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Effects of Increasing Levels of Nickel Contamination on Structure of Offshore Nematode Communities in Experimental Microcosms

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Abstract A microcosm experiment was used to examine the effects of nickel on offshore nematode communities of a Tunisian coastal zone (Southwestern Mediterranean Sea). Sediments were contaminated with three nickel concentrations [low (250 ppm), medium (550 ppm) and high (900 ppm)], and effects were examined after 30 days. Results showed significant differences between nematode assemblages from undisturbed controls and those from nickel treatments. Most univariates measures, including diversity and species richness, decreased significantly with increasing level of Ni contamination. Results from multivariate analyses of the species abundance data demonstrated that responses of nematode species to the nickel treatments were varied: *Leptonemella aphanothecae* was eliminated at all the nickel doses tested and seemed to be intolerant species to nickel contamination; *Daptonema normandicum*, *Neochromadora trichophora* and *Odontophora armata* which significantly increased at 550 ppm nickel concentration appeared to be "opportunistic" species at this dose whereas *Oncholaimus campylocercoides* and *Bathylaimus capacosus* which increased at all doses tested (250, 550 and 900 ppm) seemed to be "nickel-resistant" species.

Keywords Free living nematodes · Sediment · Nickel · Contamination · Microcosms

Nickel is the 24th element in order of natural abundance in the earth's crust and it is widely distributed in the environment. Natural sources of aqueous nickel derive from biological cycles and solubilization of nickel compounds from soils (Kasprzak et al. 2003). Nickel concentration in deep-sea water usually range from 0.1 to 0.5 ppb Ni, whereas surface water contains 15–20 ppb Ni (Norseth and Piscator 1979). The sources of environmental nickel contamination include the production and processing of nickel, the recycling of nickel-containing products and nickel containing waste disposal. Nickel components are also found in soils and exist in both insoluble forms, such as sulfides and silicates, and in a number of soluble forms (Garrett 2000). Many studies had reported toxicity effects that occur during exposure to elevated concentrations of nickel (Denkhaus and Salnikow 2002; Kasprzak et al. 2003). For example, Nicolaidou et al. (1989), and Nicolaidou and Nott (1990) had reported that an increase of nickel concentration in marine sediments altered Gastropods community of the coastal area of Larymna (north Evoikos Gulf, Greece).

Mediterranean marine sediments are well known to be contaminated with heavy metals (Mulsow et al. 2001; Rouibah 2001; Yoshida et al. 2004), but the influence of these pollutants on Mediterranean natural communities is poorly understood. No experimental study has assessed the impacts of these contaminants on Mediterranean benthic organism assemblages and field studies (Mahmoudi et al. 2002; Hedfi et al. 2003) have been, by necessity, restricted to correlative relationships between pollutant concentrations and community composition. The assessment of the potential biological impacts of heavy metals contamination often requires direct tests involving bioassays on individuals or populations of selected species. Among benthic organisms, nematode communities constitute a good biological material for environmental impact assessments. They have short generation time (mostly days to weeks),

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high density and continuous reproduction all year round (Austen and McEvoy 1997). These small animals (between 40 μm and 1 mm) are also easily maintained and, therefore, their potential for rapid response to environmental change is high (Bell 1988; Warwick et al. 1988; Coull and Chandler 1992; Mahmoudi et al. 2005). Because of these features it is easy to maintain and manipulate quite natural nematode communities in simple laboratory microcosms (Austen et al. 1994; Schratzberger et al. 2002; Millward et al. 2004; Gyedu-Ababio and Baird 2006). In the present study, we present the results of a microcosm experiment designed to incubate meiobenthic communities in offshore sediment from the bay of Bizerte (Northern Tunisia) with defaunated sediment (also collected offshore) contaminated with nickel. The investigation focused on the comparison of densities, diversity and species composition of nematode assemblages from control microcosm and nickel treatments.

Materials and Methods

Offshore sediment and meiofauna were collected from the bay of Bizerte (Northern Tunisia) (37° 17.654′N 09° 52.476′E) using hand cores (10 cm^2 sampling surface area) to a depth of 10 cm. At the prospected site, water depth was 1.20 m and salinity was 36 PSU. The sediments had a median particle diameter of 22 μm and 1.32% of organic carbon content.

