals, some (e.g., Cr and Cu) are essential to most organisms due to their active participation in carbohydrate metabolism and protein synthesis; however, elevated doses Cr and Cu interfere with osmoregulation and oxidative stress pathways (Brooks and Mills, 2003; Lushchak, 2011; Olmedo et al., 2013). Other metals (e.g., Pb and Hg) are nonessential metals, and are known neurotoxins because of their potential to damage

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gonad and brain cell membranes (Verstraeten et al., 2008; Sinkus et al., 2017). Although heavy metals are present in low concentrations in the environment, they may be biomagnified via food webs, accumulating in top predators, and eventually threatening human health (Lavoie et al., 2010; Liu et al., 2019a).

Benthic assemblages are in direct contact with pollutants in the sediment and often rapidly respond to adverse ecological conditions. Therefore, they are recommended for use in monitoring and detection of environmental changes (Pearson and Rosenberg, 1978). Generally classified as protists and metazoans between 31 and 500 μm (Higgins and Thiel, 1988; Giere, 2009), meiofauna have certain key advantages as biological indicators. For example, they usually exhibit high diversity, high abundance, high turnover rates, and spend their entire lifecycles within sediment environments (Higgins and Thiel, 1988). Marine nematodes are dominant meiobenthic communities and are sensitive biological indicators of pollution, including heavy metal pollution (Gyedu-Ababio et al., 1999; Mahmoudi et al., 2007; Monteiro et al., 2018). The sensitivity and responses of nematodes to increased metal pollutants have been reported by numerous studies based on microcosm experiments or field investigations, and changes in nematode community features have commonly been interpreted in the context of metal pollution (Austen and Somerfield, 1997; Gyedu-Ababio and Baird, 2006; Mahmoudi et al., 2007; Monteiro et al., 2018). In many cases, elevated doses of metals (e.g., Pb, Cu, Zn, Ni, or Cr) have had clear effects on the structure of marine nematode communities, and nematode diversity decreases with increasing metal pollution due to the elimination of sensitive species and the dominance of tolerant species (Austen et al., 1994; Korthals et al., 1996; Austen and McEvoy, 1997; Mahmoudi et al., 2007; Heininger et al., 2007; Monteiro et al., 2018).

In recent decades, increasing interest has been directed towards the development of nematode indices to monitor environmental changes. Nematode indices, such as diversity indices, index of trophic diversity (ITD), maturity index (MI), and colonizer-persister (c-p) classes were analyzed and specific thresholds were developed by Moreno et al. (2011) and Semprucci et al. (2014a, 2014b). These metrics have been applied to evaluate ecological quality in the Mediterranean Sea and the Black Sea (Moreno et al., 2011; Semprucci et al., 2014a, 2015; Ürkmez et al., 2014; Bianchelli et al., 2016, 2018; Franzo et al., 2018), the Indian Ocean (Semprucci et al., 2014b), Malaysian coastal habitats (Chen et al., 2018), and the Gulf of Mexico (Soto et al., 2017). Among these metrics, the Shannon–Wiener diversity index (H'), MI, and c-p classes were recommended as the optimal descriptors for monitoring marine ecological quality in coastal habitats (Semprucci and Balsamo, 2014; Chen et al., 2018). Low H' and MI values often reflect the most impacted environments (Semprucci and Balsamo, 2014). However, their application in environmental quality assessment remains controversial. Diversity indices may not always discriminate between environmental stressors because they are highly influenced by sample size and habitat type or complexity (Gaston, 1996; Gray, 2000). The MI was proposed based on the classification of nematode families or genera into c-p classes (Bongers, 1990; Bongers et al., 1991; Bongers and Bongers, 1998). Extreme colonizers (c-p = 1) are represented by nematodes with a generation time of only a few days, while extreme persisters (c-p = 5) are nematodes with a generation time of 1 yr (Bongers et al., 1991). However, the current nematode c-p index still needs to be updated and modified for marine nematodes (Moreno et al., 2011; Moens et al., 2013). The ITD is another potentially useful metric in environmental monitoring, and is calculated based on the classification of species into trophic groups (Heip et al., 1985). However, the complexity of feeding habitats cannot be adequately represented by trophic groups (Moens and Vincx, 1997; Moens et al., 2005). Therefore, the assessments given by ITD deviate from rankings of environmental quality based on H' and MI in several studies (Semprucci et al., 2015; Bianchelli et al., 2018). The potential application of nematode metrics in the assessment of environmental quality in coastal waters still requires extensive testing in different habitats.

Environmental quality assessments of Bohai Bay associated with several metals (Cu, Cd, As, Pb, Zn, Cr, Hg, and Ni) have been examined in previous studies (Zhan et al., 2010; Xu et al., 2012; Gao and Chen, 2012; Gao and Li, 2012; Gao et al., 2014; Liu et al., 2015; Li et al., 2018). In these studies, the mean metal concentrations were compared with those prescribed by the guidelines of National Standard of China for Marine Sediment Quality GB 18668–2002 (SEPA, 2002), or other marine sediment assessment methods (e.g., the geoaccumulation index [I_{geo}], enrichment factor [EF], and potential ecological risk index) to evaluate the ecological situation of Bohai Bay. In general, the north-western coast of Bohai Bay faces severe pollution threats (Meng et al., 2008; Gao and Li, 2012; Zhou et al., 2015; Li et al., 2018). Most metals posed limited risk of toxicity, except Hg and Cd which posed high potential ecological risks to the structure and function of the Bohai Bay ecosystem (Liu et al., 2015; Li et al., 2018).

Bioassessment indicators have been applied to evaluate the ecological quality of Bohai Bay. Macrofaunal indicators revealed a slight disturbance status in the mouth of bay (Ni et al., 2019), and meiofaunal diversity indices and the nematode to copepod ratio indicated the contamination of sediments in Bohai Bay (Zhang et al., 2011, 2012; Liu et al., 2015). The application of meiofauna or nematode parameters in environmental quality assessment activities could enhance our understanding of the ecological quality of the bay. However, only a few meiofaunal studies have been conducted in Bohai Bay (Zhang et al., 2009, 2011, 2012; Hu and Zhang, 2012; Liu et al., 2015), and their features in environmental assessment activities remain poorly understood.

In the present study, we investigated nematode community features, including taxonomic and functional diversity, life strategy traits, and the key factors influencing these features in the coastal waters of Bohai Bay. To the best of our knowledge, this is the first attempt to investigate the effects of heavy metals on the nematode community in Bohai Bay. Owing to their potential effectiveness as an environmental quality indicator, information on nematode features was used to evaluate the environmental quality of Bohai Bay in relation to metal pollution. To assess the effectiveness of these features, the ranked results were compared to results derived from the Hakanson's Ecological Risk Index method, which was applied to assess metal contamination and potential ecological risk (Hakanson, 1980). The aims of the present study were to: (1) understand intra-bay variability in nematode community features; (2) determine whether variations in nematode features indicate environment changes and metal pollution, in particular; and (3) investigate the suitability of nematodes as effective environmental quality indicators.

2. Materials and methods

2.1. Study area

Bohai Bay encompasses an area of $1.47 \times 10^4 \text{ km}^2$ and has a mean water depth of 10 m (Nie and Tao, 2009). The coastline has a gentle slope, the width of the tideland is 3–5 km, and the area experiences an irregular semidiurnal tide (Gao and Chen, 2012). The Bohai Sea is situated in a typical monsoon region. It is hot and rainy in summer, and cold and dry in winter. The mean sea surface temperature is 26.1 °C in summer and 0.4 °C in winter (Song and Duan, 2019). The prevailing wind in summer is from the southeast, while a northwest wind dominates in winter (Lv et al., 2014). The annual mean precipitation is 545 mm and the majority of rainfall (approximately 55.3%) occurs during July and August (Wang et al., 2019). The sea surface salinity varies between 26 psu, with an increasing trend observed from the near shore area moving eastwards (Qing et al., 2013). Salinity increases with a decrease in fresh water discharge from rivers and is highest in winter and lowest in summer (Wei et al., 2017). Several rivers flow into the bay, delivering considerable amounts of fresh water and sediment. Industrial and domestic waste water discharges are also transported into the bay

by rivers (Song and Duan, 2019). The Haihe River, situated in Tianjin, is the largest river entering the bay, with annual water discharges of approximately $18.3 \times 10^8 \text{ m}^3$ (Song and Duan, 2019). Clay and silt are distributed throughout this region and are the most prominent sediment types. The surface sediments of the western Bohai Bay are mainly derived from terrigenous detritus which are transported by the rivers flowing into the sea (Feng et al., 2015). Low water exchange occurs between Bohai Bay and the central Bohai Sea (i.e., mean velocity of the residual current is $< 0.1 \text{ m s}^{-1}$), and the self-cleaning capacity is poor (Song and Duan, 2019). High salinity water from the central Bohai Sea flows into Bohai Bay along both the left and right sides of the mouth of the bay, and outflows from the middle, forming a two-loop structure in the mouth of the bay (Liu et al., 2019b). In the western coastal waters, because of the high discharge of fresh river water and strong stratification, two coastal currents occur during summer; however, the current is negligible in winter because of strong mixing induced by winds and waves (Zhao and Lei, 1995; Wan et al., 2004).

2.2. Field sampling

In May 2011, field sampling was conducted at 35 stations in Bohai Bay ($38^\circ 42' - 39^\circ 08' \text{ N}$ and $117^\circ 42' - 118^\circ 08' \text{ E}$, Fig. 1). A modified 0.05 m^2 Gray-O'Hara box corer was used to collect sediment samples. Sub-samples were collected using a sawn-off syringe (2.9-cm inner diameter) placed in each box corer. Three sub-samples were obtained separately from each of three box corers and sectioned vertically into 0–2 cm, 2–5 cm, and 5–8 cm fractions for meiofauna analyses. The sub-samples were fixed using 5% formalin solution. Four other sub-samples were obtained to analyze sediment grain-size, chlorophyll-a (Chl-a) and pheophytin-a (Pheo-a) concentrations, heavy metals concentrations, and the organic matter (OM) content. All samples for analyses of environmental variables were collected to a 5-cm depth and preserved frozen at -20°C until subsequent analyses. Bottom water temperature (Temp), salinity, and dissolved oxygen (DO) concentrations were measured *in situ* using a YSI 600XLM sonde (Yellow Springs Instruments, Yellow Springs, Ohio, USA).

2.3. Nematodes

Sieves with 500- and 31- μm mesh sizes were used to wash the sediment samples in the laboratory, and the sediment retained in the 31- μm sieve was collected. Subsequently, the Ludox TM-50 (gravity of 1.13 g/cm^3) centrifugation technique was used to extract meiofauna from the sediment (Higgins and Thiel, 1988; Giere, 2009). All meiofauna specimens were sorted and counted manually. Nematodes were mounted on permanent slides with anhydrous glycerol for subsequent identification

(Zhang and Platt, 1983; Somerfield et al., 2005). Each nematode was identified to the species level (indicated by the genus name followed by sp. 1, sp. 2, etc.) following the pictorial keys of Platt and Warwick (1983), Platt and Warwick (1988), Warwick et al. (1998), and NeMys online identification keys (Bezerra et al., 2020) using an Olympus BHS compound microscope (Olympus, Tokyo, Japan).

