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Nematode community structure in relation to metals in the southern of Caspian Sea

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Abstract

Spatial distribution and structure of nematode assemblages in coastal sediments of the southern part of the Caspian Sea were studied in relation to environmental factors. By considering metals, organic matter, Shannon diversity index (H), maturity index (MI) and trophic diversity (ITD), ecological quality status of sediment was also determined. Fifteen nematode species belonging to eleven genera were identified at the sampling sites. Average density of nematode inhabiting in sediment of the studied area was 139.78±98.91 (ind. per 15.20 cm^2). According to redundancy analysis (RDA), there was high correlation between metals and some species. Based on biological indicators, the studied area had different environmental quality. Generally, chemical and biological indices showed different results while biological indices displayed similar results in more sites.

Key words: free-living nematodes, metal, ecological quality status, the Caspian Sea

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1 Introduction

The Caspian Sea, which regards as 40% to 44% of the total lacustrine waters of the world, is still under debate to be ascribed as a sea or a lake. Owing to high biodiversity, the sea is regarded as one of the most valuable ecosystems on the earth. The Caspian Sea, as a closed water body, has a high level of endemism which is remarkably evident in its fauna. Various features of the sea, such as the presence of shallow areas, several deep depressions and a wide range of salinities from 0.1‰ to 13‰ offer different ecological niches which give rise to high species diversity.

In most marine meiobenthic habitats, nematodes are numerically the dominant animal group and have wide distribution, inhabiting in pristine to extremely polluted habitats. Usually, 70%–90% of the meiobenthic metazoan abundance is dedicated to nematodes in marine sediments, where they play major ecological functions (Austen, 2004).

Nematodes, with a daily and/or weekly generation time, represent high density and continuous reproduction all over the year (Austen and McEvoy, 1997). The highly abundance and diversity of nematodes, in addition to their wide distribution often provide more strong data than what can be acquired for most larger-sized organisms (review by Heip et al., 1985; Bongers and van de Haar, 1990; Leduc et al., 2015).

Biological activities of nematodes like as absorption of dissolved organic compounds, consumption of fungi and other organisms, nutrients regeneration, changes in sediment texture by mucus secretion, improvement of gas diffusion as well as serving as a food source for other predators from their own taxon make their presence essential within the benthic network of organisms (Bongers and van de Haar, 1990; Taheri et al., 2015).

Until now, although many researchers have focused on the ecology and biodiversity of the nematodes in many parts of the world (e.g., Heip et al., 1985; Giere, 2009; Gambi and Danovaro, 2016), their species richness especially in less investigated coastal areas such as the Caspian Sea is still unknown.

Only a few studies have described nematodes in the Caspian Sea (Zenkevich, 1963; Birshtein et al., 1968; Chesunov, 1980; Tchesunov, 1981) especially in the southern part in Iranian border (Riyazi, 2002; Mirzajani et al., 2003).

The objective of this study was to investigate the composition of the nematode community from the southern part of Caspian Sea, and to evaluate the correlations between nematode abundance, composition and distribution and the main environmental contaminants.

2 Materials and methods

2.1 *Nematode sampling and identification*

Sampling was conducted in six shallow water stations (Fig. 1) with different sediment types and depth ranged from 7 m to 15 m in autumn (November 2013). At each sampling location, three replications of the cores (id=4.4 cm) were collected to a depth of

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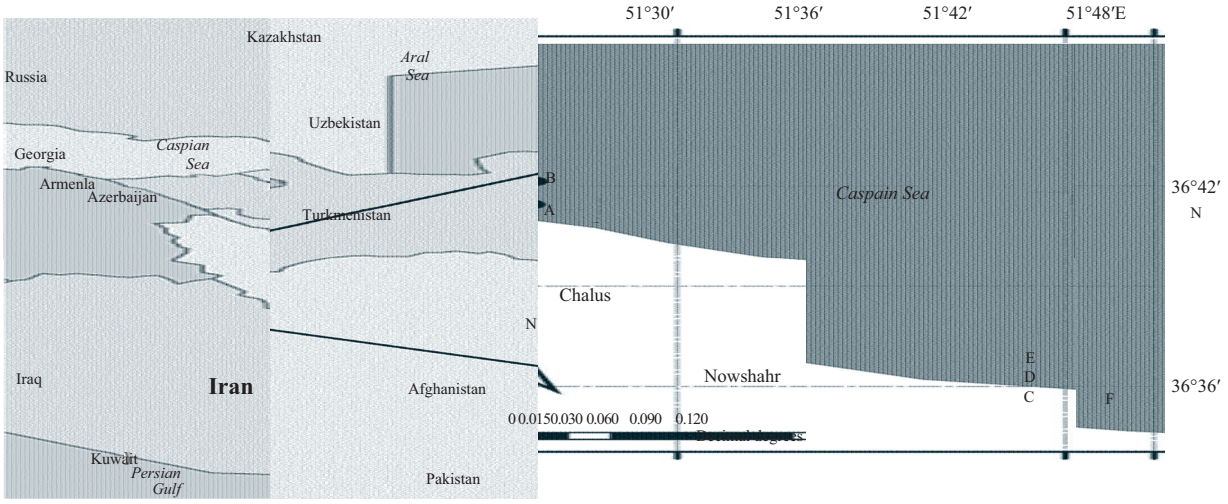

Fig. 1. The locations of the sampling sites at the south Caspian Sea.

4 cm. Samples were then fixed in 4% neutral formaldehyde seawater solution in different containers.

Each sample after transferring to the laboratory was firstly sieved through a 1-mm and then a 38-μm mesh size sieve. Thereafter, the remaining fraction was centrifuged three times with Ludox (colloidal silica) to separate organisms from sediments. The observed animals were stained with Rose Bengal and then counted.

The first 120 nematodes from each replicate were selected, placed in glycerin and then mounted on slides for identification to species level. In case of individual number lower than 120, all the nematodes were isolated. Species name was harmonized according to NeMys online identification system (Vanaverbeke et al., 2004).

