The use of meiofauna diversity as an indicator of pollution in harbours

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We assessed several meiofaunal indices in sediments of three Mediterranean harbours differing in environmental contamination to evaluate their usefulness as indicators of pollution and to identify those that best described environmental quality. In general, indicators based on meiofaunal taxa demonstrated a significant correlation with the concentration of contaminants, especially the polycyclic aromatic hydrocarbons (PAHs). Nematode genus-based indicators correlated with contaminant concentrations at similar levels of significance, suggesting that a high taxonomic resolution does not improve the information content of meiofauna diversity indicators for assessing the environmental quality in these harbours. Notably, environmental variables that affect meiofaunal and nematode assemblages (e.g. water depth, grain size, and food sources) demonstrated a low spatial and temporal variability in the harbours investigated and did not represent important confounding factors. We conclude that the application of meiofaunal and nematode indices can be a useful tool for assessing the environmental quality of harbour ecosystems.

Keywords: environmental indicators, harbour, meiofauna, nematodes.

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Introduction

Harbours, as a major interface between coastal cities and the sea, are often under heavy pressure from human activities and increasingly suffer from environmental risks linked to poor water and sediment quality (Estacio *et al.*, 1997). These risks may be associated with the presence of toxic contaminants and/or with hypertrophy caused by the high input of organic-matter concentrations and the associated development of anoxia and bad odours. Typically, environmental disturbance in harbours varies considerably over small spatial scales, owing to various factors including the location and magnitude of pollution sources, river inputs, tidal regime, and water circulation (Moreno *et al.*, 2008).

To improve the ecological status of harbour ecosystems, management must first be informed, through monitoring programmes, about the spatial variation in water and sediment quality. This type of knowledge can be enhanced through the use of environmental indicators that can be related to the pressure put upon these systems. The introduction of biological features in the assessment of environmental quality is an innovation of recent monitoring programmes, as required by the Water Framework Directive (WFD) of the European Union (2000/60/EC). Benthic communities are commonly considered to be sensitive to local pressure, because they should reflect an integrated response over time. In the WFD, specific indicators that reflect the abundance and diversity of macrofauna organisms have been proposed for assessing environmental quality of coastal systems (Borja *et al.*, 2000; Simboura and Zenetos, 2002). However, in enclosed and stressed systems such as harbours, macrobenthic organisms are generally scarce and difficult to sample representatively, thereby providing little information that can be used effectively. In contrast, meiofaunal organisms appear to represent the only major metazoan component of the fauna in harbour sediments (Fichet *et al.*, 1999). Moreover, because these small and abundant organisms are bound to the sediment throughout their life history (Suderman and Thistle, 2003) and are often sensitive to many toxicants (Coull and Chandler, 1992; Long, 1992; Guo *et al.*, 2001), they are considered to be good candidate organisms for environmental quality assessment of harbours (e.g. Amjad and Gray, 1983; Lambshead, 1984; Shiells and Anderson, 1985; Lampadariou *et al.*, 1997; Fichet *et al.*, 1999; Suderman and Thistle, 2003; Vezzulli *et al.*, 2003; Moreno *et al.*, 2008).

We tested a number of widely used indicators on meiofauna and nematodes in sediments of three Mediterranean harbours that suffer different levels of pollution. The Shannon–Wiener diversity index (H'), Pielou evenness index (J'), the number of taxa/genera (S), the maturity index (MI), and the index of trophic diversity (ITD) were measured at different stations and during different periods in relation to the concentration and typology of the main environmental contaminants. Our main objective was to evaluate the use of meiofauna as an indicator of state and pollution effects by identifying the metrics that best describe the environmental quality and its spatial heterogeneity within harbour ecosystems.