The ITD was calculated using the following equation: $\text{ITD} = \sum g_i^2$, where g is the relative abundance of the i^{th} trophic group (Heip et al., 1985). Nematodes were assigned to different trophic groups based on buccal cavity morphology as selective deposit feeders 1A, non-selective deposit feeders 1B, epistratum feeders 2A, or omnivores/predators 2B (Wieser, 1953). The ITD values varied from 0.25 (i.e., the highest trophic diversity when all four groups are equally represented) to 1 (i.e., the lowest trophic diversity when one group dominates all others). The MI was computed by the following equation: $\text{MI} = \sum v(i) \cdot f(i)$, where $v(i)$ is the c-p value of the species i , and $f(i)$ is the frequency of the species i in the sub-sample (Bongers, 1990; Bongers et al., 1991). The c-p values followed the families and genera list compiled by Bongers et al. (1991), Bongers et al. (1995) and Bongers and Bongers (1998). Extreme colonizers (c-p = 1) are represented by nematodes with short generation times, high egg production rates, and an ability to form dauer larvae, while extreme persisters (c-p = 5) are nematodes with a long life span and low reproduction rates, such as *Enoplus* (Bongers and Bongers, 1998). A high MI indicates that the community is more stable or mature; a low MI means that the community is in a stressed condition. The contribution of each life-history group (c-p 1–5) to the total nematode assemblage was also calculated.

2.4. Environmental variables

Sediment grain size was analyzed using a dry sieve method (Higgins and Thiel, 1988; Liu et al., 2015). Sediment clay, silt, and sand proportions, and the medium particle diameter (Md) value corresponding to the 50%-point of the cumulative scale were estimated. The OM content in the sediment was determined using the $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ oxidation method according to Liu et al. (2007). Sediment Chl-a and Pheo-a concentrations were determined using the spectrophotofluorimetry method of Lorenzen and Jeffrey (1980) and was modified according to Liu et al. (1998) for wet sediment. The concentrations of heavy metals, namely Cd, Cu, Pb, Zn, Cr, Ni, Co, and Mn, were analyzed as described by Liu et al. (2015). The dried sediment sample (approximately 0.5 g) was placed in a plastic digestion tube with 10 mL concentrated HNO_3 in a microwave digestion system to react. After the reaction was completed, the digested samples were diluted with de-ionized water to a final volume of 50 mL and the sample solutions were analyzed for heavy metals using inductively coupled plasma-mass spectrometry (ICP-MS, Optima

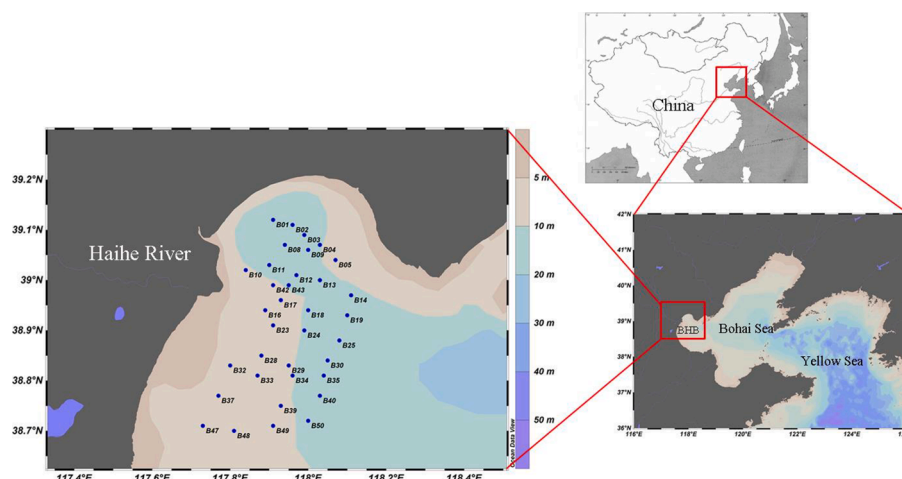


Fig. 1. Map of the study stations in Bohai Bay. BHB indicates Bohai Bay.

2100DV ICP System, Perkin Elmer).

2.5. Statistical analyses

Ocean Data View (ODV) maps were generated using environmental variables and nematodes metrics using ODV v5.3.0 (Schlitzer, 2020). IBM SPSS Statistics v20 (IBM Corp., Armonk, NY, USA) was used to perform Pearson correlation analysis of univariate variables.

The nematode diversity indices were examined using species number (S), Shannon–Wiener diversity index (H' , $\log_2$), and the Pielou evenness index (J'). All the above indices were calculated using the function DIVERSE in PRIMER v7 (Clarke and Gorley, 2006).

Nematode community metrics (abundance, taxonomic structure, and functional traits) were analyzed by means of non-parametric permutational analysis of variance (PERMANOVA) using PRIMER v7 + PERMANOVA add-on package (Anderson et al., 2008). Environmental variables were checked for uniform distributions, and the concentrations of Chl-*a*, Pheo-*a*, OM, and eight heavy metals were log transformed to compensate for skewness. Principal component analyses (PCA) were performed on normalized data and Euclidean resemblance matrix to assess the overall differences among stations. Nematode species abundance was fourth root transformed before Bray–Curtis similarities. Cluster analysis (group average) was subsequently performed to identify different station groups, along with the SIMPROF permutation test (at 1% significance level).

PERMANOVA tests were used to assess the differences among station groups. The tests of homogeneity of dispersion (PERMDISP) were performed to identify whether significant PERMANOVA results were caused by the homogeneity of dispersion of the samples within groups. These analyses were followed by pairwise comparisons of the observed significant differences. Bonferroni corrections to the p -levels of the pairwise tests were applied to reduce the Type I error rate. The sizes of effects in PERMANOVA were measured and compared by the average similarities among pairs of groups.

A distance-based linear model (DISTLM) was applied to test the relationship between abiotic environmental variables and nematode features. The models were based on the Euclidean distance matrix (derived from the values of univariate indices) and the Bray–Curtis similarity index (derived from the nematodes species abundance), using a step-wise selection procedure. The adjusted R^2 selection criterion was used to assess the goodness of model fit, which has been proven to be a more useful criterion for model selection than R^2 (Anderson et al., 2008).

2.6. Environmental quality assessment

Nematode community taxonomic structure, c–p classes and MI, ITD, and H' values were selected as environmental quality indicators in the present study. Environmental quality was ranked (High > Good >

Moderate > Poor > Bad) according to the thresholds proposed by Moreno et al. (2011) and Semprucci et al. (2014a), (2014b). The thresholds are listed in Table 1.

To assess the effectiveness of the environmental quality status of the study area based on nematode community features, we compared our results with Hakanson's Ecological Risk Index, which was applied to assess the metal contamination and potential ecological risk (Hakanson, 1980). The contamination factors (CFs) for all the studied metals, the total contamination degree (C_d), and the potential ecological risk index (RI) were calculated. Each CF was determined as the ratio between the sediment metal concentration at a given station and the background value of the metal (Hakanson, 1980). The CFs of metals were categorized according to four contamination levels: low ($CF < 1$), moderate ($1 \leq CF < 3$), considerable ($3 \leq CF < 6$), or very high ($CF \geq 6$). The C_d was computed using the following equation: $C_d = \sum CFs$. C_d was classified into four levels of contamination degree: low ($C_d < 8$), moderate ($8 \leq C_d < 16$), considerable ($16 \leq C_d < 32$), or very high ($C_d \geq 32$). The background values of Cu, Pb, Zn, Cd, Cr, Ni, Mn, and Co in Bohai Bay were 22.1, 14.0, 65.2, 0.088, 60.0, 36.1, 571.0, and 13.2 $\mu\text{g/g}$, respectively (Li et al., 1994, 1995; Wu and Li, 1985; Feng et al., 2011; Xu et al., 2014). The RI was included to assess the comprehensive potential ecological risk of metals in the sediment (Hakanson, 1980); it was defined as $RI = \sum Tr \cdot CFs$, where Tr is the toxic-response factor of each substance which was determined as $Cd = 30$, $Pb = Cu = Ni = Co = 5$, $Cr = 2$, $Mn = Zn = 1$. The RI was ranked as very high ($RI \geq 600$), considerable ($300 \leq RI < 600$), moderate ($150 \leq RI < 300$), or low ($RI < 150$).

3. Results

3.1. Environmental variables

The environmental variables of the study stations are presented in Table S1. The mean water depth (WD) was 9.2 m, ranging from 2.4 m (B42) to 15.4 m (B30), and increasing from the west inshore stations to the east offshore ones (Fig. 2). Bottom water temperature (Temp) varied from 14.0 °C (B25) to 20.3 °C (B37), and a declining trend with increasing water depth was observed (Fig. 2). Pearson correlation analysis results showed that Temp was correlated negatively with WD ($r = -0.776$, $p < 0.001$). Bottom water salinity varied from 29.8‰ (B19) to 31.1‰ (B47), and was lowest at the northern inshore stations of the study area (Fig. 2). Salinity was positively correlated with WD (Pearson's $r = 0.315$, $p = 0.065$) and sediment silt and clay content (Pearson's $r = 0.395$, $p = 0.016$). Sediment Md ranged from 10.7 to 88.7 μm , and the majority of sediments consisted of silt and clay. High proportions (>90%) of silt and clay were observed in most samples, with higher proportions in the southern part of the study area where the sediment Md was < 30 μm (Fig. 2). In the northern part of the study area, there was a decreasing gradient of silt and clay content from the area adjacent to the Haihe River estuary to the offshore area. Sediment silt and clay

Table 1
The proposed thresholds and classifications of environmental quality.

Indicator	Bad	Poor	Moderate	Good	High	Source
MI	≤ 2.2	$2.2 < MI \leq 2.4$	$2.4 < MI \leq 2.6$	$2.6 < MI \leq 2.8$	> 2.8	Moreno et al., 2011
C-P 2 & 4	c-p 2 > 80%	c-p 2 > 60% and c-p 4 < 3%	c-p 2 $\geq$ 50% and 3% < c-p 4 < 10%	c-p 2 $\geq$ 50% and c-p 4 > 10%	c-p 2 $\leq$ 50% and c-p 4 > 10%	Moreno et al., 2011
C-P 1 and 2	80–100%	60–80%	40–60%	20–40%	0–20%	Semprucci et al., 2014a,b
C-P 3 to c-p 5	0–20%	40–20%	60–40%	60–80%	80–100%	Semprucci et al., 2014a,b
H'	$0 < H' \leq 1$	$1 < H' \leq 2.5$	$2.5 < H' < 3.5$	$3.5 < H' < 4.5$	$H' > 4.5$	Moreno et al., 2011
ITD	1	$0.6 < ITD \leq 0.8$	$0.4 < ITD \leq 0.6$	$0.25 < ITD \leq 0.4$	0.25	Moreno et al., 2011
sensitive/tolerant genera (>10%)	<i>Paracomosoma</i> <i>Terschlingia</i> <i>Sabatieria</i> group	<i>Daptonema</i> / <i>Theristus</i> <i>Paralongicyatholaimus</i> <i>Parodontophora</i> <i>Odontophora</i>	<i>Anticoma</i> <i>Desmodora</i> <i>Spirinia</i> <i>Marylynnia</i> <i>Prochromadorella</i>	<i>Halalaimus</i> <i>Setosabatieria</i> <i>Ptycholaimellus</i>	<i>Desmoscolecidae</i> <i>Microlaimus</i> <i>Richtersia</i> <i>Oncholaimus</i> <i>Pomponema</i> <i>Epacanthion</i>	Moreno et al., 2011