Regarding the feeding type classification proposed by Wieser (1953), all nematodes were grouped as: (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate (diatom) feeders, and (2B) predators/omnivores. Density per feeding group (both in number and percentage) was determined for all stations.

As described by Heip et al. (1985), Index of Trophic Diversity (ITD) was calculated varying from the highest value (0.25) to the lowest one (1.0).

Maturity index (MI) was determined as the weighted average of individual colonisere-persister (c-p) values (Bongers, 1990):

$$MI = \sum v(i) \cdot f(i),$$

where $v(i)$ represents c-p value of the genus i and $f(i)$ as the frequency of that genus. As a semi quantitative value, this index indicates ecosystem conditions considering the nematode assemblage composition.

2.2 *Determination of physical and chemical parameters*

Environmental parameters including depth, temperature, dissolved oxygen and salinity were recorded at all sampling stations. These parameters were measured in water column by using a CTD probe (Ocean Seven 316, Italy).

Organic matter determination was performed by drying sediment samples at 70°C for 24 h and then combusting in an oven at 550°C for 4 h. As declared by Abrantes et al. (1999), total organic matter (TOM, %) was calculated by the following equation:

$$TOM = [(B - C)/B] \times 100,$$

where B and C are the weights of dried sediment before and after combusting in the oven, respectively.

Sediment grain size analysis was made from sediment samples collected in separate polythene bags. For that, samples were air dried and sieved through a mechanical sieve to remove shells, debris, etc. Dried sediment samples were subjected to size fraction analysis following the procedure of Wentworth (1992). Hundred grams of sample was taken and sieved through a 62 lm mesh-sized screen for 10 min in a mechanical sieve shaker. The sample that remained in the sieve was weighed and treated as sand. The sediment samples which passed through the sieve were the mud (silt and clay).

2.3 *Metals analysis*

As shown in Fig. 1, sediment samples were collected from the six stations using Van-Veen grab for metal analysis. After packing in plastic bags, samples were carried to the laboratory in iced-boxes and stored at 4°C until analysis.

Sediment samples were dried in an oven, ground by using a hand mortar and then screened through a 0.5-mm mesh size sieve to remove large particles. Sediment sample digestion (0.5 g each sample) was carried out using a mixed solution of HF-HCl-HNO_3-$HClO_4$ according to the ASTM standard practice D5258-92 (ASTM, 2013).

Samples were analyzed for Al, As, Cu, Ni, Pb, Co, Cr, V and Zn by using inductively coupled plasma-optical emission spectrometry (ICP-OES; Varian VISTA-MPX). A good agreement was found between the analytical results of the quality control samples with the certified values.

2.4 *Statistical analysis*

Univariate indices of community parameters including density (N, ind./(15.20 cm^2)), number of species (S), species diversity [Shannon diversity index, (H, $\log_2$)], species richness [Margalef's (d)] and evenness Pielou's (J) were calculated using statistical package of PRIMER Ver. 5 (Clarke and Warwick, 2001).

In order to evaluate the relationships between nematode community composition and environmental variables, first, a

Detrended Canonical Correspondence Analysis (DCCA) was applied on non-transformed abundance data to obtain the length of gradient along the first axis according to Ter Braak and Smilauer (2002); when the gradient length is less than 3, a redundancy analysis (RDA) is recommended, while for gradient length more than 4, a canonical correspondence analysis (CCA) is suggested.

Regarding to our short length gradients in DCCA analysis, the linear model of RDA was then selected as appropriate model for the dataset. All data were lg(x+1) transformed in order to reduce the influence of common taxa. Furthermore, data were centered and standardized by species to harmonize different scales (Lepš and Šmilauer, 2003).

Monte Carlo permutation test was executed to determine whether the relation between species abundances and the measured physicochemical variables was significant. These analyses were performed using the software of CANOCO 4.5 (Ter Braak and Smilauer, 2002). The relationships amongst various biological and environmental parameters were tested by Spearman correlation analysis.

3 Results

3.1 *Environmental variables*

Environmental parameters data of the studied area are presented in Table 1. The range of depth was between 6.8 m and 14.85 m with the highest and the lowest depths at Stas E and A, respectively. The average depth was (11.63±3.14) m. Concerning to the analysis, TOM content in sediment of the studied area varied from 1.92% to 7% with an average value of 3.95%±1.93%. Stations F and A exhibited the highest and the lowest TOM amounts. In addition, the average contents of sand and mud were 68.87%± 34.38% and 31.12%±34.38%, respectively (Table 1). Average concentrations of As, Co, Cr, Cu, Ni, Pb, V and Zn were (10.16±3.65) μg/g, (29.66±4.50) μg/g, (110.5±20.56) μg/g, (22.16±10.30) μg/g, (49.16±11.33) μg/g, (15.33±3.26) μg/g, (136.66±33.35) μg/g and (73±17.52) μg/g, respectively (Table 1).

3.2 *Nematode assemblage*

As presented in Table 2, 15 nematode species belonging to 11 genera were identified at the sampling sites. From total individuals, Xyalidae and Tripyloidoidea had dominantly the highest individuals with 66.54% and 12.95%, respectively.

Numerically, Genus Daptonema with five species was dominant. The highest and the lowest frequency at the sampling sites were dedicated to *D. curticauda* and *H. minusculus* (Table 2). As summarized in Table 3, *D. curticauda*, *D. tenuispiculum*, *D. robustus* were identified at all sampling sites while other species were present at some stations (Table 3). There were differences in total nematode density (N) among sampling sites. Stas D and F had the maximum and the minimum densities, respectively (Table 3). Overall mean density of nematode inhabiting in sediment of the studied area was 139.78±98.91 (ind./(15.20 cm^2)). Shannon diversity index (H) ranged between 1.11 and 2.69 with average value of 2.12±0.55. The most and the least values of Shannon diversity index was recorded at Stas A and F, respectively (Table 3). Species richness (d) ranged between 0.67 and 1.58 and displayed the highest and the lowest values at Stas C and F, respectively.

