Individual and combined effects of cadmium and diesel on a nematode community in a laboratory microcosm experiment

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Abstract

A microcosm experiment was carried out to study the influence of cadmium and diesel, individually and in a mixture, on a free living nematode community of a Tunisian lagoon. Sediments were contaminated with cadmium that ranged in concentration from 0.54 to 1.40 mg Cd kg⁻¹ (dry weight (dw)), by diesel at 0.25 mg kg⁻¹ (dw), by a cadmium–diesel mixture (Cd at 1.40 mg kg⁻¹ + Diesel at 0.25 mg kg⁻¹) and effects were examined after 90 days. Univariate analysis showed that all univariate indices did not change significantly neither at all the levels of cadmium contamination nor at 0.25 mg kg⁻¹ (dw) diesel concentration. But, at the cadmium–diesel mixture, significant differences were recorded between nematode assemblages from uncontaminated sediment control microcosm and those from cadmium–diesel mixture amended sediment treatments. Total nematode abundance (I), mean individual weight (bi), Shannon–Weaver index H', species richness (d), evenness (J') and number of species (S) decreased significantly in microcosms contaminated with both cadmium and diesel. Results from multivariate analyses of the species abundance data demonstrated that responses of nematode species to the cadmium–diesel treatments were varied: Marylynnia stekhoveni, Calomicrolaimus honestus and Oncholaimellus mediterraneus were significantly affected at the cadmium–diesel contamination but they were not eliminated. These species could be categorized as “cadmium–diesel sensitive”. Enoploides sp. and Oncholaimus campylocercoides, characterized by increased abundances in cadmium–diesel amended sediment, seemed to be “cadmium–diesel resistant” species. All these species, “cadmium–diesel sensitive” or “cadmium–diesel resistant”, were not affected by either cadmium or diesel alone.

Keywords: Nematodes; Microcosm; Cadmium; Diesel; Contaminant mixture; Sediment; Community structure

1. Introduction

The most abundant contaminants in aquatic ecosystems are heavy metals (copper (Cu), cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn)) (Hagopian-Schlekat et al., 2001). Among trace metals cadmium has been classified as a group I carcinogen by the International Agency for Research in cancer (IARC) and a probable human carcinogen (group BI) by Environmental Protection Agency (EPA) (Merrill et al., 2001).

Even though pollution and toxicity of substances in living organisms or environments cause ecological problems, and therefore toxicological studies should be community based (Gyedu-Ababio and Baird, 2006). Most studies on cadmium toxicity so far concentrated on cells (Boscolo et al., 1985; Fotakis et al., 2005) or individual species (George and Pirie, 1979; Moraïtou-Apostolopoulou and Verriopoulos, 1982; Etxeberria et al., 1994). Several approaches were already applied for the investigation of the potential risks of trace metals on the sediment meiofauna (Warwick et al., 1988; Sundelin and Elmgren, 1991; Austen et al., 1994; Austen and McEvoy, 1997; Austen and Somerfield, 1997; Gyedu-Ababio and Baird, 2006) with different results. For example, cadmium seems to have no significant impacts on estuarine meiobenthic nematode communities even at high dose (Austen et al., 1994) and copper and zinc low doses appear to have much more drastic effects on offshore meiobenthic communities than the high doses (Austen and McEvoy 1997). Metal...
contamination significantly alters the composition of meiofaunal assemblages (Burton et al., 2001; Mahmoudi et al., 2002; Gyedu-Ababio and Baird, 2006). Although aquatic sediments often contain complex mixtures of contaminants, including metals and hydrocarbons (Daskalakis and O’Conner, 1995; Kennicutt et al., 1996), the influence of contaminant mixtures on natural communities is poorly understood (Breitburg et al., 1999). Little is known about the collective action of complex contaminant mixtures because relatively few toxicity studies have been conducted (Steevens and Benson, 2001; Millward et al., 2004; Gyedu-Ababio and Baird, 2006).