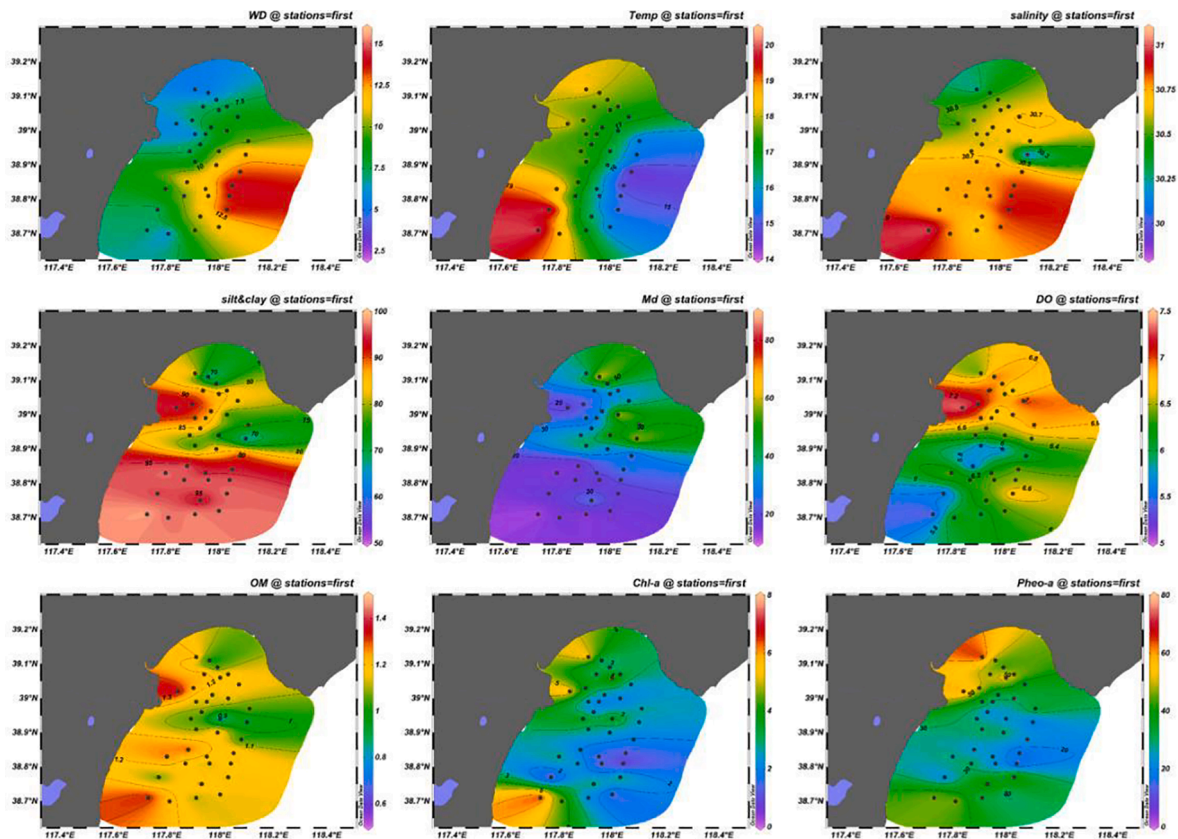


Fig. 2. Distribution patterns of mean water depth (WD), bottom water temperature (Temp), bottom water salinity, dissolved oxygen concentration (DO), sediment silt and clay content (silt&clay), sediment medium particle diameter (Md), organic matter content (OM), chlorophyll-a (Chl-a) and pheophytin-a (Pheo-a) concentrations in the study area.

content was significantly negatively correlated with Md (Pearson's $r = -0.953$, $p < 0.001$). The mean DO concentrations were 6.5 ± 0.6 mg/L, and the highest concentrations were detected at stations B10 and B11, located north of the Haihe River estuary (Fig. 2). DO was positively correlated with Md (Pearson's $r = 0.290$, $p = 0.091$), but negatively correlated with WD (Pearson's $r = -0.241$, $p = 0.163$) and salinity (Pearson's $r = -0.252$, $p = 0.145$). The OM content in the sediment ranged from 0.57% (B18) to 1.50% (B10) (Fig. 2), and a significant relationship with Md (Pearson's $r = -0.570$, $p < 0.001$) and silt and clay content (Pearson's $r = 0.635$, $p < 0.001$) was observed. Mean sediment Chl-a and Pheo-a concentrations were 2.85 ± 1.94 and 34.67 ± 16.90 $\mu\text{g/g}$, respectively, and decreased from the inshore stations to the offshore stations, with significant negative relationships with water depth (Chl-a: Pearson's $r = -0.409$, $p = 0.015$; Pheo-a: Pearson's $r = -0.530$, $p = 0.001$).

The concentrations of eight heavy metals are reported in Table S1. The mean concentrations of all eight analyzed metals in the surface sediments of the bay were higher than their background values. Mn was present at the highest concentration and Cd was present at the lowest concentration. Although the lowest concentrations of all the metals investigated were observed at the station farthest from the coast (B19), the highest concentrations were observed at different stations (Fig. 3). The distributions of the mean heavy metal concentrations exhibited similar patterns, except for Ni, and were significantly correlated with each other (Pearson's $p < 0.05$, majority $p < 0.01$). Generally, higher metal concentrations were present in sediments with higher OM, higher salinity, or higher silt and clay contents. The DISTLM results for metal datasets proved that the silt and clay content, salinity, and OM content were the most significant predictors, explaining the majority of the observed variability in metal concentrations (Table 2). Temp, water depth, Md, DO, and Chl-a concentration were also involved in the model

and exhibited weaker correlations (Table 2).

PCA was performed to detect environmental variables in relation to their locations (Fig. 4). Along the first principal component (PC1), high positive scores were mainly due to the metal concentrations, sediment silt and clay content, OM, and bottom water salinity, while high negative scores were due to Md and DO; these variables explained 42.2% of the total environmental variability. To a lesser extent, water depth, sediment Pheo-a concentration, Temp, and Chl-a concentration contributed to the differences between stations; these environmental variables dominated PC2 and explained 17.0% of the overall variation.

3.2. Nematode community structure

A total of 18 meiofauna higher taxa were identified: Nematoda, Copepoda, Polychaeta, Heteropoda, Bivalvia, Gastropoda, Kinorhyncha, Turbellaria, Halacaroida, Oligochaeta, Ostracoda, Amphipoda, Isopoda, Cumacea, Ophiura, Tardigrada, Nauplii, and Insecta. Nematodes accounted for a mean of 91.8% of total meiofaunal abundance (786.2 ± 537.3 ind/10 cm^2), followed by Copepoda (4.0%), Nauplii (2.2%), and Polychaeta (1.0%). The cumulative contribution of the remaining taxa was 1%. The highest nematode abundance (1980.8 ± 577.3 ind/10 cm^2) was observed at B08 station, and the lowest abundance (33.3 ± 5.5 ind/10 cm^2) was observed at B28 station (Table S2). Nematode abundance appeared to be higher in near shore stations and decreased with increasing water depth (Fig. 5).

Focusing on the nematode community, a total of 77 marine nematode species were identified. Thirteen species accounted for 91% of the whole nematofauna: *Dorylaimopsis rabalaisi* (20.7%), *Parodontophora deliensis* (19.6%), *Parodontophora* sp. 2 (17.3%), *Daptonema macrostoma* (12.9%), *Sphaerolaimus gracilis* (3.7%), *Sabatieria* sp. 1 (3.2%), *Daptonema* sp. 1 (3.1%), *Thalassomonhystera* sp. 1 (2.8%), *Sphaerolaimus*

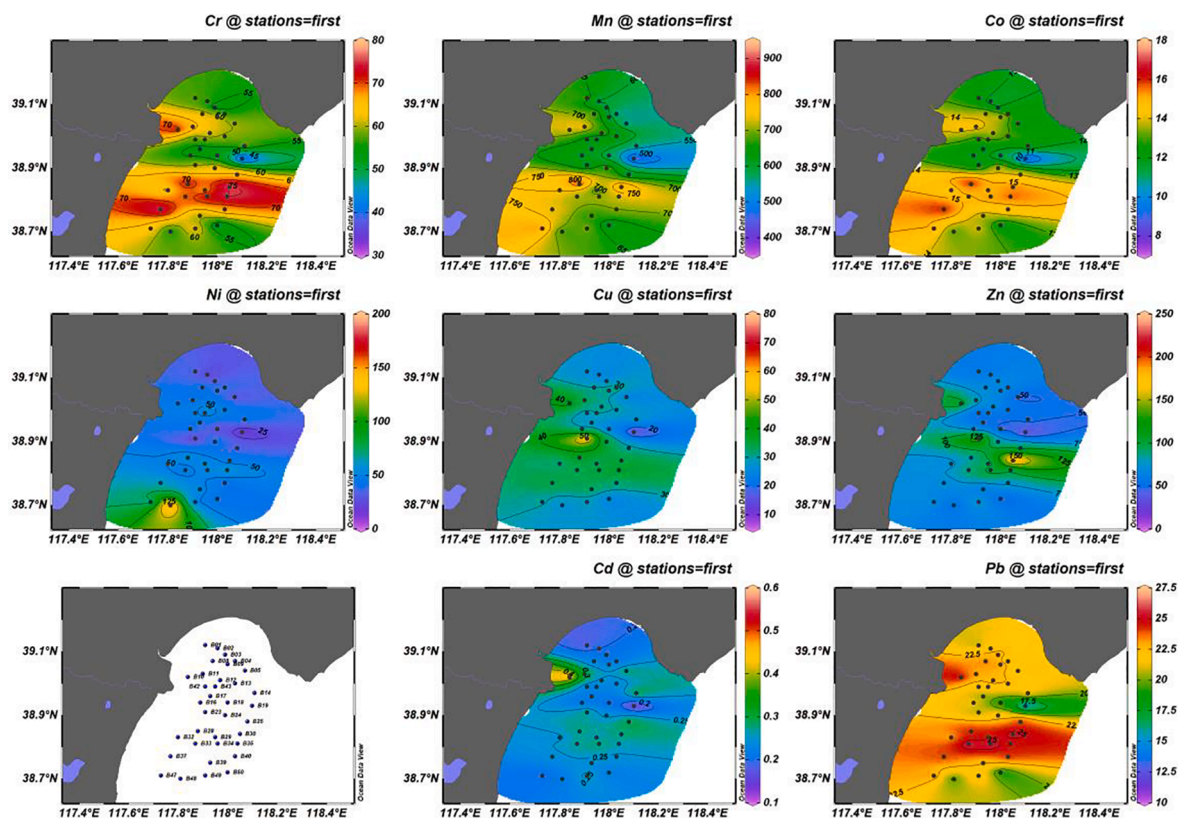


Fig. 3. Distribution patterns of the mean concentrations of eight heavy metals in the study area.

Table 2

Results of the DISTLM sequential test for metals. SS, mean square; F, F statistic; Prop, the proportion of the variability explained; Cumul, the cumulative proportion of the variability explained; WD, water depth; Temp, bottom water temperature; DO, dissolve oxygen concentration; Md, sediment medium particle diameter, Chl-a, Chlorophyll-a; OM, organic matter content. Statistically significant correlations are indicated in bold. **, $p < 0.01$; *, $p < 0.05$.