Table 1. General characteristic and metal content (average±SD) of the sediments at sampling sites

Sampling site	Depth/m	Sand/%	Mud/%	TOM/%	As/μg·g^{-1}	Co/μg·g^{-1}	Cr/μg·g^{-1}	Cu/μg·g^{-1}	Ni/μg·g^{-1}	Pb/μg·g^{-1}	V/μg·g^{-1}	Zn/μg·g^{-1}
A	6.80	91.84	8.16	1.92	8	22	75	15	34	10	89	51
B	13.25	80.65	19.35	4.90	11	30	122	23	60	15	161	60
C	8.98	91.00	9.00	2.56	7	35	137	18	45	15	184	80
D	11.84	64.50	35.50	4.72	10	28	107	21	46	15	117	72
E	14.85	83.60	16.40	2.61	8	30	113	14	45	17	129	73
F	14.11	1.65	98.35	7.00	17	33	109	42	65	20	140	102
ERL	–	–	–	–	8.2	nd	81	34	21	46.7	nd	150
ERM	–	–	–	–	70	nd	370	270	52	218	nd	410
Average±SD	11.63 ±3.14	68.87 ±34.38	31.12 ±34.38	3.95 ±1.93	10.16 ±3.65	29.66 ±4.50	110.5 ±20.56	22.16 ±10.30	49.16 ±11.33	15.33 ±3.26	136.66 ±33.35	73 ±17.57

Note: ERL represent effect range low (Long et al., 1995) and ERM effect range medium (Long et al., 1995). nd means not determined.

Table 2. Species, frequency trophic group (FT) and c-p of nematodes from sampling sites

Family	Species name	Frequency	Percentage of frequency/%	FT	c-p
Oncholaimidae	*Adoncholaimus araelensis*	0.019 4	1.94	2B	4
Leptolaimoidea Örley, 1880	*Antomicron elegans*	0.020 6	2.06	1A	3
Axonolaimoidea De Coninck, 1965	*Axonolaimus spinosus*	0.005 1	0.51	1B	2
Chromadoroidea Filipjev, 1917	*Chromadorita* sp.1	0.067 1	6.71	2A	3
Sphaerolaimoidea Filipjev, 1918, Xyalidae	*Daptonema curticauda*	0.400 2	40.02	1B	2
Sphaerolaimoidea Filipjev, 1918, Xyalidae	*Daptonema karabugasensis*	0.037 7	3.77	1B	2
Sphaerolaimoidea Filipjev, 1918, Xyalidae	*Daptonema robustus*	0.051 6	5.16	1B	2
Sphaerolaimoidea Filipjev, 1918, Xyalidae	*Daptonema setosum*	0.003 5	0.35	1B	2
Sphaerolaimoidea Filipjev, 1918, Xyalidae	*Daptonema tenuispiculum*	0.154 2	15.42	1B	2
Oxystominidae	*Halalaimus minusculus*	0.000 3	0.03	1A	4
Linhomoeidae	*Hofmaenneria brachystoma*	0.024 2	2.42	1B	2
Microlaimidae	*Microlaimus naidinae*	0.057 6	5.76	2A	2
Sphaerolaimidae	*Sphaerolaimus cuneatus*	0.009 9	0.99	2B	3
Xyalidae	*Theristus flevensis*	0.018 2	1.82	1B	2
Tripyloidoidea Filipjev, 1928	*Tripyloides marinus*	0.129 5	12.95	1B	2

Table 3. Total density (*N*), species number (*S*), species richness (*d*), Shannon diversity (*H*) and evenness (*J*), Maturity (MI) and Trophic Dominance (ITD) indices measured at each sampling station

Parameters	Station					
	A	B	C	D	E	F
Identified species	*A. elegans* *Chromadorita* sp.1 *D. curticauda* *D. karabugasensis* *D. robustus* *D. setosum* *D. tenuispiculum* *M. naidinae* *S. cuneatus* *T. marinus*	*Chromadorita* sp.1 *D. curticauda* *D. karabugasensis* *D. robustus* *D. setosum* *D. tenuispiculum* *H. brachystoma* *M. naidinae* *Th. flevensis* *T. marinus*	*A. elegans* *A. spinosus* *Chromadorita* sp.1 *D. curticauda* *D. karabugasensis* *D. robustus* *D. tenuispiculum* *H. minusculus* *H. brachystoma* *M. naidinae* *Th. flevensis*	*Chromadorita* sp.1 *D. curticauda* *D. karabugasensis* *D. robustus* *D. setosum* *D. tenuispiculum* *H. brachystoma* *M. naidinae* *Th. flevensis* *T. marinus*	*A. elegans* *D. curticauda* *D. karabugasensis* *D. robustus* *D. tenuispiculum* *M. naidinae* *Th. flevensis* *T. marinus*	*A. araelensis* *D. curticauda* *D. robustus* *D. tenuispiculum*
S	9	9	10	9	8	4
N	217.00±69.76	179.00±19.46	72.00±30.04	243.00±136.92	105.00±40.92	22.66±9.29
d	1.49±0.08	1.35±0.18	1.58±0.06	1.35±0.21	1.15±0.19	0.67±0.36
J	0.85±0.06	0.78±0.03	0.78±0.002	0.78±0.07	0.73±0.16	0.72±0.09
H ($\log_2$)	2.69±0.19	2.34±0.19	2.31±0.09	2.37±0.13	1.90±0.20	1.11±0.36
MI	2.13±0.01 2.12–2.15	2.15±0.08 2.10–2.26	2.02±0.02 2–2.05	2.09±0.03 2.05–2.12	2.00±0.005 2–2.01	3.36±0.27 3.09–3.63
ITD	0.71±0.02 0.69–0.74	0.68±0.07 0.59–0.74	0.79±0.07 0.71–0.86	0.71±0.09 0.62–0.80	0.76±0.03 0.73–0.80	0.59±0.10 0.50–0.70

As showm in Table 3, species richness averaged 1.26±0.32 and indicated differences among sampling sites (Table 3). The highest and the lowest MI value were obtained at Stas F and E, respectively. The minimum (maximum trophic diversity) and maximum (minimum trophic diversity) of ITD were measured 0.59±0.10 at Sta. F and 0.79±0.07 at Sta. C, respectively (Table 3).