The Mediterranean marine sediments are well-recognized to be contaminated with petroleum hydrocarbons (Sellali et al., 1992; Aboul-Kassim and Simoneit, 1996; Lipiatou et al., 1997; Dachs et al., 1999; Louati et al., 2001) and by heavy metals (Bernhard 1978; Donazzolo, 1984; Martinec et al., 1989; Palanques and Diaz, 1994; Rouibah, 2001; Mulso et al., 2001; Yoshida et al., 2004) but the influence of these pollutant mixtures on Mediterranean natural communities is poorly understood. Little is known about the collective action of hydrocarbons and heavy metals on Mediterranean benthic organism assemblages because no in vivo toxicity studies have been conducted, and field studies (Beyrem and Aïssa, 2000; Mahmoudi et al., 2001, 2002, 2003a) of these contaminants action have been, by necessity, restricted to correlative relationships between pollutant concentrations and community composition.

Among benthic organisms, nematode communities are well suited to microcosm experiments. They have short generation time, high density and continuous reproduction (Suderman and Thistle, 2003). These small animals are also easily maintained and sensitive to many toxicants (Coull and Chandler, 1992; Long, 1992; Guo et al., 2001). In the present study, we present the results of a microcosm experiment designed to assess the response of a meiobenthic nematode community in term of densities, mean individual weight, diversity and species composition to cadmium and diesel used separately and in a mixture.

2. Methods

2.1. Collecting site

Natural meiobenthic communities were collected from Ghar El Melh lagoon (Tunisia). Handcores of 10 cm^2 were used to a depth of 15 cm to transfer sediment into a bucket. The Ghar El Melh ecosystem was studied previously to determine the environmental variables controlling the nematofauna abundance, diversity and species composition (Mahmoudi et al., 2003b). At the prospected site (37°09.10’ N 10°13.01’ E), depth was 1.30 m and salinity was 41 PSU. The sediment had a median particle diameter of 39 μm. The organic carbon content of sediment in this area was about 1.32%, and with 40% silt and 21% clay.

On return to the laboratory, sediments were homogenized by gentle hand stirring with a large spatula before they were used for cadmium and/or diesel sediment contamination or microcosms filling.

2.2. Cadmium and diesel contamination of sediments

Sediment used for contamination was first alternately frozen and thawed three times to defaunate it (Austen et al., 1994), and then it was wet sieved to remove the larger particles (>63 μm). Next, stock cadmium chloride solutions were made in distilled water and quantities of 100 g (dry weight, dw) of sediment were contaminated with appropriate doses of cadmium in order to obtain final concentrations of 0.54 mg Cd kg\(^{-1}\) (dw) and 1.40 mg Cd kg\(^{-1}\) (dw) after being mixed with 200 g of natural (uncontaminated) sediment. Similarly, for diesel-contaminated sediment preparation, quantities of 100 g (dry weight, dw) of defaunate sediment were contaminated by appropriate doses of diesel in order to obtain final concentrations of 0.25 mg diesel kg\(^{-1}\) (dw). Sediments contaminated with both cadmium at 1.40 mg Cd kg\(^{-1}\) (dw) and diesel at 0.25 mg kg\(^{-1}\) (dw) concentrations were prepared by adding the metal (as described above) to diesel-contaminated sediment. For cadmium contamination, the doses used were similar to those employed by Austen et al. (1994) so that the results should be directly comparable. For diesel enrichment of sediment, the concentration used (0.25 mg kg\(^{-1}\) (dw)) was chosen based on previous observations that higher diesel doses (>0.25 mg kg\(^{-1}\)) altered free living nematodes community structure (Mahmoudi et al., 2005). Cadmium and/or diesel were mixed into the sediment with a food mixer and the amended sediment was left to equilibrate for 1 week at 5 °C before microcosms were assembled.

2.3. Experimental set-up

Microcosms consisted of 570 ml glass bottles. One control and five treatments with four replicates each were set up. Treated microcosms were gently filled with 300 g of homogenized sediment (200 g of natural sediment and 100 g contaminated sediment) topped up with filtered (1 μm) natural lagoon water at 41 PSU. Control consisted of uncontaminated and defaunate sediment. The treatments consisted of three levels of cadmium (0.54 mg Cd kg\(^{-1}\) (dw), 0.90 mg Cd kg\(^{-1}\) (dw) and 1.40 mg Cd kg\(^{-1}\) (dw)), one of diesel (0.25 mg kg\(^{-1}\)) and a mixture of cadmium (1.4 mg Cd kg\(^{-1}\)) and diesel (0.25 mg kg\(^{-1}\)).