Material and methods

Sampling sites

Surface sediment samples (0–2 cm) were collected from the harbours of Genoa-Voltri, Portosole, and Marina degli Aregai, Italy, which are all located in the Ligurian Sea (northwest Mediterranean). Genoa-Voltri is a heavily polluted, commercial harbour covering an area of ~34.5 ha with a depth of 7–12 m and sediment composed of fine sand and silt. Samples were collected on four occasions (June, July, and November 2002, and February 2003) at three stations representing the inner part of the harbour (Station I), the middle part (Station M), and the outer part (Station O) close to the open sea (Figure 1a). Portosole and Marina degli Aregai are both tourist marinas with areas of ~16 and ~18 ha, respectively, and sediment that is dominated by the fine-sand fraction. Portosole is located close to a commercial harbour (Sanremo) and has an average depth of 3–7 m. Marina degli Aregai has a depth of 3–12 m. In both harbours, samples were collected on two occasions (July 2005 and January 2006) at four stations (Stations 1–4; Figure 1b and c).

At each station, six PVC-core (inner diameter, 3.6 cm) sediment samples were taken by scuba divers, three for the analysis of contaminants [protein, heavy metals, and polycyclic aromatic hydrocarbons (PAHs)] and three for meiofauna and nematodes. Heavy-metal concentrations were not determined for Genoa-Voltri. A detailed description of methods and results regarding contaminants can be found in Salvo *et al.* (2005), Fabiano *et al.* (2006), and Moreno *et al.* (2006, 2008). A summary of the results is presented in Table 1.

Meiofauna

Samples were fixed with 4% buffered formaldehyde in filtered seawater. In the laboratory, all meiofaunal samples were rinsed with a gentle jet of fresh water over a 0.5 mm sieve to exclude macrofauna, decanted over a 38 μm sieve ten times, centrifuged three times with Ludox HS40 (specific density 1.18; Heip *et al.*, 1985), and stained with Rose Bengal. Meiofauna were counted and identified at the major taxon level, using a stereomicroscope, and density was recalculated as abundance 10 cm^{-2}. Diversity indices (H', J', and S) were calculated using the Primers routine (Clarke and Warwick, 1994). The nematode (Ne)–copepod (Co) ratio was also analysed, considering all (Ne:Co; Raffaelli and Mason, 1981) and epistrate-feeding nematodes only (Ne2A:Co; Warwick, 1981).

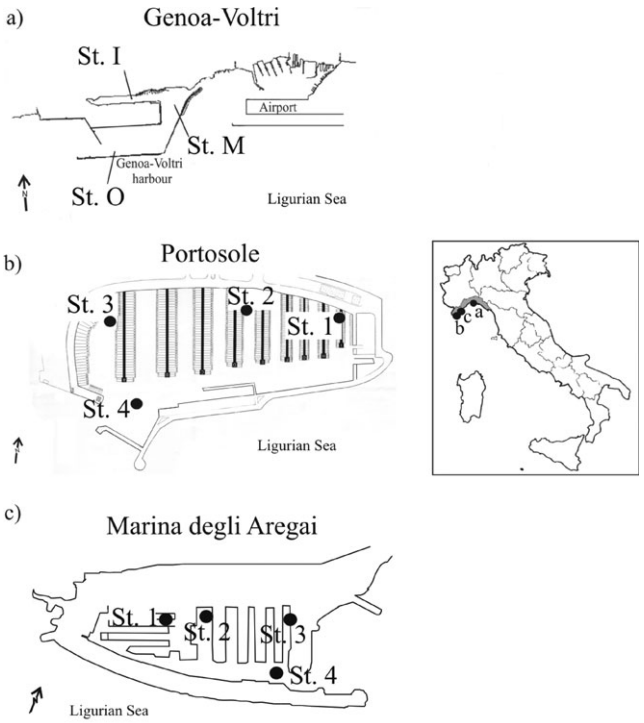

Figure 1. Layout of the harbours and stations sampled: (a) Genoa-Voltri; (b) Portosole; and (c) Marina degli Aregai.