Regarding the results of SIMPER analysis, genus Daptonema was the most impacted taxa at all stations as high abundances of *D. curticauda*, *D. tenuispiculum* and also *T. marinus* were discovered throughout the studied area (Table 4).

Percentage of each feeding type is shown in Fig 2. In most sampling sites, non-selective deposit feeders (IB) were dominant group, followed by epistrate feeders (2A).

Limited distribution of predators/omnivores (2B) was only revealed at Stas A and F while selective deposit feeders (1A) were presented at Stas A, C and E.

3.3 *Relationship between nematode and environmental variables*

As illustrated in Fig. 3, the first two RDA axes showed high eigenvalues (0.49 and 0.25, respectively) in comparison to subsequent axes and explained 49.3% and 25.8% of the variation in species composition, respectively. On the whole, both axes explained 75.1% of variance of species-environment relation (Fig. 3).

Furthermore, RDA analysis exhibited an association between

Table 4. Results of SIMER analysis carried out for nematode community at the stations

Species	Av Abu	Cum/%	Species	Av Abu	Cum/%
Station A. average similarity: 86.60			Station B. average similarity: 77.26		
D. curticauda	62.76	15.93	*D. curticauda*	76.47	23.82
T. marinus	47.85	31.10	*D. tenuispiculum*	31.43	42.22
D. tenuispiculum	28.60	44.65	*Chromadorits* sp.1	29.21	59.11
D. robustus	25.44	56.78	*T. marinus*	12.79	72.66
D. karabugasensis	16.75	68.53	*M. naidinae*	6.97	84.18
A. elegans	16.49	79.89	*D. robustus*	6.19	88.78
S.cuneatus	8.33	88.47	*Th. flevensis*	7.45	93.17
Station C. average similarity: 67.76			Station D. average similarity: 78.17		
D. tenuispiculum	23.11	24.87	*D. curticauda*	109.61	20.23
D. curticauda	22.11	48.91	*T. marinus*	37.05	36.85
M. naidinae	7.11	66.55	*D. tenuispiculum*	33.90	51.19
A. spinosus	4.28	79.71	*Chromadorits* sp.1	22.17	65.22
H. brachystoma	7.69	86.11	*M. naidinae*	15.00	79.24
D. robustus	1.94	90.81	*H. brachystoma*	9.11	90.22
Station E. average similarity: 77.44			Station F. average similarity: 78.55		
D. curticauda	59.63	27.11	*A. araelensis*	16.31	49.99
D. tenuispiculum	11.95	47.13	*D. curticauda*	5.07	90.78
M. naidinae	13.70	67.09			
T. marinus	11.15	85.39			
Th. flevensis	2.81	90.71			

Note: Av Abu represents average abundance and Cum cumulative.

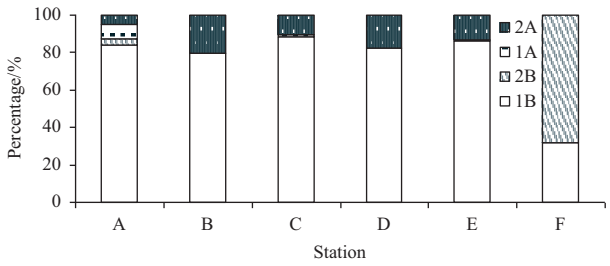

Fig. 2. Percentage of trophic groups at the various sampling stations.

A. araelensis with high values of As, Cu and Mud. Positive correlations were observed between abundances of *A. spinosus*, *H. minusculus*, *H. brachystoma* and *T. flevensis* with Cr, V and Co values. There was a negative correlation between sand and *A. realness* abundance (Fig. 3).

3.4 *Relationship between environmental variables and biological parameters*

According to Pearson's analysis, a positive correlation was detected between depth and all the elements (Table 5). The whole elements, except for Cr, were positively correlated with mud. Also, all the elements had positive correlations with TOM. There were negative correlations between *H*, *J* and *N* with the whole elements. A negative correlation was found between diversity indices and TOM (Table 5). Significantly, positive correlations were discovered between MI with As, Cu and mud (p<0.01). Moreover, ITD was negatively correlated with mud, As, Cu and TOM (p<0.05).

4 Discussion

Two sets of SQGs developed for marine and estuarine ecosystems (MacDonald et al., 1996; Long and MacDonald, 1998) were applied in this study to assess the ecotoxicological risk assessment of metals in sediments: (1) the effect range low (ERL)/effect range median (ERM) and (2) the threshold effect level (TEL)/probable effect level (PEL) values. Low range values (i.e., ERLs or TELs) are concentrations below which adverse effects upon sediment dwelling fauna would infrequent be expected. In contrast, the ERMs and PELs represent chemical concentrations