	Variable	Adjusted R ²	SS(trace)	Pseudo-F	p	Prop.	Cumul.
Cr	Silt and clay %	0.32246	11.641	17.181	0.001**	0.342	0.342
	Salinity	0.43452	4.2637	7.54	0.017*	0.125	0.468
	Md	0.46308	1.4508	2.702	0.108	0.043	0.510
	Log(OM)	0.46376	0.55739	1.0394	0.313	0.016	0.527
	Silt and clay %	0.37998	13.539	21.837	0.001**	0.398	0.398
Mn	Temp	0.41042	1.594	2.7037	0.101	0.047	0.445
	Md	0.47049	2.4516	4.6298	0.053	0.072	0.517
	WD	0.50792	1.6526	3.3584	0.090	0.049	0.566
	Salinity	0.5124	0.62198	1.2756	0.237	0.018	0.584
	Silt and clay %	0.54038	18.832	40.974	0.001**	0.554	0.554
Co	Salinity	0.63555	3.5051	9.6174	0.013*	0.103	0.657
	Md	0.65806	1.0625	3.1072	0.092	0.031	0.688
	Temp	0.66971	0.69139	2.0933	0.162	0.020	0.709
	WD	0.67047	0.35221	1.0688	0.295	0.010	0.719
	Silt and clay %	0.42571	15.048	26.204	0.001**	0.443	0.443
Ni	Md	0.4633	1.777	3.311	0.078	0.052	0.495
	WD	0.50696	1.8902	3.8338	0.054	0.056	0.550
	Salinity	0.53421	1.3106	2.8137	0.113	0.039	0.589
	Log(Chl-a)	0.53499	0.48828	1.05	0.306	0.014	0.603
	Log(OM)	0.53538	0.476	1.0245	0.334	0.014	0.617
Cu	Log(OM)	0.21793	8.1918	10.474	0.011*	0.241	0.241
	Salinity	0.36161	5.3798	8.4271	0.017*	0.158	0.399
	DO	0.36775	0.82878	1.3108	0.252	0.024	0.424
	WD	0.36931	0.67892	1.0765	0.312	0.020	0.444
	Log(OM)	0.11065	4.6513	5.23	0.034*	0.137	0.137
Zn	Salinity	0.18691	3.3296	4.095	0.067	0.098	0.235
	Salinity	0.12767	5.213	5.9759	0.035*	0.153	0.153
	Log(OM)	0.20226	3.2592	4.0855	0.059	0.096	0.249
	DO	0.2488	2.2407	2.9828	0.099	0.066	0.315
	Salinity	0.25788	9.51	12.815	0.006**	0.280	0.280
Pb	Log(OM)	0.3488	3.6515	5.6073	0.037*	0.107	0.387
	DO	0.35414	0.81686	1.2648	0.266	0.024	0.411

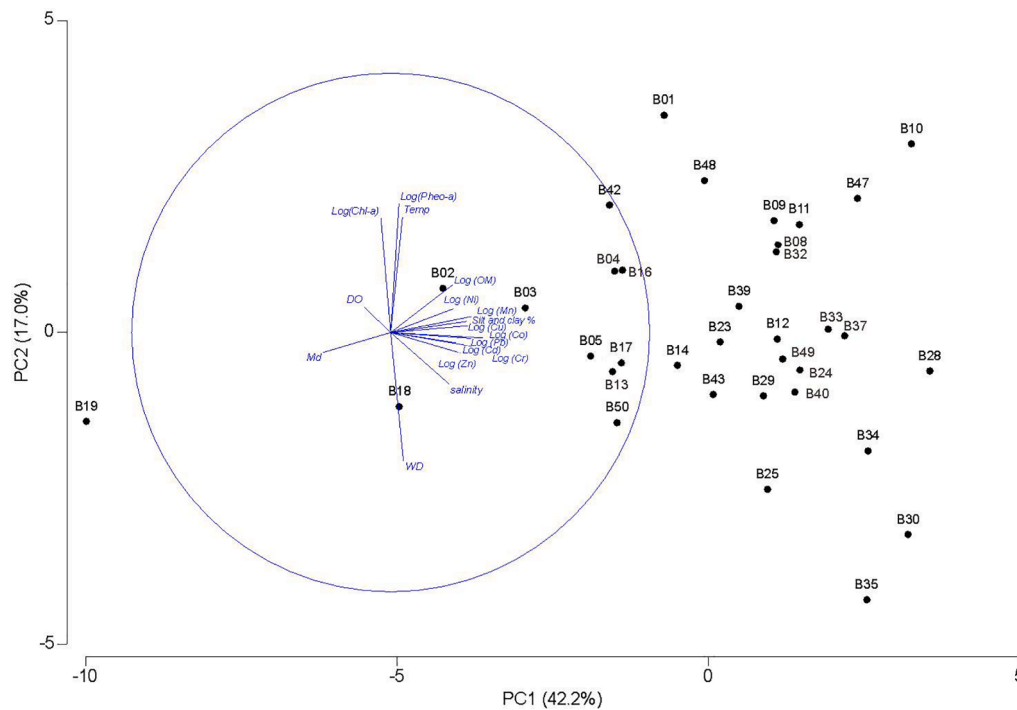


Fig. 4. Principal component analysis (PCA) plot of environmental variables. PC1 explains 42.2% of the variance (Eigenvalue: 7.18); PC2 explains 17.0% of the variance (Eigenvalue: 2.88). WD, water depth; Temp, bottom water temperature; DO, dissolve oxygen concentration; Chl-a, Chlorophyll-a; Pheo-a, pheophytin-a; OM, organic matter content, Md, sediment medium particle diameter.

balticus (2.1%), *Promonhystra* sp.1 (2.0%), *Spilophorella* sp. 1 (2.0%), and *Pseudosteinera* spp. (1.7%) (Table S3).

Cluster analysis and SIMPROF analysis (at 1% significance level) of nematode communities revealed that the studied stations could be divided into five major groups (Fig. 6): group A included the shallow water stations near the west coastal area, indicating the inshore group; group E included stations far from the coast, indicating an offshore group, and groups B, C, and D included stations located between groups A and E. Only four species (<20 nematode individuals) were recorded at station B28, which appeared to have a significantly different nematode species composition than that of other stations. Differences in the relative abundance of several dominant species (e.g., *Parodontophora* sp. 2, *Daptonema* sp. 1, *Sabatieria* sp. 1, *Thalassomonhystra* sp. 1, and *Daptonema macrostoma*) were conspicuous among the major groups (Table 3). PERMANOVA results confirmed that the species composition differed significantly among the five major groups (Table 4). Pairwise comparisons indicated significant differences in nematode species composition between groups, except between D and C, and between D and E, after a Bonferroni correction (p -level of 0.05/10 pairwise comparisons resulted in a p -level of 0.005, Table 5). SIMPER analysis revealed a 56.7% dissimilarity between group E and the other groups; 4.5%, 4.4%, 3.9%, and 3.8% of the variation were attributed to *Parodontophora* sp. 2, *Daptonema* sp. 1, *Sabatieria* sp. 1, and *Thalassomonhystra* sp. 1, respectively, which were in low abundance in group E. However, PERMDISP results showed a significant p value (<0.05) for species composition (Table 4), indicating a different distribution of data within groups. When applying PERMDISP pairwise comparison, significant p values (<0.05) were only reported between A and E, B and E, and C and E, indicating that the different distribution could be ascribable to group E. A cautious interpretation of the significant difference in the nematode species' composition in group E may be caused by the within-group dispersion.

Comparing the nematode abundances across the station groups based on the species composition, the highest mean nematode abundance was in group B (1069.0 ± 446.8 ind/10 cm²), followed by group A (inshore stations, 994.2 ± 486.4 ind/10 cm²), and the lowest in group E (offshore stations, 205.5 ± 88.6 ind/10 cm²) (Table S2). PERMANOVA

results confirmed that nematode abundance differed significantly among the station groups (Table 4). Nematode abundance showed a similar distribution in each station group according to PERMDISP results (Table 4). The results of the pairwise comparisons after a Bonferroni correction revealed that nematode abundance of group E (offshore stations) differed significantly from that of groups A and B (Table 5).

3.3. Nematode diversity

The number of nematode species ranged from 4 (B28) to 37 (B13) across the studied sites, while H' and J' ranged from 1.80 to 4.15 and 0.54–0.97, respectively (Table S2). The lowest H' and J' values were calculated at station B30, while the highest H' and J' values occurred at stations B12 and B28, respectively (Fig. 5). Generally, low H' values appeared at stations with lower abundance (Pearson correlation $r = 0.424$, $p = 0.011$), and were < 2.5 at stations B28, B30, B35, and B42. The highest H' , J' , and S were in group B, while the lowest were in group E (Table S2). PERMANOVA results confirmed that H' , J' , and S differed significantly among the station groups according to the nematode species compositions (Table 4). Pairwise comparison results revealed that H' and S significantly differed among group B and other groups after a Bonferroni correction (p -level of 0.05/10 pairwise comparisons resulted in a p -level of 0.005; Table 5). However, PERMDISP results showed significant p values (<0.05) of H' and J' (Table 4), indicating different distribution of data within groups. The PERMDISP pairwise comparison showed that significant p values (<0.05) were only reported between A and E, B and E, and D and E, also indicating that the different distribution could be ascribable to group E.

3.4. Nematode community trophic diversity and maturity index

Epistatrum feeders (2A) dominated the study area (49%), followed by non-selective deposit feeders (1B; 40%). Omnivores/predators (2B) and selective deposit feeders constituted only 6% and 5% of the nematodes, respectively. The ITD ranged from 0.28 (B49) to 0.82 (B18) (Table S2; Fig. 5). Low ITD values appeared at stations with higher H'

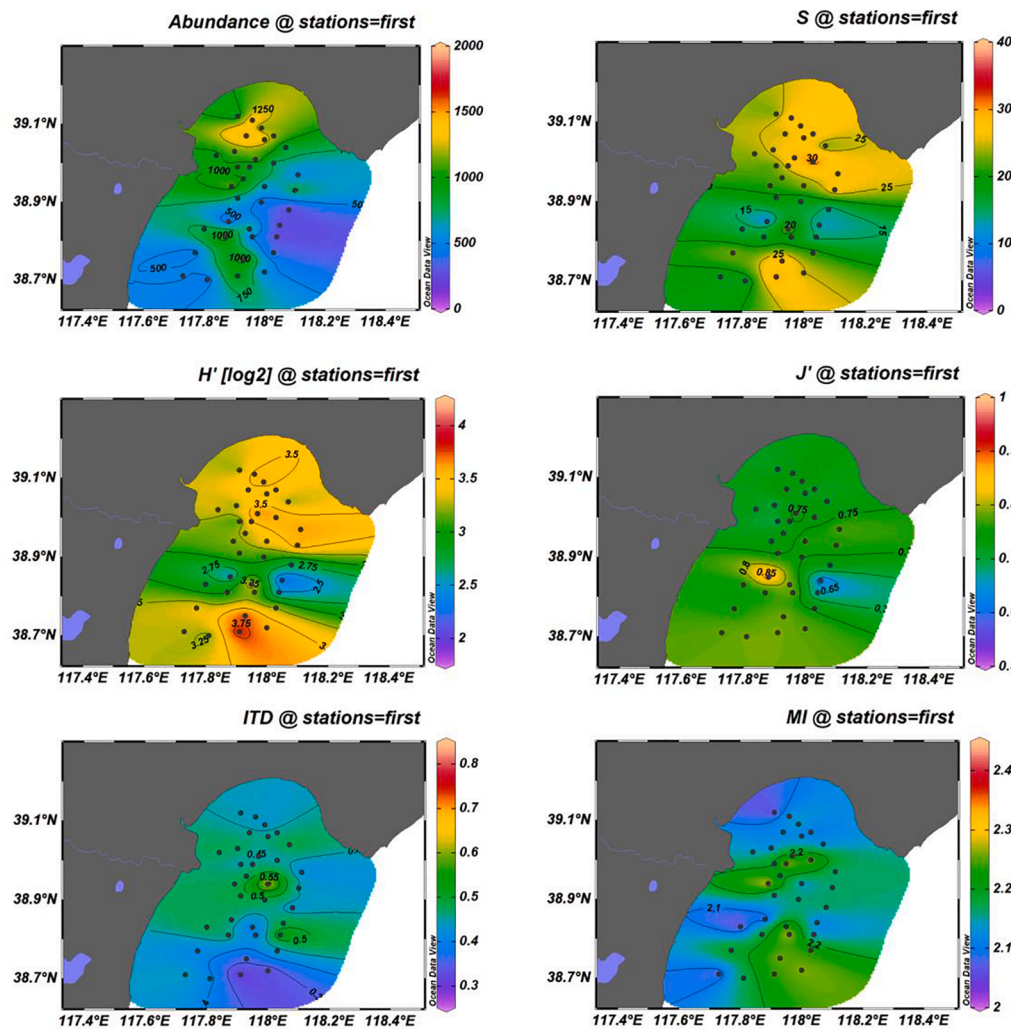


Fig. 5. Distribution of mean nematode abundance (ind/10 cm²), nematode species number (S), Shannon–Wiener diversity index (H'), Pielou evenness index (J'), index of trophic diversity (ITD), and maturity index (MI).