Nematodes

Nematodes were analysed from the surface layers (0–2 cm) of the three replicate cores. The first 100 nematodes were picked out using a fine pin under a stereomicroscope (magnification ×40), transferred from formalin to glycerol through a series of ethanol–glycerol solutions, and mounted on slides in anhydrous glycerine, following the procedure described by Heip *et al.* (1985). Nematodes were identified at the genus level, using the pictorial keys of Platt and Warwick (1983, 1988), Warwick *et al.* (1998), and Steyaert *et al.* (2005). Diversity indices (H', J', and S) were calculated as for meiofauna. Nematode genera were also classified according to Wieser (1953) into four feeding groups to investigate the trophic structure of the community: selective (1A) and non-selective (1B) deposit-feeders, epistrate-feeders (2A), and predators/omnivores (2B). The ITD was calculated according to Heip *et al.* (1985), ranging from 0.25 (highest trophic diversity) to 1.0 (lowest trophic diversity). The MI uses the classification of nematode genera into "colonizers" (c; organisms with a high tolerance to disturbance events) and "persistents" (p; organisms with little colonizing capability and low tolerance of disturbance events). Based on their specific characteristics, all nematode genera have been distributed on a c–p scale ranging from 1 (for extreme colonizers) to 5 (for extreme persistent genera). MI was calculated as the weighted average of the c–p values of the individual genera according to Bongers *et al.* (1991).

Statistical analyses

Analysis of variance (ANOVA) was used to investigate whether or not the differences in abundance of meiofauna and nematodes among sampling stations and sampling times were significant. Before the analysis, normality of data was checked and, when necessary, data were transformed appropriately. The homogeneity of variance was assessed by Cochran's test. Pearson's correlation analysis was carried out to test correlation among various indices and contaminant concentration.

Results

Meiofauna

Total meiofaunal density in Genoa-Voltri harbour revealed significant differences between stations, with higher average densities at Station I (1403 ± 658 ind. 10 cm^{-2}) than at Stations O and M (641 ± 410 and 290 ± 214 ind. 10 cm^{-2}, respectively; ANOVA, $p < 0.05$). Assemblage structure was similar across stations, with a clear dominance of nematodes (on average 52%; Table 2). S, H', and J' were lower at Station M than at the other stations, although the Ne–Co and Ne2A–Co ratios were higher (Table 3).

The Portosole marina revealed high densities of meiofauna, ranging from 1429 ± 83 (Station 1) to 2423 ± 146 ind. 10 cm^{-2}

Table 1. Average concentration of sediment contaminants and total protein content over all sampling periods and sampling stations (from Salvo *et al.*, 2005; Fabiano *et al.*, 2006; Moreno *et al.*, 2006, 2008).

Harbour	Station (ppb)	PAHs (ppm)	Cr (ppm)	Ni (ppm)	As (ppm	Cd (ppm)	Pb (ppm)	Zn (ppm)	Cu (ppm)	Total heavy metals (ppm)	Total protein (mg g^{-1})
Genoa-Voltri	I	11 468	–	–	–	–	–	–	–	–	2.1
	M	14 661	–	–	–	–	–	–	–	–	8.5
	O	6 083	–	–	–	–	–	–	–	–	1.8
Portosole	St. 1	181	18.6	17.0	10.7	0.20	46.6	183	208	484	3.0
	St. 2	78	15.7	11.7	6.1	0.01	27.5	110	74	245	1.5
	St. 3	145	11.4	9.3	5.0	0.01	20.6	90	67.	203	0.8
	St. 4	215	23.0	20.0	7.8	0.12	35.6	136	65	287	2.6
Marina degli Aregai	St. 1	87	18.3	20.8	8.6	0.11	13.7	84	64	208	2.0
	St. 2	67	12.3	12.7	6.3	0.01	9.8	53	23	117	1.0
	St. 3	96	20.6	20.5	19.4	0.15	18.6	146	118	343	1.5
	St. 4	135	20.3	18.2	9.8	0.19	18.4	124	69	260	2.3

Coefficient of variation <30% for all data.

Table 2. Average densities (ind. 10 cm^{-2}) of meiofaunal taxa identified over all sampling periods by harbour and sampling station.