Each microcosm bottle was stoppered with a rubber bung with two holes and aerated via an air stone diffuser. All experiments were ended after 90 days and the sediments fixed in 4% formaldehyde.

2.4. Sample processing

Meiofaunal taxa, defined here as metazoa that pass through a 1 mm mesh sieve and are retained on a 40 μm sieve (Vitiello and Dinet, 1979), were sieved following the resuspension-decantation methodology (Wieser, 1960) and stained with Rose-Bengal (0.2 g l\(^{-1}\)). All nematodes were counted under a stereo dissecting microscope, and preserved for later biomass determination. The body volume of nematodes was derived from measurements of body length and width using the Andrassy formula \(V = LW^2/16 \times 10^{3}\) (Andrassy, 1956), where \(V\) is the volume in nanolitres, \(L\) the length and \(W\) the maximum width (\(L\) and \(W\) expressed in micrometers). The wet weight of each nematode was obtained from multiplying volume by specific gravity (assumed to be 1.13, Wieser, 1960). Dry weight was assumed to be 25% of the wet weight (Juarió, 1975). Nematodes were identified to genus or species using the pictorial keys of Platt and Warwick (1983, 1988), and Warwick et al. (1998).

2.5. Data processing

The majority of data analysis followed standard community analysis methods described by Clarke (1993) and Clarke and Warwick (2001) using the PRIMER (plymouth routines in Multivariate Ecological Research) software package. Univariate indices were computed: total nematode abundance \(N\), mean individual weight \(m\), number of species \(S\), diversity (Shannon-Weaver index \(H\)), species richness (Margalef’s \(d\) and...
evenness (Pielou’s $J$) were calculated for each microcosm. The one-way ANOVA was used to test for overall differences between these indices and the Tukey HSD multiple comparisons test was used in pairwise comparisons of treatments and control. In all the above statistical significance testing a significant difference was assumed when $p<0.05$.

Species abundance data were presented in $k$-dominance plots, in which species were ranked in decreasing order of dominance. The percentage cumulative abundance ($k$-dominance) was then plotted against the species rank $k$ (Lambshead et al., 1983).

Multivariate data analysis was by non-parametric multi-dimensional scaling (MDS) ordination with the Bray–Curtis similarity measure performed on square-root transformed species abundance data to determine whether the nematode assemblages responded to the contaminations by changes in the relative abundance of species. Pairwise analysis of similarities (ANOSIM) was carried out to determine if there were any significant differences between nematode assemblages in different treatments. SIMPER (similarity percentages) was used to determine the contribution of individual species towards dissimilarity between treatments and control.

Throughout the text, the shorthand codes given in Table 1 are used to identify microcosms.

### 3. Results

#### 3.1. Univariate indices

The graphical summary of univariate indices for nematode assemblages from each microcosm (Fig. 1) illustrates clear treatment effects only in the microcosm treated with a cadmium–diesel mixture.

The results of significance testing using the one-way ANOVA for overall differences between univariate indices indicate that only cadmium–diesel contamination resulted in significant changes of univariate community attributes. Results from multiple comparisons tests show that all univariate indices did not change significantly neither at all the levels of cadmium contamination nor at 0.25 mg kg$^{-1}$ (dry) diesel concentration. But at the cadmium–diesel mixture significant differences were recorded between nematode assemblages from uncontaminated sediment control microcosm and those from cadmium–diesel amended sediment treatments ($p<0.05$). Total nematode abundance ($I$), mean individual weight ($bi$), Shannon–Weaver index $H'$, species richness ($d$), evenness ($J$) and number of species ($S$) decreased significantly in microcosms contaminated with the cadmium–diesel mixture.