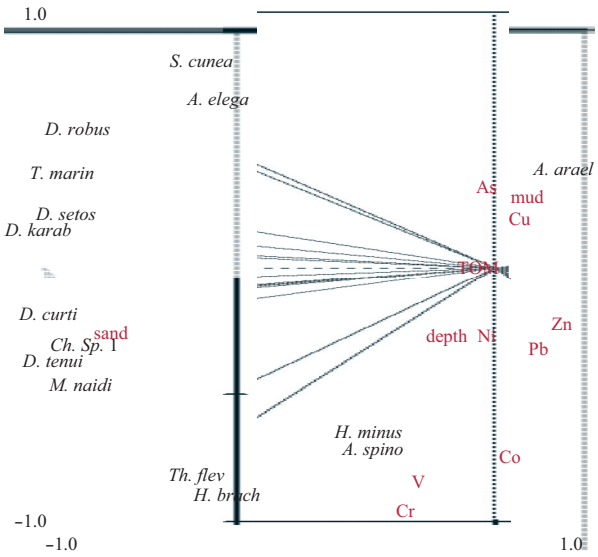

Fig. 3. Results of redundancy analysis (RDA) between annelids assemblage, environmental parameters and heavy metals in the Gorgan Bay. The arrows indicate the direction of increase for the variables studied. The angles between variables reflect their correlations (angles near 90° indicate no correlation, angles near 0° indicate high positive correlation and angles near 180° indicate high negative correlation). *A. arael*: *Adoncholaimus araelensis*, *A. elega*: *Antomicron elegans*, *A. spino*: *Axonolaimus spinosus*, *Ch.* sp.1: *Chromadorita* sp.1, *D. curti*: *Daptonema curticauda*, *D. karab*: *Daptonema karabugasensis*, *D. robus*: *Daptonema robustus*, *D. setos*: *Daptonema setosum*, *D. tenui*: *Daptonema tenuispiculum*, *H. minus*: *Halalaimus minusculus*, *H. brach*: *Hofmaenneria brachystoma*, *M. naidi*: *Microlaimus naidinae*, *S. cunea*: *Sphaerolaimus cuneatus*, *Th. fleve*: *Theristus flevensis*, and *T. marin*: *Tripyloides marinus*.

Table 5. Correlation between metals, biological and physicochemical parameters measured at each sampling station

Parameter	Depth	Sand	Mud	TOM	As	Co	Cr	Cu	Ni	Pb	V	Zn
Sand	–0.500											
Mud	0.500	–1.000**										
TOM	0.607	–0.887*	0.887*									
As	0.524	–0.956**	0.956**	0.941**								
Co	0.458	–0.344	0.344	0.389	0.271							
Cr	0.381	0.017	–0.017	0.179	–0.033	0.905*						
Cu	0.430	–0.957**	0.957**	0.921**	0.971**	0.424	0.101					
Ni	0.712	–0.731	0.731	0.898*	0.834*	0.605	0.454	0.833*				
Pb	0.836*	–0.751	0.751	0.720	0.681	0.757	0.521	0.705	0.792			
V	0.236	–0.002	0.002	0.184	0.027	0.889*	0.954**	0.184	0.495	0.440		
Zn	0.500	–0.811	0.811	0.650	0.663	0.758	0.430	0.767	0.628	0.889*	0.403	1.000
N	–0.374	0.512	–0.512	–0.306	–0.417	–0.775	–0.488	–0.536	–0.508	–0.735	–0.559	–0.811
d	–0.704	0.911*	–0.911*	–0.765	–0.880*	–0.297	0.049	–0.822*	–0.704	–0.813*	0.064	–0.740
J	–0.888*	0.616	–0.616	–0.577	–0.535	–0.722	–0.529	–0.538	–0.687	–0.974**	–0.408	–0.806
H	–0.694	0.875*	–0.875*	–0.710	–0.800	–0.571	–0.220	–0.812*	–0.728	–0.920**	–0.210	–0.894*
MI	0.355	–0.957**	0.957**	0.799	0.941**	0.299	–0.087	0.957**	0.697	0.647	0.013	0.749
ITD	–0.363	0.856*	–0.566*	–0.854*	–0.950**	0.021	0.291	–0.879*	–0.702	–0.435	0.196	–0.414

Note: ** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level. *T* represents temperature, TOM total organic matter, *S* number of species, *N* number of individual, *d* species richness (Margalef's index), *j* evenness Pielou's, *H* species diversity (Shannon diversity index), MI Maturity index, and ITD index of trophic diversity.

above which adverse effects are likely to occur (Long and MacDonald, 1998). At all sampling sites, concentrations of Pb and Zn were lower than the relevant values of effects range low (ERL). A decrease in As, Cr and Cu contents were in the range between ERL and ERM, proposing that these metals would be anticipated rarely to cause adverse biological effects on biota (Long et al., 1995) in the studied areas. Furthermore, Ni levels were higher than ERM at some sampling sites, implicating that negative eco-risk effects often occur at Stas B and F. All elements, except for Cr and V, showed high positive correlations with mud, depth and TOM. The results presented here, suggested the impact of sediment grain size, depth and TOM content on metal distributions (Pb, Zn, As, Cu, Co and Ni) in the studied area. Generally, sediment grain size becomes finer as depth increases. Sandy sediments are made in coastal areas due to their much weight, and the amount of large-sized sediments decreases off shore, while fine-sized sediments increase when coming on shore. The fine grains, representing the higher rate of surface to volume and ionic absorption power, are more capable in the absorption of contaminated organic and inorganic materials (McCave, 1984; Horowitz and Elrick, 1987). Generally, fine-grained sediments carrying lots of organic matter are more contaminated than coarse-grained sediments. By contrast, the presence of a week correlation between Cr and V with sediment grain size, depth and TOM might indicate the possible effect of other parameters on their concentrations. Sediment grain size and organic matters has been proposed as dominant factors elucidating a significant part of the variance in species composition of nematode assemblages (Heip et al., 1985). Additionally, water depths seemed to be potentially important which influence the structure of nematode assemblage, probably by determining other factors like as TOM and the stability of physico-chemical factors. Both the amount of organic matter and oxygen content are also determined by the sediment grain size, as the highest oxygen penetration depth with the lowest organic matter is generally observed in coarser sediment (Castro and Huber, 2003). Several investigators have reported the increased density and diversity of marine nematodes with increased sediment grain size (Vanaverbeke et al., 2011; Fonseca et al., 2014), though contradictory result has also been published elsewhere (Maria et al., 2013), implying that their response is species-specific. In our survey, the abundance and diversity indices of nematodes were negatively affected by depth, mud and TOM while they showed a positive correlation with sand. This is probably due to the organic matter enrichment in muddy station which can decrease total density and species number of nematode by creating a hypoxia inside the sediment; thus change their community structure (Armenteros et al., 2009, 2010). But a higher density and species number is commonly observed in coarser sediments, where a deep penetration of oxygen is occurred by active flows through the sediments (de Beer et al., 2005).