Taxon	Genoa-Voltri			Portosole				Marina degli Aregai			
	I	O	M	St. 1	St. 2	St. 3	St. 4	St. 1	St. 2	St. 3	St. 4
Nematodes	423.6	346.4	168.7	860.3	1 602.6	1 452.0	1 519.3	771.8	1 265.1	702.6	212.6
Copepods	305.9	99.8	49.1	174.9	286.4	114.7	110.3	175.6	163.2	73.8	67.7
Nauplii	346.4	111.9	39.4	362.7	398.0	133.1	63.0	200.8	376.0	77.0	55.1
Polychaetes	150.3	46.4	6.5	2.7	64.7	43.3	29.1	9.6	7.4	9.2	10.2
Bivalves	2.9	2.2	3.1	0.0	0.9	0.0	2.8	0.0	0.0	0.0	0.9
Ostracods	14.2	0.3	4.6	0.7	1.9	0.0	3.0	10.9	1.2	0.0	4.0
Kinorhynchs	3.2	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0
Turbellarians	68.0	13.8	12.3	15.8	55.8	45.1	21.6	10.8	9.4	8.2	20.5
Oligochaetes	12.3	0.1	2.2	7.4	2.3	7.5	3.5	9.2	21.2	12.9	3.3
Gastrotrichs	75.3	6.5	0.9	1.2	6.5	64.9	0.0	0.0	0.0	0.0	0.0
Tanaids	0.0	2.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.5	0.0
Mites	0.6	0.0	2.7	3.3	3.0	0.6	1.4	1.6	0.0	0.9	0.0
Others	1.0	2.3	0.1	0.0	0.5	7.2	0.0	0.6	0.0	3.5	0.0

Table 3. Average values of meiofauna taxon-based indicators over all sampling periods by harbour and sampling station.

Harbour	Stations	H'	J'	S	Ne:Co	Ne2A:Co
Genoa-Voltri	I	1.48	0.64	10	1.9	0.8
	M	0.89	0.51	7	6.5	2.2
	O	1.23	0.54	10	2.0	1.9
Portosole	St. 1	0.99	0.54	7	26.7	11.1
	St. 2	1.02	0.45	10	7.9	4.8
	St. 3	0.82	0.41	8	12.8	7.3
	St. 4	0.57	0.27	8	14.9	10.9
Marina degli Aregai	St. 1	0.96	0.46	8	6.2	1.5
	St. 2	0.85	0.46	7	11.0	2.0
	St. 3	0.83	0.37	10	11.5	2.0
	St. 4	1.23	0.61	8	3.6	1.0

Coefficient of variation <10% for all data. S, species richness.

(Station 2), with no significant differences detected between stations (ANOVA). The meiofauna were dominated by nematodes (on average, 73%; Table 2). H' and J' indices were lower at the outer Station 4, although S was lower at the inner Station 1 (Table 3). The Ne–Co and Ne2A–Co ratios were higher at Stations 1 and 4.

Meiofaunal densities of Marina degli Aregai were highest at Station 2 in January (3288 ± 2185 ind. 10 cm^{-2}) and lowest at Station 3 in July (338 ± 6 ind. 10 cm^{-2}; ANOVA, $p < 0.05$). The taxa composition of the community is given in Table 2. H' and J' indices revealed lower values at Station 3 and higher values at Station 4. In contrast, the higher S was found at Station 3. The Ne–Co and Ne2A–Co ratios revealed higher values at Station 3 and the lowest values at Station 4 (Table 3).

Clearly, there were no consistent trends in the various indicator values along the inner–outer gradient in any of the harbours. However, the three biodiversity indices (H', J', and S) were negatively correlated with the concentrations of total PAHs ($p < 0.05$), and S was positively correlated with the redox potential (Eh) values at the water–sediment interface, whereas all correlations with heavy metals were not significant (see Table 6 later). In contrast, the Ne–Co and Ne2A–Co ratios revealed a significant positive correlation with total heavy-metal concentrations, with

Table 4. Average percentage distribution of nematode genera identified over all sampling periods by harbour and sampling station.