#### 3.2. Distributional plots

The $k$-dominance plots (Fig. 2) graphically illustrate a clear effect of the cadmium–diesel mixture (Cd (H)+Dies 0.25) on nematode community. An increase of dominance and a decrease of diversity was obvious at this metal–hydrocarbon contamination. All the other amended sediment microcosms (Cd (L), Cd (M), Cd (H), Dies 0.25) had similar dominance and diversity to the controls (C).

#### 3.3. Multivariate indices

MDS results (Fig. 3) indicate that only the cadmium–diesel contamination affects the nematode assemblages. The replicates of all treatments except those of the cadmium–diesel mixture are grouped with the controls.

ANOSIM results show that only nematodes assemblages from microcosms treated with the cadmium–diesel mixture are significantly impacted. Only these treatments are significantly different from controls and from all the others treatments (Table 2).

SIMPER results reveal that the average dissimilarity between microcosms is very high in the samples treated with the cadmium–diesel mixture when compared to controls or to all the others treatments (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncontaminated control</td>
<td>C</td>
</tr>
<tr>
<td>Sediment with a final cadmium concentration of 0.54 mg kg$^{-1}$ (dry)</td>
<td>Cd (L)</td>
</tr>
<tr>
<td>Sediment with a final cadmium concentration of 0.90 mg kg$^{-1}$ (dry)</td>
<td>Cd (M)</td>
</tr>
<tr>
<td>Sediment with a final cadmium concentration of 1.40 mg kg$^{-1}$ (dry)</td>
<td>Cd (H)</td>
</tr>
<tr>
<td>Sediment with a final diesel concentration of 0.25 mg kg$^{-1}$ (dry)</td>
<td>Dies 0.25</td>
</tr>
<tr>
<td>Sediment contaminated with a mixture of cadmium (1.40 mg kg$^{-1}$) and diesel (0.25 mg kg$^{-1}$ (dry))</td>
<td>Cd (H)+Dies 0.25</td>
</tr>
</tbody>
</table>

Fig. 1. Graphical summary of univariate indices for nematode assemblages from each microcosm ($I$ = abundance, $bi$ = mean individual weight, $H'$ = Shannon–Weaver index, $d$ = species richness, $J$ = evenness, $S$ = number of species.)
The control microcosm (C) was mainly dominated by *Oncholaimus campylocercoides* (13%), *Marylynnia stekhoveni* (12.61%), *Calomicrolaimus honestus* (12.44%), *Oncholaimellus mediterraneus* (12.37%), *Hypodontolaimus colesi* (9.65%), *Neochromadora trichophora* (5.05%), *Daptonema trabeculosum* (4.46%), *Odontophora wieseri* (3.74%), *Mesacanthion hirsutum* (3.02%) and *Paracomesoma dubium* (2.59%). In rank order of abundance there was a group of common species with similar abundances (2.29%) to each other: *Chromadorina metulata*, *Metalinhomoeus numidicus*, *Latronema* sp., and a less abundant species *Nannolaimus fucus* (1.57%). All the cadmium treatments (Cd (L), Cd (M) and Cd (H)) and the diesel treatments (Dies 0.25) were dominated by *Marylynnia stekhoveni*, *Oncholaimellus mediterraneus*, *Hypodontolaimus colesi* and *Oncholaimus campylocercoides*. The cadmium-diesel amended sediment microcosms were mainly dominated by *Oncholaimus campylocercoides* (19.56%), *Enoploides* sp. (12.38%), *Mesacanthion hirsutum* (8.88%) and *Bathylaimus australis* (5.65%). All these species were dominant (abundance >1%) in the control microcosm except *Enoploides* sp. and *Bathylaimus australis* which were present at very minor populations (abundance <1%).

Significant differences between the cadmium–diesel mixture microcosms and all the other replicates mainly resulted from changes in the abundances of the dominant species (Table 4). Decreasing abundances of *Marylynnia stekhoveni*, *Calomicrolaimus honestus* and *Oncholaimellus mediterraneus* and increasing number of *Enoploides* sp. and *Oncholaimus campylocercoides* were responsible for significant difference between the cadmium–diesel mixture treatments (Cd (H) + Dies 0.25) and all the other microcosms (C, Cd (L), Cd (M), Cd (H) and Dies 0.25).