Some typical effects of metals exposure and hydrocarbons contamination including changes in nematode assemblage structure, mortality of the most sensitive species and decreased diversity have been demonstrated by several authors (e.g., Boucher, 1980; Tietjen, 1980; Somerfield et al., 1994; Danovaro et al., 1995; Austen and Somerfield, 1997; Beyrem and Aissa, 2000; Mahmoudi et al., 2002; Hedfi et al., 2007, 2008; Boufahja et al., 2011). Although nematode abundance might not be affected, a great deal of studies have informed a significantly metal-induced decrease in nematode abundance.

As previously reported by Somerfield et al. (1994), there were less abundance, lower richness and species diversity of nematode fauna in copper-contaminated sites as compared to the one from less contaminated areas. Correspondingly, negative correlation was found between *N*, *J* and *H* with all the metal contents in this study. Metal accumulation in the sediments might change the associated microbial communities; consequently affecting the meiobenthose through changes in food supply (Austen and McEvoy, 1997). Millward and Grant (1995) documented that a certain selection for more tolerant species occurs only after longer periods of exposure to metals, which affects the community composition: the number of tolerant nematode species against Cu increased along an estuary enriched with copper in comparison to unpolluted reference sites.

Many researchers exhibited that there are adapted species with a high tolerance to metal compounds among nematodes (e.g., *Molgolaimus demani*, *Sabatieria pulchra*, *Axonolaimus paraspinosus*, *Oncholaimus campylocercoides*, *Bathylaimus capacosus*; Warwick, 1988; Somerfield et al., 1994; Hedfi et al., 2007). Moreover, as detected by Somerfield et al. (1994), adaptive effects may expand the tolerance range within the same species like as *Enoplus brevis* (Nematoda) from a contaminated site was more tolerant than specimens from uncontaminated sites. In the present study, the association between *A. araelensis* with high values of As, Cu and mud infer that this species can tolerate medium levels of As and Cu. As demonstrated by laboratory studies, in addition to the chemical form of a metal, other parameters including temperature (Lehtinen et al., 1984; Vranken et al., 1989), salinity (Bengtsson and Bergström, 1987) and food (Verriopoulos and Moraitou-Apostolopoulou, 1989) can influence on acute and sublethal toxicity of metals to a variety of meiofauna. Nematodes can uptake metals through the cuticle (Howell, 1983) and the secretion of mucous substances makes them capable of binding metals. Riemann and Schrage (1978) proposed mucus as a part of feeding mechanism in many nematode species. Another explanation for metals assimilation in meiofauna is metal binding capability of acid mucopolysaccharides secreted by the bacteria, as many nematodes and copepods are bacterial feeders (Jensen, 1987). According to our results, some species, such as *A. spinosus*, *H. minusculus*, *H. brachystoma* and *T. flevensis* can tolerate high range of Cr, V and Co; thus they posses some form of tolerance mechanism. Increased metals led to a decrease in abundances of *A. elegans*, *Chromadorita* sp.1, *D. curticauda*, *D. karabugasensis*, *D. robustus*, *D. setosum*, *D. tenuispiculum*, *M. naidinae*, *S. cuneatus*. Hence, they seemed to be intolerant species to metal. In previous study by Moreno et al. (2011), who compared the use of different ecological indices, including *H*, MI, c-p%, ITD and the presence of sensitive/tolerant genera at many different sites in the Mediterranean Sea, the use of nematodes has been proved as the best tool for efficient evaluation of ecological quality status.

Regarding TOM content, all sites showed a high/good EQS (Tables 6 and 7). Based on metal concentration, the majority of sites displayed a moderate EQS, except for B and F, which revealed a poor/bad EQS (Table 7). Summarizing the presented results based on these variables, it can be inferred that A, C and D were the ones described by greater environmental quality, whereas Stas B and F were characterized by lower environmental quality.

The maturity index is also used to assess the EQS at different sites, even though its utilization is arguable in marine and brackish ecosystems (Bongers et al., 1991; Neilson et al., 1996; Essink and Keidel, 1998; Mirto et al., 2002; Fraschetti et al., 2006; Gyedu-Ababio and Baird, 2006; Moreno et al., 2008a, b). Principally, the maturity index is based on various strategies of nematode as-

Table 6. Thresholds proposed to evaluate the ecological quality status (Moreno et al., 2011)

Indicator	High	Good	Moderate	Poor	Bad
Organic matter		<5%	5%–10%	>10%	
Metals		<ERL	ERL<conc<ERM	>ERM	
MI	>2.8	2.8≤MI<2.6	2.6≤MI<2.4	2.4≤MI<2.2	≤2.2
ITD	0.25	0.25<ITD≤0.4	0.4<ITD≤0.6	0.6<ITD≤0.8	1
H	>4.5	3.5<*H*<4.5	2.5<*H*<3.5	1<*H*≤2.5	0<*H*≤1

Note: Conc represents concentration.

Table 7. Results of the EQS on the considered study sites

Indicator	High	Good	Moderate	Poor	Bad
Organic matter	A, B, C, D, E, F				
Metals			A, C, D, E	B, F	
MI	F				A, B, C, D, E
ITD			F	A, B, C, D, E	
H			A	B, C, D, E, F	

semblage in relation to different disturbances. Considering this index, Sta. F represented a high EQS whilst a poor EQS was discovered at Stas A, B, C, D and E. In order to correlate the trophic diversity of nematodes with pollution levels, the index of trophic diversity is usually used (Heip et al., 1985; Mirto et al., 2002). The application of this index revealed a poor/bad EQS at Stas A, B, C, and D while Sta. F displayed moderate EQS (Table 7). In accord with the Shannon diversity index, a poor EQS was discovered at Stas B, C, D, E and F while a moderate EQS was assigned to Sta. A (Table 7). In stressed environments subjected to organic enrichment, human disturbance and physical stressors, values of the Shannon diversity index decreased which is implicated to a reduction in biodiversity (Fraschetti et al., 2006; Bianchelli et al., 2008; Danovaro et al., 2008). ITD, *H* and MI indices indicated that Stas B, C, D, E have lower environmental quality. Generally, chemical and biological indices displayed different results while biological indices showed similar findings in more sites.