Family	Genus	c–p	Trophic group	Genoa-Voltri			Portosole				Marina degli Aregai			
				I	O	M	St. 1	St. 2	St. 3	St. 4	St. 1	St. 2	St. 3	St. 4
Anoplostomatidae	*Anoplostoma* spp.	2	1B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	1.1	1.4	0.4
Anticomidae	*Anticoma* spp.	2	1A	0.3	8.8	1.0	0.5	2.0	0.2	0.3	7.3	1.9	0.8	1.5
Axonolaimidae	*Axonolaimus* spp.	2	1B	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0
	Parodontophora spp.	2	1B	12.3	0.0	0.0	0.0	2.5	0.0	5.2	0.8	0.0	9.4	0.0
	Odontophora spp.	2	1B	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	2.7	2.4	0.9
Ceramonematidae	*Pselionema* spp.	3	1A	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chromadoridae	*Prochromadorella* spp.	2	2A	6.0	5.3	3.7	3.1	2.5	15.4	2.3	6.3	3.6	2.8	11.4
	Chromadorina spp.	3	2A	0.3	0.3	0.3	0.0	3.5	0.0	0.0	0.5	1.3	0.4	0.0
	Chromadorita spp.	3	2A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.6	0.7	0.7
	Chromadora spp.	2	2A	0.0	0.0	0.0	0.9	1.0	0.0	0.0	9.5	0.0	0.0	0.5
	Chromadorella spp.	3	2A	0.0	0.0	0.0	1.9	0.3	0.0	0.0	3.3	1.8	0.0	2.5
	Dichromadora spp.	2	2A	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.0	0.3	1.0	0.7
	Metachromadora spp.	2	2A	0.0	0.0	0.0	0.0	0.5	3.6	5.3	0.0	0.0	0.0	0.0
	Neochromadora spp.	2	2A	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Ptycholaimellus spp.	3	2A	0.0	1.2	9.8	18.2	0.3	1.3	2.2	0.3	0.3	1.0	0.0
	Graphonema spp.	3	2A	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Comesomatidae	*Sabatieria* spp.	2	1B	0.6	9.8	5.9	0.0	2.3	0.3	7.8	2.0	20.6	4.7	1.3
	Paracomesoma spp.	2	2A	36.3	0.5	13.8	10.8	4.9	13.5	7.5	0.0	0.0	0.0	0.0
	Comesoma spp.	2	1B	0.0	0.0	0.6	0.0	1.5	0.0	0.3	0.0	0.0	0.0	0.0
	Metacomesoma spp.	2	1B	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Setosabatieria spp.	2	1B	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0	4.7	0.0
	Laimella spp.	2	2A	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyatholaimidae	*Marylynnia* spp.	3	2A	0.2	7.9	0.6	0.7	0.8	0.2	0.0	3.7	6.1	2.9	2.4
	Paracanthonchus spp.	2	2A	0.0	0.0	0.2	0.2	0.6	4.5	0.0	0.3	0.3	0.3	0.8
	Longicyatholaimus spp.	2	2A	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
	Paralongicyatholaimus spp.	3	2A	0.0	0.0	0.0	1.5	45.0	21.0	55.9	0.0	0.0	0.0	0.0
	Paracyatholaimus spp.	2	2A	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.8	0.0	0.0	0.0
	Pomponema spp.	4	2B	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Desmodoridae	*Desmodora* spp.	2	2A	2.7	15.3	4.5	0.7	4.3	0.2	0.0	0.5	1.3	1.5	2.1
	Molgolaimus spp.	3	1A	0.0	0.3	0.3	0.0	0.0	0.0	0.0	3.4	4.1	3.3	7.2
	Polysigma spp.	2	2A	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Spirinia spp.	3	2A	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.7
Desmoscolecidae	*Quadricoma* spp.	4	1A	2.8	1.6	0.1	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
	Tricoma spp.	4	1A	0.7	0.9	0.2	0.0	0.0	0.0	0.6	0.3	0.3	0.5	0.4
	Desmoscolex spp.	4	1A	0.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diplopeltidae	*Campylaimus* spp.	3	1B	3.0	0.0	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.0	0.0
Draconematidae	*Draconema* spp.	4	1A	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enchelidiidae	*Bathyeurystomina* spp.	4	2B	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Calyptronema spp.	4	2B	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
	Eurystomina spp.	4	2B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
Ethmolaimidae	*Neotonchus* spp.	2	2A	0.3	6.4	0.0	0.0	0.0	0.0	1.1	0.5	2.2	5.0	4.1
	Comesa spp.	3	1B	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
	Filitonchus spp.	3	1A	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.9	2.2	0.8	0.0
	Nannolaimus spp.	3	2A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	1.1
Epsilonematidae	*Epsilonema* spp.	4	1A	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leptolaimidae	*Leptolaimus* spp.	2	1A	0.3	3.0	0.2	0.0	0.0	1.5	0.5	0.0	0.0	0.0	0.0
Linhomoeidae	*Terschellingia* spp.	3	1A	24.3	7.7	15.8	17.8	14.7	4.0	0.8	5.5	5.5	6.4	4.4
	Metalinhomoeus spp.	2	1B	0.5	4.5	1.6	0.0	0.2	1.0	1.7	2.6	3.8	8.8	1.9
	Paralinhomoeus spp.	2	1B	0.0	0.9	3.0	0.0	0.0	1.0	0.0	1.3	1.1	1.1	8.8