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>R value</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>C–Cd (L)</td>
<td>0.125</td>
<td>0.657</td>
</tr>
<tr>
<td>C–Cd (M)</td>
<td>−0.156</td>
<td>0.886</td>
</tr>
<tr>
<td>C–Cd (H)</td>
<td>0.083</td>
<td>0.314</td>
</tr>
<tr>
<td>C–Dies 0.25</td>
<td>−0.198</td>
<td>0.971</td>
</tr>
<tr>
<td>C–Cd (H) + Dies 0.25</td>
<td>0.879*</td>
<td>0.029</td>
</tr>
<tr>
<td>Cd (L)–Cd (M)</td>
<td>0.010</td>
<td>0.371</td>
</tr>
<tr>
<td>Cd (L)–Cd (H)</td>
<td>0.135</td>
<td>0.200</td>
</tr>
<tr>
<td>Cd (L)–Dies 0.25</td>
<td>0.135</td>
<td>0.743</td>
</tr>
<tr>
<td>Cd (L)–Cd (H) + Dies 0.25</td>
<td>0.906*</td>
<td>0.029</td>
</tr>
<tr>
<td>Cd (M)–Cd (H)</td>
<td>−0.063</td>
<td>0.629</td>
</tr>
<tr>
<td>Cd (M)–Dies 0.25</td>
<td>−0.198</td>
<td>0.857</td>
</tr>
<tr>
<td>Cd (M)–Cd (H) + Dies 0.25</td>
<td>0.938*</td>
<td>0.029</td>
</tr>
<tr>
<td>Cd (H)–Dies 0.25</td>
<td>−0.146</td>
<td>0.714</td>
</tr>
<tr>
<td>Cd (H)–Cd (H) + Dies 0.25</td>
<td>0.983*</td>
<td>0.029</td>
</tr>
<tr>
<td>Dies 0.25–Cd (H) + Dies 0.25</td>
<td>0.893*</td>
<td>0.029</td>
</tr>
</tbody>
</table>

*Denotes significant differences when $p < 0.05$.

### Table 3

<table>
<thead>
<tr>
<th>Average dissimilarity %</th>
<th>C</th>
<th>Cd (L)</th>
<th>Cd (M)</th>
<th>Cd (H)</th>
<th>Dies 0.25</th>
<th>Cd (H) + Dies 0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>22.12</td>
<td>25.11</td>
<td>27.50</td>
<td>26.76</td>
<td>27.39</td>
<td></td>
</tr>
<tr>
<td>Cd (L)</td>
<td>25.11</td>
<td>27.50</td>
<td>28.18</td>
<td>25.05</td>
<td>25.05</td>
<td></td>
</tr>
<tr>
<td>Cd (M)</td>
<td>27.67</td>
<td>29.64</td>
<td>28.18</td>
<td>25.05</td>
<td>25.05</td>
<td></td>
</tr>
<tr>
<td>Dies 0.25</td>
<td>26.76</td>
<td>29.64</td>
<td>28.18</td>
<td>25.05</td>
<td>25.05</td>
<td></td>
</tr>
<tr>
<td>Cd (H) + Dies 0.25</td>
<td>59.12</td>
<td>61.96</td>
<td>62.84</td>
<td>64.72</td>
<td>60.49</td>
<td></td>
</tr>
</tbody>
</table>