This study showed the differences in marine nematode communities caused by environmental parameters and indicated that the nematode communities were notably influenced by metals contamination. Because in the present work, the nematode assemblages were investigated only in autumn, more studies during other seasons, within at least two or three successive years and with increasing the sample size, are needed to fully understand how nematode respond to metals contamination and other environmental parameters. Furthermore, the use of different ecological indices showed various results; hence determination of the best indicator would be important in future studies for the evaluation of environmental quality status.

References

Abrantes A, Pinto F, Moreira M H. 1999. Ecology of polychaete *Nereis diversicolor*, in the Cannal de Mira (Ria de Averio, Portugal). population dynamics, production and oogenic cycle. Acta Oecologica, 20(4): 267–283

Armenteros M, Pérez-García J A, Ruiz-Abierno A, et al. 2010. Effects of organic enrichment on nematode assemblages in a microcosm experiment. Mar Environ Res, 70(5): 374–382

Armenteros M, Ruiz-Abierno A, Fernández-Garcés R, et al. 2009. Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. Estuar Coast Shelf Sci, 85(2): 179–189

ASTM. 2013. D4698–92, 2013, Standard Practice for Total Digestion of Sediment Samples for Chemical Analysis of Various Metals. West Conshohocken, PA: ASTM International. www.astm.org

Austen M C, McEvoy A J. 1997. The use of offshore meiobenthic communities in laboratory microcosm experiments: response to heavy metal contamination. J Exp Mar Biol Ecol, 211(2): 247–261

Austen M C. 2004. Natural nematode communities are useful tools to address ecological and applied questions. Nematol Monogr Perspect, 2: 1–17

Austen M C, Somerfield P J A. 1997. Community level sediment bioassay applied to an estuarine heavy metal gradient. Mar Environ Res, 43: 315–328

Bengtsson B E, Bergström B. 1987. A flowthrough fecundity test with *Nitocra spinipes* (harpacticoidea crustacea) for aquatic toxicity. Ecotoxicol Environ Saf, 14(3): 260–268

Beyrem H, Aissa P. 2000. Les nématodes libres, organismes-sentinelles de l'évolution des concentrations d'hydrocarbures dans la baie de Bizerte (Tunisie). Cah Biol Mar, 41(3): 329–342

Bianchelli S, Gambi C, Pusceddu A, et al. 2008. Trophic conditions and meiofaunal assemblages in the Bari Canyon and the adjacent open slope (Adriatic Sea). Chem Ecol, 24(S1): 101–109

Birshtein Y A, Vinogradov L G, Kondakov N N, et al. 1968. Atlas of Invertebrates of the Caspian Sea. Moscow: Pishchevaya Promyshlennost

Bongers T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83: 14–19

Bongers T, Alkemade R, Yeates G W. 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar Ecol Prog Ser, 76: 135–142

Bongers T, van de Haar J. 1990. On the potential of basing an ecological typology of aquatic sediments on the nematode fauna: an example from the river Rhine. Hydrobiol Bull, 24(1): 37–45

Boucher G. 1980. Impact of *Amoco Cadiz* oil spill on intertidal and sublittoral meiofauna. Mar Poll Bull, 11(4): 95–101

Boufahja F, Hedfi A, Amorri J, et al. 2011. An assessment of the impact of chromium-amended sediment on a marine nematode assemblage using microcosm bioassays. Biol Trace Elem Res, 142(2): 242–255

Castro P, Huber M. 2003. Marine Biology. 4th ed. Boston: McGraw Hill

Chesunov A V. 1980. New data on free-living nematodes of the superfamily Monhysteroidea in the Caspian Sea. Zool Zh, 59(7): 973–985

Clarke K R, Warwick R M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed. Plymouth: PRIMER-E Ltd

Danovaro R, Fabiano M, Vincx M. 1995. Meiofauna response to the *Agip Abruzzo* oil spill in subtidal sediments of the Ligurian Sea. Mar Poll Bull, 30(2): 133–145

Danovaro R, Gambi C, Dell'Anno A, et al. 2008. Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Curr Biol, 18(1): 1–8

De Beer D, Wenzhöfer F, Ferdelman T G, et al. 2005. Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnol Oceanogr, 50(1): 113–127

Essink K, Keidel H. 1998. Changes in estuarine nematode communities following a decrease of organic pollution. Aquat Ecol, 32(3): 195–202

Fonseca G, Maria T F, Kandratavicius N, et al. 2014. Testing for nematode-granulometry relationships. Mar Biodiv, 44(3): 435–443

Fraschetti S, Gambi C, Giangrande A, et al. 2006. Structural and functional response of meiofauna rocky assemblages to sewage pollution. Mar Pollut Bull, 52(5): 540–548

Gambi C, Danovaro R. 2016. Biodiversity and life strategies of deep-sea meiofauna and nematode assemblages in the Whittard Canyon (Celtic margin, NE Atlantic Ocean). Deep Sea Res I Oceanogr Res Papers, 108: 13–22

Giere O. 2009. Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments. 2nd ed. Berlin Heidelberg: Springer-Verlag

Gyedu-Ababio T K, Baird D. 2006. Response of meiofauna and nematode communities to increased levels of contaminants in a laboratory microcosm experiment. Ecotoxicol Environ Saf, 63(3): 443–450

Hedfi A, Mahmoudi E, Beyrem H, et al. 2008. Réponse d'une communauté de nématodes libres marins à une contamination par le cuivre: étude microcosmique. Bull de la Soc Zool de France, 133(1–3): 97–106

Hedfi A, Mahmoudi E, Boufahja F, et al. 2007. Effects of increasing levels of nickel contamination on structure of offshore nematode communities in experimental microcosms. Bull Environ Contam Toxicol, 79(3): 345–349

Heip C H R, Vincx M, Vranken G. 1985. The ecology of marine nematodes. Oceanogr Mar Biol Ann Rev, 23: 399–489