Continued

Table 4. *Continued*

Family	Genus	c–p	Trophic group	Genoa-Voltri			Portosole				Marina degli Aregai			
				I	O	M	St. 1	St. 2	St. 3	St. 4	St. 1	St. 2	St. 3	St. 4
	Eleutherolaimus cf. *r*	2	1B	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microlaimidae	*Microlaimus* spp.	2	2A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.5	0.0
Oncholaimidae	Oncholaimidae	4	2B	0.0	0.6	3.7	0.0	0.3	0.4	0.0	0.3	0.0	0.0	0.0
	Viscosia spp.	3	2B	1.4	0.7	2.3	6.2	1.5	0.7	1.1	2.1	0.0	0.5	1.1
	Metoncholaimus spp.	4	2B	0.0	0.0	10.0	7.3	0.0	0.0	0.0	1.8	0.0	0.0	0.4
	Oncholaimus spp.	4	2B	0.3	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxystominidae	*Halalaimus* spp.	4	1A	0.2	7.3	0.3	0.0	0.2	0.2	0.3	0.3	0.0	0.0	0.0
	Oxistomina spp.	4	1A	0.9	0.0	0.1	0.0	0.0	0.0	0.6	0.3	0.0	0.0	1.1
	Thalassolaimus spp.	4	1A	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Selachinematidae	*Richtersia* spp.	3	1B	0.2	1.3	0.1	0.0	0.0	0.5	1.1	0.5	0.0	0.3	0.4
	Halichoanolaimus spp.	3	2B	0.5	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Siphonolaimidae	*Siphonolaimus* spp.	3	2B	0.0	0.0	0.0	0.0	0.2	0.0	0.3	0.0	0.0	0.0	0.0
Sphaerolaimidae	*Sphaerolaimus* spp.	3	2B	0.0	0.0	0.0	0.9	0.0	0.9	0.0	0.0	0.0	0.5	0.0
Thoracostomopsidae	Thoracostomopsidae	2	2B	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
	Mesacanthion spp.	3	2B	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.4
	Halanonchus spp.	4	1B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Tripyloididae	*Tripyloides* spp.	2	1B	0.0	0.0	0.0	0.3	0.0	7.3	0.3	0.0	0.3	0.8	0.0
Tylenchidae	Tylenchidae	2	F	0.0	0.0	0.0	0.0	0.7	0.2	0.0	0.0	0.0	0.0	0.0
Xyalidae	*Daptonema* spp.	2	1B	2.1	10.8	19.1	25.7	5.5	17.3	2.9	39.0	32.4	32.1	34.5
	Metadesmolaimus spp.	2	1B	0.0	0.3	0.0	1.8	0.3	0.0	0.0	0.8	0.0	0.0	0.7
	Stylotheristus spp.	2	1B	0.0	0.0	0.0	0.0	0.8	3.6	0.0	0.5	5.0	2.8	5.2
	Paramonhystera spp.	2	1B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	1.4	0.4
	Theristus spp.	2	1B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1

Pb specifically, and with PAHs. Although no significant correlation was found between biodiversity indices and protein concentrations for all pooled data, a negative correlation was found for H' and S in Genoa-Voltri harbour ($n = 12$; $p < 0.05$).

Nematodes

Nematodes represented the dominant group at all sampling stations, accounting on average for >60% of the total meiofaunal abundance in the three harbours (Table 2).