(Pearson correlation $r = -0.386$; $p = 0.022$). Nematodes with a c–p class of 2 dominated (85%), followed by nematodes with c–p classes of 3 (13%) and 4 (2%). No extreme colonizers (c–p = 1) and extreme persisters (c–p = 5) were observed. The MI ranged from 2.00 (B28) to 2.44 (B43), exhibiting the opposite distribution pattern to that of ITD (Pearson correlation $r = -0.441$, $p = 0.008$; Table S2, Fig. 5).

Comparing the ITD values across the station groups based on the species composition, ITD was lowest in group B (0.39 ± 0.06), and highest in groups A (0.47 ± 0.02) and C (0.47 ± 0.04 , Table S2). PERMANOVA results confirmed that nematode ITD differed significantly among the station groups according to the species composition (Table 4). ITD showed a similar distribution in each station group according to PERMDISP results (Table 4). Pairwise tests showed that the ITD of group B differed significantly from that of group A after a Bonferroni correction (Table 5). The MI was not significantly different among station groups (Table 4). However, the abundances of c–p classes differed significantly among station groups (Table 4). As indicated by the pairwise test outputs (after a Bonferroni correction), the abundances of the c–p 2 class from group E (offshore stations) differed significantly from that of other stations, as did the abundance of the c–p 4 class from that of group B (Table 5). However, the PERMDISP results showed different distributions of the abundances of c–p 3 and c–p 4 classes within groups ($p < 0.05$; Table 3). Significant p values (< 0.05) of c–p 3 class were only reported between B and E, C and E, and D and E, while significant p values of c–p 4 class were reported between A and B, A and

D, B and C, B and E, and D and E, according to the PERMDISP pairwise comparison.

3.5. Relationships between nematode community features and environmental variables

The DISTLM analyses results based on 10 environmental variables (i. e., water depth, Mn, Temp, DO, Co, OM, salinity, Md, Chl-a and Pheo-a concentrations) explained 44.1% of the observed variability of the nematode species composition (Table 6). Among these, water depth, Mn, and Temp explained 22.7% of the variability, significantly influencing the nematode species composition (Table 6).

In addition, the DISTLM results of the sequential test performed on nematode community univariate descriptors indicated that the Mn concentration was a strong predictor of nematode abundance and H' , explaining 9.5% and 19.2% of the observed variances, respectively (Table 7). The Mn concentration was also involved in the species number and ITD and MI models, but exhibited a weaker correlation (Table 7). Pb, Cr, Ni, and Co also exhibited weaker correlations with MI, while together with Mn they explained 31.7% of the observed variance of MI. The Cd concentration was identified also as a significant predictor of J' , explaining 11.6% of the observed variance (Table 7). In addition to the metal concentrations, water depth, Md, and DO were significant predictors of the variances of nematode abundance, species number, and J' , explaining 27.0%, 21%, and 13.1%, respectively (Table 7). The

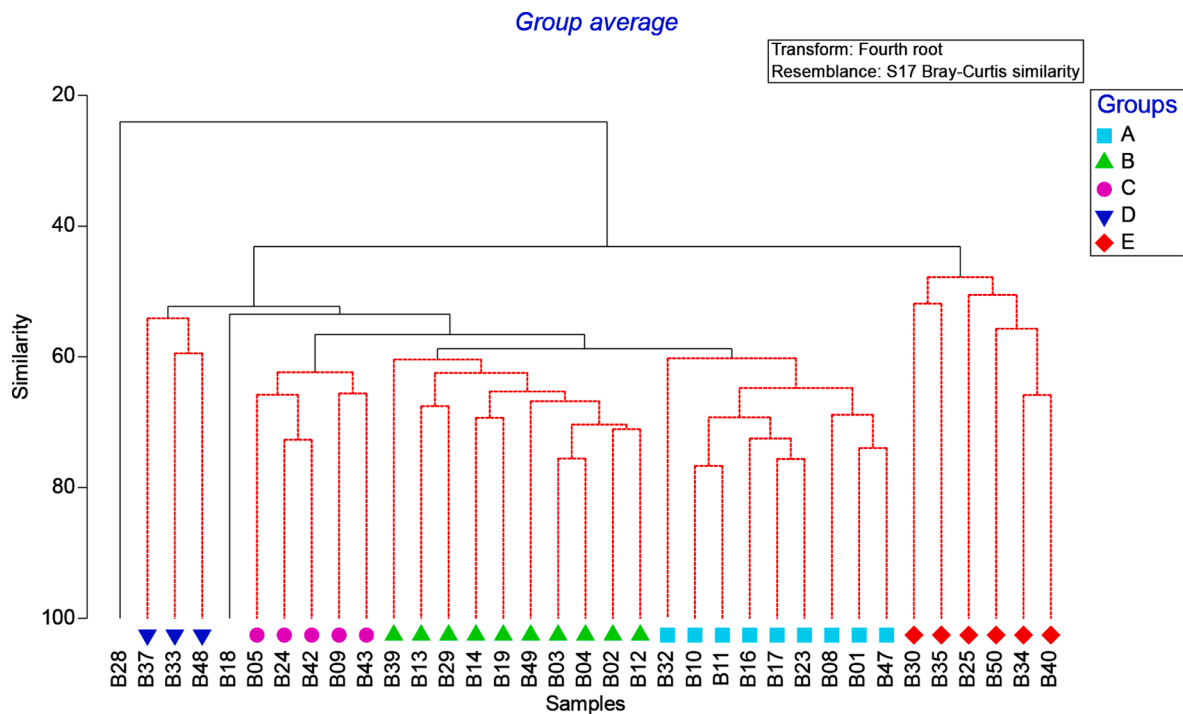


Fig. 6. Cluster analysis of the nematode community.

DISTLM results of the sequential test performed on the abundances of the c-p groups revealed that the variability in the abundance of c-p 2 group was significantly explained by WD and Ni concentrations (Table 7). The observed variations of the abundance of the c-p 3 and c-p 4 classes were significantly explained by the Mn concentration and the sediment Md, respectively.

3.6. Benthic environmental quality assessment

The environmental quality in the studied area was ranked from bad to good based on the percentage of the c-p classes, and the overall MI, ITD, and H' values. Different rankings were observed depending on the indicators used. According to the H' values, the environmental quality of the stations was ranked from poor to good; the majority of stations were ranked as having a moderate or good status (Table 8). Poor environmental quality based on H' was indicated only at stations B28, B30, B35, and B42 (Table 8). Based on the ITD, environmental quality could be ranked from bad to good; most stations were ranked as being of moderate quality. The environmental quality at stations B03, B14, B25, B29, B34, B37, B39, B49, and B50 was assessed as good, and as bad and poor at stations B18 and B35, respectively (Table 8). The environmental quality at most study stations were ranked as bad or poor except for two stations, according to MI (B16 and B43, Table 8). The classification results based on MI and the percentages of c-p 2 and 4 classes were highly consistent except at stations B13, B39, B49, and B37, which were assessed as having poor environmental quality according to MI, but moderate or bad according to the percentages of all c-p classes (Table 8). The ranking results according to MI also matched with those based on the percentage of all c-p classes at all stations except B16, B37, and B43 (Table 8).

The environmental quality of the stations was assessed based on the species belonging to sensitive/tolerant genera and was ranked from bad to poor. Species indicating poor status, including *Parodontophora del-tensis*, *Parodontophora* sp. 2, and *Daptonema macrostoma*, were observed at all study stations. The relative abundances of some or all of these species were > 10% at all study stations excluding station B37, where the relative abundance of all of these three species was < 10%.

Dorylaimopsis rabalaisi was dominant at station B37 with a relative abundance of 38% (Table S3). The environmental quality status of study stations B10, B17, and B32 was bad, as the relative abundance of *Sabatieria* sp. 1 was > 10% (10–25%).

To understand the degree of heavy metal contamination and potential ecological risk in the sediments of Bohai Bay, the CFs of all eight metals and the RI values were calculated (Table S4). The CFs ranged from 0.23 to 6.48, and contamination in most sediments was low or moderate (CFs < 6) (Table S4). The mean CFs of the metals decreased in the following order: Cd (2.82) > Pb (1.60) > Cu (1.42) > Ni (1.30) > Mn (1.16) > Zn (1.15) > Co (1.02) > Cr (0.98), indicating a high contamination pressure attributed to Cd. The total contamination degree (C_d) ranged from 4.99 to 17.62. All stations were assessed as being moderately contaminated except B10 (assessed as being considerably contaminated) and B18 and B19 (assessed as having low contamination) (Table 8). The RI values for the eight studied metals were < 150 at all stations except B10 (Table 8), suggesting an overall low ecological risk, but a moderate potential ecological risk at B10.

4. Discussion

4.1. Variations in nematode community features and indications of habitat heterogeneity

Several studies have been conducted on marine nematode communities in Bohai Bay (Zhang et al., 2009, 2011, 2012; Hu and Zhang, 2012; Liu et al., 2015). Our results were similar to those of previous research (Zhang et al., 2011, 2012). In the present study, the nematode community was dominated by epistratum feeders and general opportunists (c-p = 2), indicating a higher fraction of tolerant species or colonizers. However, intra-bay variability of nematode community features was observed in Bohai Bay. The least abundant and least diverse nematode assemblage was found at offshore stations, reflecting the most isolated nematode assemblage in the study area. However, the PERMDISP results showed significant p values for species composition, indicating different distribution of the data within groups. In addition, PERMDISP pairwise comparisons indicated that the different distribution of the data could be

Table 3

Relative abundance of dominant species of the marine nematode community in different groups. Species with a relative abundance higher than 1% in each group are shown.

	Group A	Group B	Group C	Group D	Group E	St. B18
<i>Parodontophora</i> sp. 2	20.9	17.5	12.8	12.6	4.0	1.6
<i>Parodontophora deltensis</i>	18.5	11.6	23.7	9.1	13.5	4.3
<i>Sabatieria</i> sp. 1	9.0	2.5	1.6	3.9		9.6
<i>Daptonema macrostoma</i>	8.0	6.7	14.3	1.3	23.8	10.7
<i>Dorylaimopsis rabalaisi</i>	6.6	15.2	14.9	31.1	21.1	
<i>Eumorpholaimus</i> sp. 1	6.2					
<i>Daptonema</i> sp. 1	6.0	3.1	2.3	1.5		3.2
<i>Thalassomonhystera</i> sp. 1	5.1	4.2		7.4		20.9
<i>Metadesmolaimus</i> sp. 1	4.4	2.3	1.1			
<i>Pseudosteinaria</i> sp. 1	3.0			1.5	1.9	11.8
<i>Daptonema</i> sp. 2	1.9					
<i>Spilophorella</i> sp. 1	1.2	4.8	3.8	2.2	2.6	5.9
<i>Sphaerolaimus gracilis</i>	1.1	3.3	4.0	4.8	6.8	
<i>Belbolla</i> sp. 1					1.4	
<i>Cervonema</i> sp. 1			2.2	3.3		
<i>Cobbia</i> sp. 1		1.0		2.8		
<i>Halalaimus</i> sp. 1		1.1				
<i>Leptolaimus</i> sp. 1		1.4				
<i>Linhystera</i> sp. 1		1.5	4.6			
<i>Linhystera</i> sp. 2		3.0				
<i>Oxystomina</i> sp. 1		1.0				
<i>Paracanthochus</i> sp. 1				1.1		
<i>Paramarylynna stenocervica</i>		1.2	2.3		2.9	
<i>Paramonohystera</i> sp. 1		1.0				17.6
<i>Parasphaerolaimus</i> sp. 1				1.3	1.5	
<i>Promonhystera</i> sp. 1		2.5			6.4	
<i>Sphaerolaimus balticus</i>		1.2	2.9	3.9	4.6	
<i>Synonchiella</i> sp. 1					1.2	
<i>Terschellingia</i> sp. 1		1.5		3.9		
<i>Trochamus</i> sp. 1		1.1	2.1			
<i>Vasostoma articulum</i>				3.3		
<i>Cobbia</i> sp. 3						2.7
<i>Eleutherolaimus</i> sp. 1						2.7
Cumulative %	92.1	88.6	92.6	95.0	91.7	90.9

ascribable to the group of offshore stations. Therefore, a cautious interpretation of the significant difference of nematode species composition at those offshore stations may be caused by the within-group dispersion.