The control microcosm (C) was mainly dominated by *Oncholaimus campylocercoides* (13%), *Marylynnia stekhoveni* (12.61%), *Calomicrolaimus honestus* (12.44%), *Oncholaimellus mediterraneus* (12.37%), *Hypodontolaimus colesi* (9.65%), *Neochromadora trichophora* (5.05%), *Daptonema trabeculosum* (4.46%), *Odontophora wieseri* (3.74%), *Mesacanthion hirsutum* (3.02%) and *Paracomesoma dubium* (2.59%). In rank order of abundance there was a group of common species with similar abundances (2.29%) to each other: *Chromadorina metulata*, *Metalinhomoeus numidicus*, *Latronema* sp., and a less abundant species *Nannolaimus fucus* (1.57%). All the cadmium treatments (Cd (L), Cd (M) and Cd (H)) and the diesel treatments (Dies 0.25) were dominated by *Marylynnia stekhoveni*, *Oncholaimellus mediterraneus*, *Hypodontolaimus colesi* and *Oncholaimus campylocercoides*. The cadmium-diesel amended sediment microcosms were mainly dominated by *Oncholaimus campylocercoides* (19.56%), *Enoploides* sp. (12.38%), *Mesacanthion hirsutum* (8.88%) and *Bathylaimus australis* (5.65%). All these species were dominant (abundance >1%) in the control microcosm except *Enoploides* sp. and *Bathylaimus australis* which were present at very minor populations (abundance <1%).

Significant differences between the cadmium–diesel mixture microcosms and all the other replicates mainly resulted from changes in the abundances of the dominant species (Table 4). Decreasing abundances of *Marylynnia stekhoveni*, *Calomicrolaimus honestus* and *Oncholaimellus mediterraneus* and increasing number of *Enoploides* sp. and *Oncholaimus campylocercoides* were responsible for significant difference between the cadmium–diesel mixture treatments (Cd (H) + Dies 0.25) and all the other microcosms (C, Cd (L), Cd (M), Cd (H) and Dies 0.25).

### 4. Discussion

The Ghar El Melh lagoon nematofauna seemed to be resistant to all the cadmium doses tested, not impacted by diesel at 0.25 mg kg$^{-1}$ (dw) but very sensitive to cadmium–diesel mixture contamination.

Univariate analysis showed that all cadmium doses tested appeared to have no significant effects on the free living nematode community. *Austen et al.* (1994) had also reported that estuarine free living nematode communities were not sensitive to cadmium. The same result was
Table 4

<table>
<thead>
<tr>
<th>Species</th>
<th>Cd (H) + diesel 0.25</th>
<th>C</th>
<th>Cd (L)</th>
<th>Cd (M)</th>
<th>Cd (H)</th>
<th>Dies 0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marylynnia stekhoveni</td>
<td>0.25 (−)</td>
<td>12.61</td>
<td>16.85</td>
<td>11.97</td>
<td>12.47</td>
<td>11.97</td>
</tr>
<tr>
<td>Enoploides sp.</td>
<td>12.38 (+)</td>
<td>0.12</td>
<td>0.21</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Oncholaimus campylocercoides</td>
<td>19.56 (+)</td>
<td>13.00</td>
<td>9.31</td>
<td>9.94</td>
<td>10.18</td>
<td>11.18</td>
</tr>
<tr>
<td>Calomicrulaimus honestus</td>
<td>3.25 (−)</td>
<td>12.44</td>
<td>11.37</td>
<td>6.90</td>
<td>8.15</td>
<td>6.65</td>
</tr>
<tr>
<td>Oncholaimellus mediterraneus</td>
<td>4.11 (−)</td>
<td>12.37</td>
<td>12.62</td>
<td>15.19</td>
<td>12.94</td>
<td>12.90</td>
</tr>
</tbody>
</table>

(+: more abundant; −: less abundant. Species accounting for ~45% of overall dissimilarity between groups are ranked in order of importance of their contribution to this dissimilarity.)