At Genoa-Voltri, 43 genera belonging to 21 families were identified (Table 4). The diversity indices (H', J', and S) were higher at Station O than at the other two stations (Table 5). MI values were similar across stations, with high percentages of nematodes with c–p values of 2 (60%; Table 4). Station I was characterized by a dominance of epistrate-feeders (2A: 46%) and selective-feeders (1A: 33%), Station M by a dominance of epistrate-feeders (2A: 33%), and non-selective-feeders (1B: 31%), whereas at Station O, the trophic groups were more evenly represented by epistrate-feeders (2A: 38%), selective-feeders (1A: 31%), and non-selective-feeders (1B: 28%). Despite these differences in food selection among the three stations, the ITD demonstrated similar values (Table 5).

The assemblage at Portosole marina was represented by 46 genera belonging to 21 families (Table 4). S was lowest at Station 1 and highest at Station 3, whereas Station 4 displayed lower values of H' and J'. MI values were similar at Stations 1, 2, and 4, and the minimum was observed at Station 3. In all, 60% of the community was characterized by a c–p value of 2 (Table 5). The dominant trophic group was formed by epistrate-feeders (2A: 59%), followed by non-selective deposit-feeders (1B: 24%; Table 4). The ITD was relatively low at Station 1 as a consequence of the high densities of predators found in July (2B: 27%; Tables 4 and 5).

Table 5. Average values of nematode genus-based indicators over all sampling periods by harbour and sampling station.

Harbour	Stations	H'	J'	S	MI	ITD
Genoa-Voltri	I	1.82	0.66	16	2.46	0.39
	M	2.25	0.77	17	2.62	0.33
	O	2.37	0.83	18	2.44	0.36
Portosole	St. 1	1.97	0.75	20	2.63	0.29
	St. 2	2.01	0.66	28	2.69	0.47
	St. 3	1.94	0.65	29	2.31	0.46
	St. 4	1.71	0.59	24	2.66	0.60
Marina degli Aregai	St. 1	2.24	0.71	24	2.28	0.36
	St. 2	2.04	0.71	18	2.24	0.52
	St. 3	2.33	0.78	21	2.19	0.53
	St. 4	2.24	0.74	21	2.26	0.41

Coefficient of variation <10% for all data. S, species richness.

The assemblage at Marina degli Aregai was characterized by 44 genera belonging to 19 families (Table 4). Station 2 exhibited relatively low values of S, H', and J', whereas the highest value of S was recorded at Station 1, and higher values of H' and J' were found at Station 3 (Table 5). MI values were consistently lower than at the other harbours at all stations. The dominant trophic group was represented by the non-selective deposit-feeders (1B: 61%),

Table 6. Correlation coefficients (*$p < 0.05$) between meiofaunal indicators and environmental parameters for ($n = 16$ for heavy metals; $n = 28$ for others).

Environmental variables	Meiofauna taxon-based indices					Nematode genus-based indices				
	H'	S	J'	Ne:Co	Ne2A:Co	H'	S	J'	MI	ITD
Eh	0.20	**0.37***	0.13	0.09	−0.07	0.00	0.05	−0.07	−0.30	0.10
Σ PAHs	**−0.53***	**−0.40***	**−0.41***	**0.48***	**0.47***	**−0.37***	**−0.37***	**−0.37***	0.35	**−0.37***
Cr	−0.12	0.15	−0.23	0.41	0.21	−0.24	−0.17	−0.11	0.14	−0.08
Ni	−0.04	0.14	−0.12	0.30	0.08	−0.13	−0.10	0.02	−0.12	−0.13
As	0.02	0.22	−0.06	0.35	0.06	0.16	0.10	0.43	−0.26	−0.08
Cd	0.00	−0.11	0.00	0.18	0.11	−0.23	−0.25	0.07	−0.04	−0.03
Pb	−0.17	−0.17	−0.14	**0.57***	**0.74***	**−0.52***	−0.42	−0.39	**0.69***	−0.16
Zn	−0.09	−0.06	−0.10	0.35	0.42	−0.41	−0.34	−0.16	0.22	−0.20
Cu	0.16	−0.09	0.16	0.15	0.39	−0.35	−0.36	0.03	0.26	**−0.43***
Σ heavy metals	0.01	−0.06	0.00	**0.50***	**0.49***	−0.39	−0.36	−0.08	0.26	−0.31
Total protein	0.05	−0.24	−0.15	−0.30	−0.20	0.11	−0.04	0.16	0.34	−0.29

S, species richness.

followed by the epistrate-feeders (2A: 23%). ITD was lowest at Station 1 (Table 5).