Apart from differences that may have resulted from stochastic causes, environmental variations may explain the observed variations of nematode community features in Bohai Bay to a certain extent. The results of this study showed that the differentiation in nematode species composition was attributed to changes in water depth, temperature, DO, OM, salinity, Md, Chl-a and Pheo-a concentrations, and Mn and Co concentrations. Metals together with other environmental variables influenced diversity indices, ITD and MI. According to the present study, the sediment silt and clay content, Md, OM, bottom water salinity, DO, and metal concentrations were the main variables causing the environmental heterogeneity in Bohai Bay. High fresh water, sediment, and pollutant discharges were input by rivers and submarine groundwater discharge (Song and Duan, 2019; Wang et al., 2019). The overall structure of the tidal residual current in Bohai Bay is clockwise circulation in the south, counterclockwise circulation in the north, and outflow from the middle (Zhao and Lei, 1995; Wan et al., 2004). Under

the action of circulation, terrestrially-derived fresh water, sediments, and metal pollutants are transported, which resulted in the variation trends of these variables from the near shore area moving eastwards (Qing et al., 2013; Feng et al., 2015). Previous studies conducted in Bohai Bay proved that sediment grain size played an important role in structuring meiofaunal assemblage in this area (Hu and Zhang, 2012; Liu et al., 2015). Guo et al. (2001) demonstrated that the nematodes abundance or diversity increased with increasing sediment grain size in the Bohai Sea. The results of this study also revealed that sediment grain size (namely Md or silt and clay content) was one of the main predictors of nematode community species composition and species number. Sediment grain size could play an important role in structuring nematode communities by increasing habitat heterogeneity, affecting the morphotypes of nematodes (Warwick, 1971). At the same time, the structuring role of sediment grain size is not straightforward either. Sediment grain size was correlated with other environmental variables, such as DO, OM content, salinity, etc., which in turn were determined by hydrodynamic features.

According to the results of our study, nematode abundance and species composition were also significantly correlated with water depth. Water depth has a potentially critical influence on the meiofaunal community structure because it affects the quantity and quality of carbon being deposited on the seafloor (Schratzberger et al., 2004). In this study, Chl-a and Pheo-a concentrations decreased with increasing water depth. Chl-a and Pheo-a are essential for nematodes and indicate food availability. Generally, nematodes appeared abundant and diverse in areas with high Chl-a concentration (Hua et al., 2009). The results of the present study showed that Chl-a and Pheo-a concentrations correlated with nematodes abundance, species composition, diversity indices, and MI, suggesting important effects on the nematode community in Bohai Bay. Moreover, water depth increased from the near shore area in an eastward direction in the study area, indicating the decreasing effects of terrestrial materials, including fresh water, nutrients, and pollutants. The mean concentrations of all heavy metals, except Ni, were significantly correlated with each other indicating that they had common sources. Rivers are the most important sources of heavy metals in Bohai Bay (Song and Duan, 2019). Due to the discharges from Haihe River, the heavy metal concentrations in surface sediments decrease with increasing distance from the Haihe River Estuary and then further decrease to the open sea (Xu et al., 2012). In this study, correlation signals between water depth and Cu, Mn, Co, and Ni concentrations were observed. The concentrations of these four metals were high in near shore shallow water and low in deeper water. In addition, WD correlated with temperature, salinity, and DO, which were also important in structuring nematode community in this study. These water depth-related factors may have stronger influences on the variations in nematode abundance and species composition than WD.

It is critical to note that metal concentrations and distributions related with other environmental variables. Firstly, water circulation is an important factor affecting the distribution of metals in the present study. Under the action of circulation, terrestrially-derived metal pollutants are transported to the northwest and central areas of Bohai Bay where they are deposited (Chen et al., 2017). Secondly, the metal concentrations are also influenced by sediment particle size. Metals are attracted to the surface of suspended particles and are deposited in sediment. These metals tend to accumulate in the fine silt-clay sediment (Xu et al., 2016). In the present study, the Cr, Mn, Co, and Ni concentrations were significantly related to sediment particle size and were much higher in the fine fraction than in the coarse fraction. Furthermore, total and dissolved organic carbon has been shown to absorb metals in both aerobic and anaerobic sediment horizons (Luoma & Davis, 1983; Hagopian-Schlekat et al., 2001). All metals were positively correlated with the OM content in the present study. In addition, higher levels of water salinity facilitated the diffusion of heavy metals in sediments (Liu et al., 2019c). This could explain the positive correlations between concentrations of all eight metals and salinity in this study.

Table 4

Results of the PERMANOVA main test and PERMDISP test performed on nematode datasets. Significant differences are indicated in bold. df, degree of freedom; SS, sum of squares; MS, mean squares. Groups refer to those based on nematode species compositions.

	PERMANOVA					PERMDISP		
	Source	df	SS	MS	Pseudo-F	P(permanova)	F	p
Species composition	Groups	6	20,930	3488.3	4.6051	0.001	6.6734	0.003
	Residual	28	21,210	757.49				
	Total	34	42,140					
Nematode abundance	Groups	6	2910	484.99	10.643	0.001	1.7177	0.255
	Residual	28	1275.9	45.568				
	Total	34	4185.9					
S	Groups	6	1495.4	249.23	13.338	0.001	2.8841	0.050
	Residual	28	523.2	18.686				
	Total	34	2018.6					
J'	Groups	6	0.072079	0.012013	2.8594	0.049	3.8014	0.021
	Residual	28	0.11764	0.0042013				
	Total	34	0.18971					
H'	Groups	6	6.5342	1.089	6.3782	0.001	4.8708	0.002
	Residual	28	4.7808	0.17074				
	Total	34	11.315					
ITD	Groups	6	0.18655	0.031092	8.2487	0.005	2.5814	0.073
	Residual	28	0.10554	0.0037694				
	Total	34	0.2921					
MI	Groups	6	0.086268	0.014378	1.23	0.324	1.1611	0.615
	Residual	28	0.32729	0.011689				
	Total	34	0.41356					
Abundance of c-p 2 group	Groups	6	1831.9	305.32	19.946	0.001	2.4711	0.097
	Residual	28	428.61	15.307				
	Total	34	2260.5					
Abundance of c-p 3 group	Groups	6	10,195	1699.1	13.955	0.029	3.4182	0.018
	Residual	28	3409.2	121.76				
	Total	34	13,604					
Abundance of c-p 4 group	Groups	6	5.4174	0.9029	2.6462	0.032	3.3744	0.037
	Residual	28	9.5537	0.3412				
	Total	34	14.971					

Table 5

Pairwise test results of nematode community species composition and univariate indices among station groups according to the species composition by PERMANOVA. Bold numbers show significant differences after Bonferroni corrections (*p*-level of 0.05/10 pairwise comparisons resulted in a *p*-level of 0.005). Only paired test between major groups are listed.

Species composition		H'		J'		S		ITD	
Groups	t	p	t	p	t	p	t	p	t
A, B	2.1866	0.001	6.5460	0.001	2.1677	0.062	7.3689	0.001	4.2217
A, C	2.1500	0.002	0.4418	0.678	0.5334	0.616	0.0881	0.950	0.1916
A, D	1.8107	0.004	0.3956	0.722	0.6990	0.553	1.0521	0.340	1.5479
A, E	2.8251	0.001	1.6016	0.142	0.5575	0.576	2.3014	0.047	0.7510
B, C	2.0637	0.001	3.7435	0.002	1.8413	0.088	4.6293	0.001	2.8481
B, D	1.9839	0.003	4.5649	0.003	0.8050	0.518	5.4402	0.006	1.4576
B, E	2.7349	0.001	4.2142	0.002	1.6007	0.142	6.8160	0.001	1.3865
C, D	1.5176	0.020	0.0865	0.927	0.6874	0.612	0.5371	0.536	0.8475
C, E	1.8941	0.002	0.8130	0.433	0.1006	0.948	1.4259	0.187	0.4714
D, E	1.9129	0.012	0.7766	0.475	0.5817	0.694	0.7402	0.528	0.0485
Nematode abundance		Abundance of c-p 2		Abundance of c-p 3		Abundance of c-p 4			
Groups	t	p	t	p	t	p	t	p	t
A, B	0.3631	0.720	2.3505	0.035	1.0802	0.305	2.7768	0.014	
A, C	1.0186	0.342	1.3084	0.234	1.1679	0.258	0.67446	0.493	
A, D	1.2489	0.224	5.7531	0.005	0.64185	0.542	0.20654	0.861	
A, E	6.4213	0.001	7.1062	0.001	0.33316	0.785	0.51032	0.614	
B, C	1.2969	0.221	0.53862	0.597	0.66099	0.532	1.6924	0.091	
B, D	1.4748	0.213	2.1906	0.050	0.14226	0.959	4.4942	0.003	
B, E	6.7562	0.001	4.1752	0.002	1.3587	0.195	4.3083	0.001	
C, D	0.3063	0.707	2.3197	0.048	0.74659	0.615	0.42959	0.744	
C, E	3.6292	0.011	3.7447	0.008	1.317	0.248	1.1781	0.269	
D, E	2.5048	0.039	1.3103	0.272	0.80107	0.475	0.78759	0.587	

However, total metal concentrations in sediment are often not predictive of sediment toxicity because many physicochemical characteristics of sediments govern metal bioavailability and mobility to pore water (Luoma, 1989). Bioavailability is regulated partly by several metal-absorption phases in sediments, for example Fe-Mn oxides, which regulate the release of other divalent cations from sediments (Luoma

and Davis, 1983). Salinity, dissolved oxygen, pH, temperature, redox potentials, sediment grain size, organic matter are also known as the important variables regulating the bioavailability and toxicity of heavy metals bound to sediments (Tietjen, 1980; Austen et al., 1994; Banks et al., 2012; Zhao et al., 2013; Wolanski and Elliot, 2016; Liu et al., 2019c). Previous studies found that bioavailability of metals in

Table 6

Results of the DISTLM sequential test for multivariate data sets of nematode species composition based on species. SS, mean square; F, F statistic; Prop, the proportion of the variability explained; Cumul, the cumulative proportion of the variability explained; WD, water depth; Temp, bottom water temperature; DO, dissolve oxygen concentration; Chl-a, Chlorophyll-a; OM, organic matter content; Md, sediment medium particle diameter. Statistically significant correlations are indicated in bold. **, $p < 0.01$; *, $p < 0.05$.