observed by Austen and McEvoy (1997) for offshore meioiobenthic assemblages. However, Hagopian-Schlekat et al. (2001) demonstrated that cadmium is very toxic to the meioiobenthic copepod Amphiascus tenuiremis. Sundelin and Elmgren (1991) detected dose response relationships between meiofauna abundances and cadmium but in their experiments the low dose level corresponded to or was above our high dose levels. Fabiano et al. (1994) found negative correlations between bacterial biomass and cadmium at similar concentrations to the dose levels we used. Toxicants may have more direct impact on associated microbial communities in the sediment than the meioiobenthos. This may be reflected in meioiobenthic community structure because of alteration of food supply or altered decomposition processes (Austen and McEvoy, 1997). Many factors can contribute to these seemingly conflicting results. The nature of the sediment and seawater characteristics affect metal bioavailability (Depledge et al., 1994; Langston and Spence, 1994). Bioavailability and hence toxicity of contaminants depends on their partitioning between the sediment, pore water and overlying water (Austen and McEvoy, 1997) and this can also be dependant on sediment organic carbon content (Di Toro et al., 1991). At lower metal doses some metals will be adsorbed onto particulate organic carbon (POC) or the sediment and then may not be bioavailable. At higher doses the uptake sites for metal binding to POC and to the sediment itself will be saturated. Hence more metal will be found as free ions in the pore water and, because the microcosms are closed systems, in the overlying water (Austen and McEvoy, 1997). Furthermore, in anoxic/suboxic horizons, acidvolatile sulfides (AVS) are the most important binding phase for divalent metals (Di Toro et al., 1990). Thus, for infauna generally, total sediment metal concentrations are often not predictive of sediment toxicity (Hagopian-Schlekat et al., 2001).

The Ghar El Melh lagoon nematofauna did not appear to be particularly sensitive to diesel at 0.25 mg diesel kg⁻¹ (dw). This was not observed at higher concentrations for the nematofauna in the same ecosystem sediments (Mahmoudi et al., 2005) and is discussed in that paper.

Even though the Ghar El Melh lagoon nematofauna did not seem to be sensitive neither to diesel at 0.25 mg kg⁻¹ (dw) nor to cadmium even at high concentration (1.4 mg kg⁻¹ (dw)), the diesel–metal mixture treatment appeared to significantly influence the free living nematode assemblages. All nematode community univariate indices were affected in the cadmium–diesel contamination. This suggests that the assemblages in this treatment became in an organized structure different from those of the control and the cadmium amended sediment microcosms. The multivariate species-dependent MDS confirmed that the dominance relationship among species changed in the cadmium–diesel treatment compared to the control and to the cadmium contaminated microcosms.

Multivariate analysis of the data revealed significant differences between cadmium–diesel microcosms and control and all cadmium treated samples. This indicates that the free living nematode community was only affected by the cadmium–diesel mixture contamination.

In the MDS plot for the nematode assemblages, all cadmium–diesel replicates were clearly separated from all the other microcosms (Fig. 3) indicating a significant change in community composition in sediment contaminated with cadmium–diesel mixture.

The responses of the Ghar El Melh lagoon nematofauna to metal–diesel combination were distinct from the responses to either metals or diesel alone. Total nematode abundance was not significantly influenced by either cadmium or diesel alone, but decreased significantly in the metals–diesel treatment. This result is suggestive of a toxicological synergism between Cd and diesel, whereby these contaminants alone have no demonstrable effect, but in combination result in acute toxic stress. Diesel could stimulated production of mucus-exopolymers from algal blooms that are typical of diesel-contaminated sediments (Carman et al., 1997). Exopolymers are known to have a strong affinity for a variety of metals (Decho, 1990), and are readily consumed by benthic organisms (Decho and Lopez, 1993). Thus, complexation of metals with exopolymers increases exposure of animals to metals. In a microcosm study of the effects of metals and diesel mixture on a benthic community, Millward et al. (2004) analysed meiofauna assemblages in microcosm experimental bioassays and found that nematodes were not sensitive to these pollutants. The exposure duration that they have used (only 30 days) might explain this result.
Marylynnia stekhotenli, Calomicrolaimus honestus and Oncholaimellus mediterraneus were significantly affected at the cadmium-diesel contamination but they were not eliminated. These species could be categorized as “cadmium-diesel sensitive”. Enoplodips sp. and Oncholaimus campylocercoides which significantly increased in cadmium-diesel amended sediment seemed to be “diesel-resistant” species. All the species cited above were not affected by either cadmium or diesel alone.

Our findings indicate very strongly that complex effects are taking place when mixtures of pollutants are introduced to the marine environment. Prediction of combined impacts of metals and diesel contamination on benthic communities can not be based on results obtained with the toxicants applied separately.

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