The H', J', and S indices revealed a significant negative correlation with PAHs, and also H' with heavy metals (Table 6). MI was positively correlated with Pb concentrations, and the ITD index revealed negative correlations with PAHs and Cu concentrations (Table 6).

Discussion

All three meiofaunal taxon-based diversity indicators revealed a significant correlation only with the concentration of PAHs, whereas the two nematode–copepod ratios revealed a correlation with PAHs and with total heavy-metal concentrations, specifically Pb. Although the use of the latter indices in relation to the environmental quality assessment appears to be controversial (Coull *et al.*, 1981; Warwick, 1981; Lambshead, 1984; Shiells and Anderson, 1985), our results suggest that the Ne–Co ratio is potentially useful as an indicator of pollution in harbours. This might be explained by the low variability within and among the harbours studied in the environmental parameters that affect the Ne–Co ratio in coastal marine systems, such as water depth and sediment grain size (Warwick, 1981; Gee *et al.*, 1985), and which therefore did not represent major confounding factors. Also, organic-matter concentrations of protein were relatively high in sediments at all stations (average = 2.5 mg g^{-1}; Table 1), suggesting that food resources did not limit meiofaunal abundance and distribution. The lack of large spatio-temporal changes in food supply might explain the absence of significant correlations between any of the indicators and the concentrations of total protein, which represent the fraction of organic matter available for these benthic consumers (Vezzulli and Fabiano, 2006). Only in Genoa-Voltri harbour has a negative correlation between meiofaunal indices and protein content been found, which might be explained by the high levels of organic enrichment affecting oxygen concentrations in the sediment (Moreno *et al.*, 2008).

The nematode genus-based diversity indicators vs. concentration of PAHs exhibited correlation coefficients that were comparable with those for meiofauna taxon-based indicators (Table 6). Therefore, the application of biodiversity indices based on higher taxonomic resolution does not seem to yield higher discriminatory power in evaluating environmental quality. The correlations with environmental contaminants observed for MI and ITD are difficult to interpret, because their sign is opposite to expectations (Heip *et al.*, 1985; Bongers *et al.*, 1991).

The presence of sensitive or tolerant meiofaunal taxa and nematode genera appears to be particularly informative in highlighting the state of sediment pollution and allows a better assessment of the spatial heterogeneity of environmental disturbance within each harbour. Generally, in heavily polluted sediments characterized by a low redox potential, the total meiofauna abundance was lower, and kinorhynchs and tanaids were absent (Table 2), whereas the nematode assemblage was dominated by the genera *Terschellingia* spp., *Sabatieria* spp. (*pulchra* group), *Paracomesoma* spp., and *Daptonema* spp. (Table 4). These nematode genera are typically found in organically rich, muddy sediment (Heip, *et al.*, 1990; Schratzberger *et al.*, 2006) and have been proposed to be representative of a community that is well adapted to disturbed conditions (Vanreusel, 1990). In particular, the genus *Sabatieria* survives low oxygen and high sulphide concentrations and often persists under conditions that are unsuitable for most other nematode species (Tietjen, 1980; Hendelberg and Jensen, 1993; Steyaert *et al.*, 1999). In contrast, in harbour sediments characterized by lower levels of environmental pollution, meiofaunal abundance was higher, kinorhynchs and tanaids were present (Table 2), and nematodes were dominated by other genera, such as *Desmodora* spp. and *Anticoma* spp. (Table 4).

At a local scale, meiofaunal indicators revealed the least correlation with the concentration of contaminants in the least-polluted Marina degli Aregai, probably because of the even spread of the pollution pressure over the four sampling stations at this site. In contrast, most meiofauna and nematode diversity indices, as well as the nematode–copepod ratios at the Portosole marina, performed well in assessing the marked heterogeneity of pollution pressure within the harbour, identifying Stations 1 and 4 as the most polluted (Marin *et al.*, 2008).

We conclude that the application of meiofaunal and nematode indices can be a useful tool for assessing the pollution pressure in harbour ecosystems, as long as there are no confounding factors, such as differences in water depth, grains size, and food sources that critically affect abundance and distribution of these creatures.

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