Variable	Adjusted R ²	SS (trace)	Pseudo-F	p	Prop.	Cumul.
WD	0.097949	5245.5	4.6919	0.001**	0.124	0.124
Log (Mn)	0.13078	2420.1	2.2464	0.017*	0.057	0.182
Temp	0.1527	1919.4	1.8278	0.034*	0.046	0.227
DO	0.16711	1586.1	1.5365	0.099	0.038	0.265
Log (Co)	0.17791	1420.4	1.3941	0.136	0.034	0.299
Log (OM)	0.18624	1308	1.2969	0.204	0.031	0.330
Salinity	0.19431	1278.5	1.2804	0.237	0.030	0.360
Md	0.19976	1174.1	1.1838	0.289	0.028	0.388
Log (Chl-a)	0.20434	1133.9	1.1499	0.322	0.027	0.415
Log (Pheo-a)	0.20768	1085.3	1.1052	0.352	0.026	0.441

sediments was elevated under the effects of hypoxia (Banks et al., 2012; Liu et al., 2019c). In some cases, the toxicity of heavy metals increased at low values of pH and salinity (Riba et al., 2004). In other cases, the bioavailability of Zn, Pb, Cd, Cu, As, Mn, and Cr in sediments was

elevated under a high level of water salinity (Zhao et al., 2013; Liu et al., 2019c). At the same time, metal toxicity can be lowered by the presence of organic carbon which binds metals to reduce their availability (Austen et al., 1994; Wolanski and Elliot, 2016). The metal release was also weakened due to the powerful effect of metal-sulfide precipitation (Banks et al., 2012). Evidently, metal accumulation and toxicity are affected and governed by environmental variables.

It is also worth noting that the variations in nematode community features are likely to be a response to the anthropogenic impacts in Bohai Bay. The bay has been subjected to increasing anthropogenic impact including climate change, pollution, and eutrophication (Song and Duan, 2019). On the one hand, these human-induced stresses may play a role in structuring the nematode community (Alves et al., 2013). On the other hand, these stresses may influence biogeochemical processes with consequences that nematode community features changed. For instance, ocean acidification may affect organic matter degradation processes (Gao et al., 2020). Organic matter is essential to nematode communities, which has both direct (i.e., provision of food) and indirect (e.g., altering the oxic conditions) effects on nematode individuals (Soetaert et al. 2009). Nematode community composition variability is often ascribed to the complex interaction among different variables (Moens et al., 2013). According to the present study, interactions between environmental variables drive the patterns of nematode community features in Bohai Bay.

Table 7

Results of the DISTLM sequential test for univariate features of nematode community. SS, mean square; F, F statistic; Prop, the proportion of the variability explained; WD, water depth; Temp, bottom water temperature; DO, dissolve oxygen concentration; Chl-a, Chlorophyll-a; Pheo-a, Pheophytin-a; OM, organic matter content; Md, sediment medium particle diameter. Statistically significant correlations are indicated in bold. **, $p < 0.01$; *, $p < 0.05$.

	Variable	Adjusted R ²	SS(trace)	Pseudo-F	p	Prop.	Cumul.
Abundance	WD	0.25730	1168.50	12.7790	0.001**	0.279	0.279
	Log (Mn)	0.33499	397.50	4.8551	0.031*	0.095	0.374
	DO	0.37065	217.98	2.8133	0.095	0.052	0.426
	Log (Chl-a)	0.38615	134.73	1.7828	0.193	0.032	0.458
S	Md	0.18603	423.85	8.7708	0.007**	0.210	0.210
	Log (Pheo-a)	0.23797	146.99	3.2491	0.078	0.073	0.283
	Log (Mn)	0.27938	121.46	2.8390	0.093	0.060	0.343
	Log (Chl-a)	0.31403	104.49	2.5656	0.113	0.052	0.395
J'	Silt and clay %	0.31766	46.98	1.1597	0.285	0.023	0.418
	Temp	0.32680	55.71	1.3938	0.243	0.028	0.446
	DO	0.10501	0.02	4.9891	0.032*	0.131	0.131
	Log (Zn)	0.13461	0.01	2.1289	0.154	0.054	0.186
H'	Log (Cd)	0.23371	0.02	5.1383	0.027*	0.116	0.301
	Log (Mn)	0.16787	2.18	7.8588	0.010*	0.192	0.192
	Log (Pheo-a)	0.21662	0.80	3.0539	0.094	0.070	0.263
	Temp	0.21828	0.28	1.0677	0.305	0.025	0.287
ITD	WD	0.22033	0.28	1.0815	0.277	0.025	0.312
	Log (Chl-a)	0.23688	0.42	1.6508	0.207	0.037	0.349
	Log (OM)	0.25612	0.43	1.7500	0.201	0.038	0.387
	Log (OM)	0.06938	0.03	3.5346	0.071	0.097	0.097
MI	Log (Mn)	0.11087	0.02	2.5399	0.137	0.066	0.163
	Log (Chl-a)	0.06957	0.04	3.5421	0.056	0.097	0.097
	Log (Mn)	0.08777	0.02	1.6584	0.204	0.044	0.141
	Log (Pb)	0.16225	0.04	3.8451	0.068	0.095	0.236
	Log (Cr)	0.23604	0.04	3.9942	0.053	0.090	0.326
	Log (Ni)	0.27724	0.02	2.7104	0.097	0.058	0.384
	Log (Co)	0.28785	0.01	1.4320	0.248	0.030	0.414
	WD	0.29976	0.01	1.4761	0.239	0.030	0.444
c-p 2	WD	0.24936	613.58	12.2940	0.002**	0.271	0.271
	Log (Ni)	0.34814	260.06	6.0005	0.023*	0.115	0.386
	DO	0.35784	63.35	1.4838	0.251	0.028	0.415
	Log (Cd)	0.37294	72.82	1.7466	0.196	0.032	0.447
c-p 3	Log (OM)	0.39749	89.01	2.2221	0.152	0.039	0.486
	Salinity	0.40716	58.06	1.4729	0.239	0.026	0.512
	Log (Mn)	0.08023	1459.50	3.9658	0.035*	0.107	0.107
	DO	0.11761	846.58	2.3979	0.109	0.062	0.170
c-p 4	Md	0.16349	2.82	7.6448	0.010*	0.188	0.188
	Log (Ni)	0.16393	0.37	1.0174	0.323	0.025	0.213
	Log (Cr)	0.17025	0.45	1.2438	0.269	0.030	0.243
	Log (Zn)	0.20146	0.78	2.2118	0.158	0.052	0.295
	DO	0.21401	0.51	1.4789	0.254	0.034	0.330
	Log (OM)	0.21714	0.38	1.1160	0.276	0.026	0.355

Table 8Results of environmental quality assessment according to different features. C_d , the average contamination degree; RI , the potential ecological risk of metals.

Station	Sensitive / Tolerant genera	H' (log2)	ITD	MI	c-p 2% & c-p 4%	c-p 1–2% & c-p 3–4%	C_d	RI
B01	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B02	Poor	Good	Moderate	Bad	Bad	Bad	Moderate	Low
B03	Poor	Good	Good	Bad	Bad	Bad	Moderate	Low
B04	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B05	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B08	Poor	Good	Moderate	Bad	Bad	Bad	Moderate	Low
B09	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B10	Bad	Moderate	Moderate	Bad	Bad	Bad	Considerable	Moderate
B11	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B12	Poor	Good	Moderate	Bad	Bad	Bad	Moderate	Low
B13	Poor	Good	Moderate	Poor	Moderate	Poor	Moderate	Low
B14	Poor	Good	Good	Bad	Bad	Bad	Moderate	Low
B16	Poor	Moderate	Moderate	Moderate	Moderate	Poor	Moderate	Low
B17	Bad	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B18	Poor	Good	Bad	Bad	Bad	Bad	Low	Low
B19	Poor	Good	Moderate	Bad	Bad	Bad	Low	Low
B23	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B24	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B25	Poor	Moderate	Good	Bad	Bad	Bad	Moderate	Low
B28	Poor	Poor	Moderate	Bad	Bad	Bad	Moderate	Low
B29	Poor	Good	Good	Poor	Poor	Poor	Moderate	Low
B30	Poor	Poor	Moderate	Bad	Bad	Bad	Moderate	Low
B32	Bad	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B33	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B34	Poor	Moderate	Good	Poor	Poor	Poor	Moderate	Low
B35	Poor	Poor	Poor	Bad	Bad	Bad	Moderate	Low
B37	Poor	Moderate	Good	Poor	Bad	Bad	Moderate	Low
B39	Poor	Good	Good	Poor	Moderate	Poor	Moderate	Low
B40	Poor	Moderate	Moderate	Poor	Poor	Poor	Moderate	Low
B42	Poor	Poor	Moderate	Bad	Bad	Bad	Moderate	Low
B43	Poor	Good	Moderate	Moderate	Moderate	Poor	Moderate	Low
B47	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B48	Poor	Moderate	Moderate	Bad	Bad	Bad	Moderate	Low
B49	Poor	Good	Good	Poor	Moderate	Poor	Moderate	Low
B50	Poor	Good	Good	Poor	Poor	Poor	Moderate	Low

4.2. The effects of heavy metals on nematode features

Many studies have demonstrated the effects of heavy metals on nematode communities, and have proven that heavy metals are important predictors of differences in nematode community features (Austen et al., 1994; Korthals et al., 1996; Austen and McEvoy, 1997; Gyedu-Ababio et al., 1999; Monteiro et al., 2018). The present DISTLM approach indicated that the Mn concentration was a strong predictor of nematode abundance, species composition, and H' . The effect of the Mn concentration was also evident in species number and ITD and MI models, but exhibited a weaker correlation signal. Mn is an essential metal that plays important roles in many physiological processes (Tuschl et al., 2013) and the functioning of many enzymes (Chen et al., 2015). However, environmental exposure to Mn may lead to its accumulation in organisms. Several studies have revealed that Mn exposure can lead to neurodegeneration and reduction of growth, reproduction, feeding, locomotion, and lifespan in *Caenorhabditis elegans* (Anderson et al., 2001; Harada et al., 2007; Tang et al., 2019). To the best of our knowledge, there is a lack of literature on the responses of free-living marine nematode species or communities to accumulated Mn. However, the present results indicated that nematode species composition and H' significantly decreased with increasing Mn concentration, and that the Mn concentration had the strongest influence on the nematode diversity in Bohai Bay.

In addition to Mn, other studied metals may also affect nematode features. Pb is a nonessential metal which is widely used in industries. It is the most common environmental contaminant and poses great risks to the developing nervous system in human (Andrade et al., 2017). Zullini and Peretti (1986) investigated moss nematode communities from the River Po (Italy) and found that nematode biomass, the number of species, and H' significantly decreased with increasing Pb concentrations. The microcosm results also showed a clear effect of Pb on the nematode

community structure (Korthals et al., 1996; Austen and McEvoy, 1997). However, community responses varied according to the presence of tolerant or sensitive indicator species. *Oncholaimus campyloceroideus* has been demonstrated as tolerant to low and medium Pb concentrations (<1400 µg/g), while *Calomicrolaimus honestus*, *Litoditis marina*, and some members of Dorylaimidae and Monhysteridae were sensitive indicators of Pb contamination (Heininger et al., 2007; Mahmoudi et al., 2007; Monteiro et al., 2018). In the present study, DISTLM results revealed that Pb was only involved in the MI model but exhibited a weak correlation signal. Thus, the Pb concentration found in this study (<30 µg/g) may not have a significant influence on nematode community features in Bohai Bay.

Another nonessential metal that is also associated with malfunction of the nervous system is Cd (Antonio et al., 2003). Several studies have revealed that both Cd alone and Cd in combination with Mn, Pb, Cu, or Zn induced changes in growth, reproduction, and feeding of *Caenorhabditis elegans* (Anderson et al., 2001; Harada et al., 2007; Moyson et al., 2018; Tang et al., 2019). However, other studies demonstrated no effects of Cd on marine nematode communities (Austen et al., 1994; Korthals et al., 1996; Austen and McEvoy, 1997). In this study, the Cd concentration appeared as one of the important predictors for differences in J' and the abundance of c-p 2 nematodes. The weak positive correlation between c-p 2 nematodes abundance and Cd indicated a possible effect of Cd on the composition of the assemblage in terms of c-p groups.