Horowitz A J, Elrick K A. 1987. The relation of stream sediment surface area, grain size and composition to trace element chemistry. Appl Geochem, 2: 437–451

Howell R. 1983. Heavy metals in marine nematodes: uptake, tissue distribution and loss of copper and zinc. Mar Pollut Bull, 14(7): 263–268

Jensen P. 1987. Feeding ecology of free-living aquatic nematodes. Mar Ecol Prog Ser, 35: 187–196

Leduc D, Nodder S D, Berkenbusch K, et al. 2015. Effect of core surface area and sediment depth on estimates of deep-sea nematode genus richness and community structure. Mar Biodiv, 45(3): 349–356

Lehtinen K J, Bengtsson B E, Bergstrom B. 1984. The toxicity of effluents from a TiO_2 plant to the harpacticoid copepod *Nitocra spinipes* Boeck. Mar Environ Res, 12(4): 273–283

Lepš J, ŠMilauer P. 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge: Cambridge University Press

Long E R, MacDonald D D, Smith S L, et al. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manag, 19(1): 81–97

Long E R, MacDonald D D. 1998. Recommended uses of empirically derived, sediment quality guidelines for marine and estuarine ecosystems. Hum Ecol Risk Assess Int J, 4(5): 1019–1039

MacDonald D D, Carr R S, Calder F D, et al. 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology, 5(4): 253–278

Mahmoudi E, Beyrem H, Baccar L, et al. 2002. Response of free-living Nematodes to the quality of water and sediment at Bou Chrara Lagoon (Tunisia) during winter 2000. Mediterr Mar Sci, 3(2): 133–146

Mahmoudi E, Essid E, Beyrem H, et al. 2007. Individual and combined effects of lead and zinc on a free-living marine nematode community: results from microcosm experiments. J Exp Mar Biol Ecol, 343(2): 217–226

Maria T F, Paiva P, Vanreusel A, et al. 2013. The relationship between sandy beach nematodes and environmental characteristics in two Brazilian sandy beaches (Guanabara Bay, Rio de Janeiro). An Acad Bras Ciênc, 85(1): 257–270

McCave I N. 1984. Size spectra and aggregation of suspended particles in the deep ocean. Deep-Sea Res, 31: 329–352

Millward R N, Grant A. 1995. Assessing the impact of copper on nematode communities from a chronically metal-enriched estuary using pollution-induced community tolerance. Mar Pollut Bull, 30(11): 701–706

Mirto S, La Rosa T, Gambi C, et al. 2002. Nematode community response to fish-farm impact in the western Mediterranean. Environ Pollut, 116(2): 203–214

Mirzajani A R, Yosefzad E, Sayad Rahim M, et al. 2003. Investigation on meiofauna and substrate characteristic in the southern Caspian Sea (Guilen province). Iran Sci Fish J, 11(4): 119–132

Moreno M, Ferrero T J, Gallizia I, et al. 2008a. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuar Coast Shelf Sci, 77(4): 565–576

Moreno M, Semprucci F, Vezzulli L, et al. 2011. The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecol Indic, 11(2): 328–336

Moreno M, Vezzulli L, Marin V, et al. 2008b. The use of meiofauna diversity as an indicator of pollution in harbours. ICES J Mar Sci, 65(8): 1428–1435

Neilson R, Boag B, Palmer L F. 1996. The effect of environment on marine nematode assemblages as indicated by the maturity index. Nematologica, 42(2): 232–242

Riemann F, Schrage M. 1978. The mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. Oecologia, 34(1): 75–88

Riyazi B. 2002. Investigation on benthos invertebrates of the Gomishan wetland. Iran J Nat Res, 55(2): 211–223

Somerfield P J, Gee J M, Warwick R M. 1994. Soft sediment meiofaunal community structure in relation to a long-term heavy metal gradient in the Fal estuary system. Mar Ecol Prog Ser, 105: 79–88

Taheri M, Grego M, Riedel B, et al. 2015. Patterns in nematode community during and after experimentally induced anoxia in the northern Adriatic Sea. Mar Environ Res, 110: 110–123

Tchesunov A V. 1981. Free-living nematodes of the group of *Theristus flevensis* (Monhysterida) species in the Caspian sea. Byulleten' Mosk Obshch Isput Prir (Otd Biol), 86: 63–70

Ter Braak C J F, Smilauer P. 2002. CANOCO Reference Manual and CanoDraw for Windows User'S Guide: Software for Canonical Community Ordination (version 4.5). Ithaca, New York: Microcomputer Power

Tietjen J H. 1980. Population structure and species composition of the free-living nematodes inhabiting sands of the New York Bight apex. Estuar Coas Shelf Sci, 10(1): 61–73

Vanaverbeke J, Merckx B, Degraer S, et al. 2011. Sediment-related distribution patterns of nematodes and macrofauna: two sides of the benthic coin?. Mar Environ Res, 71(1): 31–40

Vanaverbeke J, Soetaert K, Vincx M. 2004. Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition. Mar Ecol Prog Ser, 273: 139–146

Verriopoulos G, Moraitou-Apostolopoulou M. 1989. Toxicity of zinc to the marine copepod Tisbe holothuriae; the importance of the food factor. Arch Hydrobiol, 114(3): 457–463

Vranken G, Tiré C, Heip C. 1989. Effect of temperature and food on hexavalent chromium toxicity to the marine nematode *Monhystera disjuncta*. Mar Environ Res, 27(2): 127–136

Warwick R M. 1988. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. Mar Pollut Bull, 19(6): 259–268

Wentworth C K. 1992. A scale of grade and class terms for clastic sediments. J Geol, 30(5): 377–392

Wieser W. 1953. Die Beziehung zwischen Mundhoehlengestalt, Ernaehrugsweise und Vorkommen bei freilebenden marinen Nematoden. Zool Arch, 4: 439–484

Zenkevich L A. 1963. Biology of the Seas of the U.S.S.R. Botcharskaya S, trans. New York: Interscience Publishers