Elevated doses of some essential metals (e.g., Cu, Zn, Ni, and Cr) affect the structure of marine and terrestrial nematode communities, and nematode diversity has been reported to be inversely proportional to these metals (Austen et al., 1994; Korthals et al., 1996; Austen and McEvoy, 1997; Monteiro et al., 2018). In the present study, J' was negatively related to the Zn concentration, suggesting a decrease in nematode community evenness due to Zn accumulation. It has been

reported that an elevated Zn concentration would cause a decrease in abundance or the disappearance of sensitive species (e.g., *Hypodontolaimus colesi*), and an increase in resistant species (e.g., *Oncholaimus campylocercoides*) (Mahmoudi et al., 2007). Although these possible indicator species of Zn contamination were not observed in Bohai Bay, c-p 4 nematodes abundance was low at sites with high Zn concentrations.

Regarding the nematode species composition, it was difficult to distinguish metal effects from other variables in terms of the structuring of the nematode community in this study. However, a significantly different species composition was observed at station B28, which had the highest Co and Mn concentrations, implying a close correlated between these metal concentrations and species composition. Changes in community structure as a consequence of metal pollution was also revealed via the relative abundance of sensitive/tolerant species. *Parodontophora deltensis*, *Parodontophora* sp. 2, *Daptonema macrostoma*, and *Sabatieria* sp. 1 were present in high abundance in this study. *Sabatieria*, *Daptonema*, and *Parodontophora* were revealed to be tolerant to metal contamination (Gyedu-Ababio et al., 1999), and have been widely suggested as indicators of bad or poor ecological quality status (Dano-varo et al., 2009; Moreno et al., 2011; Bianchelli et al., 2018). In the present study, these meiofaunal key indicators of environmental stress were observed in high abundance at all the study stations, suggesting an overall stressed environment. On this basis, relationships between the species compositions and pollutants tended to be slightly less clear than that using other variables. Similarly, although the Mn concentration was involved in the nematode ITD model, it exhibited a weaker effect on the variance of the ITD.

The influence of metals on the nematode community was also reflected in the composition of life-history strategists (c-p classes) and MI. Low c-p value nematodes, which often exhibit relatively short generation time, high colonization ability, and high tolerance to disturbance or stress, are equivalent to r-strategists (Bongers, 1990). Disturbance or stress causes a decrease in abundance or disappearance of sensitive species, and their resources are exploited by r-strategists (Warwick, 1986). The c-p value of the key species influencing taxonomic structure in the present study was 2 following Bongers (1990) and Bongers et al. (1991), also suggesting a stressed environment. The DISTLM results revealed that Mn, Ni, Cd, Zn, and Cr were involved in the c-p abundance models, suggested a possible effect of these metals on the composition of the assemblage in terms of c-p groups. At the same time, the DISTML results also showed that the Mn, Pb, Cr, Ni, and Co concentrations could explain 31.7% of the observed differentiation in MI. The composition of life-history strategists appeared be affected more by metals than that by other environmental variables.

We would like to emphasize that the metal accumulation and toxicity are affected and governed by environmental variables. Previous studies showed that sites characterized by the highest metal concentrations in the bioavailable phase had the lowest biodiversity (Ramirez et al., 2005). Thus, the variations in nematode community features in this study indicated the combined effects of environment changes.

4.3. Effectiveness of nematode community features in environmental quality assessment

The environmental quality status has been discussed extensively with regard to metal pollution in Bohai Bay (Long et al., 1995; Zhan et al., 2010; Xu et al., 2012, 2016; Gao and Chen, 2012; Gao and Li, 2012; Gao et al., 2014). Due to anthropogenic disturbances, the mean As, Cd, Cu, Hg, Pb, Zn, Cr, and Ni concentrations in sediments increased compared with their background values, and some exceeded the satisfactory levels, indicating potential harm to organisms (Gao et al., 2014). According to the criteria presented by Hakanson (1980), the Bohai Bay area had low or moderate contamination levels of most studied metals, which may be reflected by their CF values in the present study. Based on the criteria of C_d , all stations were moderately contaminated and posed

an overall low ecological risk; an exception was station B10 (i.e., the station nearest to the Haihe River estuary), which had a high contamination pressure attributed to Cd and a moderate potential ecological risk.

Liu et al. (2015) previously used the nematode to copepod ratio in assessing pollution in the study area and revealed a close relationship between this ratio and Ni contamination. Here, we extended the assessment using information on nematode community responses which may be more insightful. However, we noticed that ranked results of environmental quality obtained according to different nematode features were not consistent in this study. Furthermore, the rankings based on nematode features often suggested a more severely polluted status than the results obtained from the Hakanson's Ecological Risk Index method.

Environmental quality was assessed as poor at most stations, and bad at three stations (B10, B17, and B32) based on the presence and relative abundance of sensitive/tolerant genera (e.g., *Sabatieria*, *Daptonema*, and *Parodontophora*). The stations with bad environmental quality were located near the coastline and were among the most polluted sites in Bohai Bay. In particular, the sediments at station B10 were considerably contaminated, reflected by the total contamination degree (C_d). It suggested a poor overall environment compared with the low sediment contamination shown by the results of the Hakanson's method, indicating a more severe environmental quality status. Indeed, there is evidence that the metal concentrations of sediments have increased compared with their background values in the last century (Gao et al., 2014). Since nematodes are in direct contact with pollutants in sediment, assessments of their responses may be more insightful than abiotic assessments.

Nematode diversity is sensitive to environmental changes, and declines as a result of metal pollution in shallow water ecosystems (Gyedu-Ababio et al., 1999; Gyedu-Ababio and Baird, 2006; Franzo et al., 2018). Therefore, it is widely recommended as a useful indicator for environmental quality assessments (Moreno et al., 2011; Semprucci et al., 2014a, 2014b; Bianchelli et al., 2018; Chen et al., 2018). In the present study, the environmental quality ranking based on H' in the study area ranged from poor to good. There were conflicts between the ranking results based on H' and those of metal CFs for some stations, most of which were ranked as having a worse environmental status according to H' than according to CFs. However, H' failed to indicate the considerably contaminated status of the B10 station, which was identified by the Hakanson's method (C_d) as the most polluted station. On the other hand, H' did highlight the poor status at the B30 station, which had the highest Cr and Zn concentrations, and the B28 station, which had the highest Co and Mn concentrations. Additionally, changes in H' as a consequence of Mn pollution were revealed via their close relationship in the present study, whereby H' appeared sensitive to changes in metal concentrations and was effective in detecting the worst environmental quality in Bohai Bay. Nevertheless, we suggest that caution should be exercised when applying H' as the only indicator in environmental quality assessments due to the complexity of the relationships between H' and environmental factors. Semprucci et al. (2015) also suggested using H' as a complementary tool along with taxonomic and functional structure trait analyses because natural environmental factors always influence H' . Furthermore, enhanced nematode H' has been observed under intermediate disturbance levels (Gheskiere et al., 2004).

The c-p classes and MI values have been reported as the optimal indicators for detecting stress or disturbance induced by river discharges (Semprucci et al., 2010), OM enrichment (Semprucci et al., 2013; Mirto et al., 2014), and pollution (Chen et al., 2018). In the present study, the results based on MI and c-p categories were highly consistent, indicating bad environmental quality status at the majority of stations. The assessment based on c-p classes and on the analysis of sensitive/tolerant genera were consistent in assessing that stations B10, B17, and B32 had the worst environmental quality, and that eight stations were of poor environmental quality. However, inconsistent assessments were

observed at other stations, most of which were classified as having a bad status based on the assessment of c–p classes. The ranking results based on MI and c–p categories also deviated from the rankings of metal total C_d at most stations. Some scientists remain concerned about the effectiveness of c–p classification analyses and MI values in environmental quality assessments (Balsamo et al., 2012; Semprucci et al., 2015; Bianchelli et al., 2018). On the one hand, limited direct evidence of the influence of environmental factors on marine nematode life strategies has been documented to date. On the other hand, the present c–p classification system assumes that nematodes from one family (or some limited genera) exhibit similar life strategies. However, the current nematode classification approach does not always reflect monophyletic groups. Therefore, the c–p scale requires updating and modification. Nevertheless, MI and life-history strategists appeared sensitive to changes in metal concentrations and could provide insightful assessments in Bohai Bay.

Based on the ITD, the ecological quality of Bohai Bay was classified as ranging from poor to good, with most sites classified as moderate, which seemed more consistent with the results obtained from Hakanson's C_d , indicating overall moderate contamination. The worst environmental quality status (rank of bad) was identified by ITD at station B18. A distinct nematode community dominated by *Thalassomonhystera* sp. 1 (a non-selective deposit feeder with a c–p class of 2) was observed at this station. The metal concentrations were not very high, while the surface sediments mainly consisted of sand (32.8%) with the lowest OM content in the study area. Sand assemblages are usually more vulnerable than communities in muddy sediments because the presence of OM reduces metal bioavailability by binding metals (Wolanski and Elliot, 2016). However, the role of *Thalassomonhystera* as an indicator is worthy of further study. The ITD is calculated based on the classification of species into trophic groups (Heip et al., 1985) and is recommended as a potentially useful metric in environmental monitoring. However, the assessments given by the ITD deviated from rankings of environmental quality based on H' and MI in several studies (Semprucci et al., 2015; Bianchelli et al., 2018). As demonstrated by previous research, the complexity of feeding habitats cannot be adequately represented by the four ITD trophic groups (Moens and Vincx, 1997; Moens et al., 2005). The OM content and Mn concentration were responsible for the ITD variations, but exhibited a weak correlation. Hence, the effects of metal concentrations on the ITD is worthy of further study. Based on the present results, however, we cannot deny the effectiveness of the index in assessing environmental quality, which effectively highlighted the lowest environmental quality status in Bohai Bay.

In general, nematode community features did not always result in the same assessments at some stations in the present study, although they were able to provide a clear indication of the lowest environmental quality status from different aspects. The deviations were also observed among assessments given by different features in previous studies (Semprucci and Balsamo, 2014; Bianchelli et al., 2018; Chen et al., 2018). Chen et al. (2018) combined the results for c–p%, MI and H' by averaging when the values led to three different quality classes. However, the merging procedure may obscure the important indicating information. Instead, it is possible that the threshold proposed for the features should be recalibrated according to different habitats (Franzo et al., 2018). Nematodes have been proven to be effective elements of environmental quality assessment, however their reliability in determining environmental quality status requires further investigation.

5. Conclusions

Overall, intra-bay variability of nematode community parameters was observed in Bohai Bay. This variability reflected the observed variations in the studied environmental variables, including the metal concentrations. According to the present results, environmental variables in combination with metals influenced nematode community features. Mn has the strongest influence on nematode diversity. The

differentiation in nematode species composition may be attributed to changes in water depth, Mn, temperature, DO, Co, OM, salinity, Md, Chl-a and Pheo-a concentrations. The composition of life-history strategists appeared to be affected more by metals than by environmental variables.

When the different nematode community features were considered to assess the environmental quality of the studied stations, different (and sometimes contradictory) results were obtained, even in relation to metal stress. The features also suggested a more severely polluted status than the assessment based on the Hakanson's Ecological Risk Index method. The ranked results based on different features conflicted for some stations, because each feature reflected the different responses of the nematode community to metal stress. Taxonomic structure, life strategy traits, ITD, and H' provided more comprehensive assessments. Overall, the results of the assessment demonstrated that marine nematode communities could provide a practical and effective environmental quality evaluation that may be more sensitive than the physico-chemical parameters that are currently used.

CRedit authorship contribution statement

Er Hua: Writing - review & editing, Software, Validation. **Yanmei Zhu:** Data curation, Writing - original draft. **Deming Huang:** Visualization, Investigation. **Xiaoshou Liu:** Conceptualization, Methodology, Supervision.

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

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