In laboratory, sediment used for nickel contamination was first alternately frozen to a temperature –20°C for 12 h and then thawed at room temperature for 48 h. This process was repeated three times before sediment was stored frozen at –20°C (Schratzberger et al. 2002), and then it was wet sieved to remove the larger particles (>63 μm). Next, stock nickel chloride solutions were made in distilled water and quantities of 100 g (dry weight, dw) of sediment were contaminated with appropriate doses of nickel in order to obtain final concentrations of 250 ppm [low dose, Ni(L)], 550 ppm [medium dose, Ni(M)] and 900 ppm [high dose, Ni(H)] after being mixed with 200 g of natural (uncontaminated) sediment. Nickel was 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.

At the end of the experiment, a small aliquot of sediment from one replicate from each treatment was analysed for the dosed metal. Sediment samples were dried at 80°C, digested in concentrated HNO_3 and, after evaporation, the residues were dissolved in 1 M HCl. Metal concentrations were determined by flame atomic absorption using a Varian Spectra AA20 atomic absorption spectrometer with air/acetylene flame and autosampler.

Microcosms consisted of 570 mL glass bottles. One control and three 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 offshore water at 36 PSU. Control (C) consisted of uncontaminated and defaunated sediment. The treatments [Ni(L), Ni(M) and Ni(H)] consisted of three levels of nickel contamination (250, 550 and 900 ppm).

Each microcosm bottle was stoppered with a rubber bung with two holes and aerated via an air stone diffuser. The experiment was maintained for 30 days at a temperature of 18°C. Sediment colour and water conductivity, salinity, pH and temperature were monitored at regular intervals. At the end of the experiment, all samples were preserved in 4% formalin.

Meiofaunal taxa 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 in order to evaluate total nematode abundance. Nematodes were identified to genus or species using the pictorial keys of Platt and Warwick (1983, 1988), and Warwick et al. (1998).

The majority of data analysis followed methods described by Clarke (1993) and Clarke and Warwick (2001) using the PRIMER software package. 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 different levels of nickel contamination by changes in the relative abundance of species. 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). 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. Univariate indices were computed: total nematode abundance (I), number of species (S), diversity [Shannon–Weaver index (H′)], species richness [Margalef's (d)] and evenness [Pielou's (J′)]. The 1-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 controls. In all the above statistical significance testing a significant difference was assumed when $p < 0.05$.

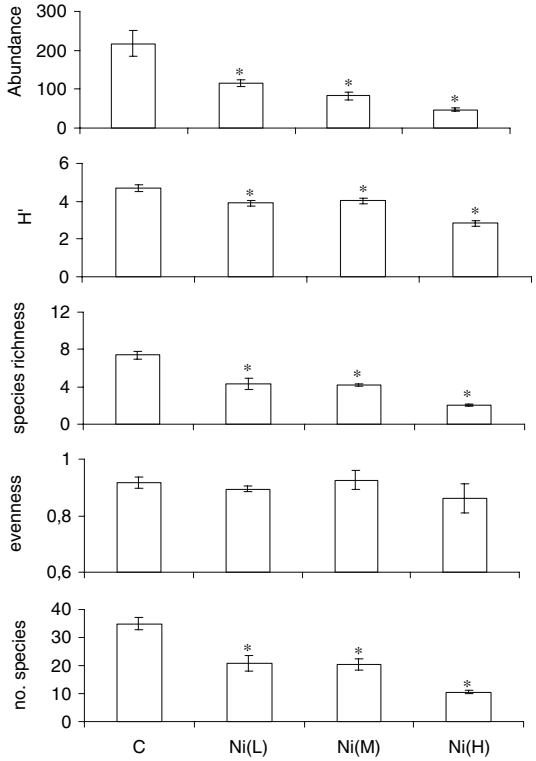

Fig. 1 Graphical summary of means and 95% pooled confidence intervals of univariate indices for nematode assemblages from each microcosm. H′ = Shannon–Weaver index, species richness = Margalef's d, evenness = Pielou's J, no. species = number of species (S). *Asterisk* indicates a significant difference ($p < 0.05$) of the univariate measure in the contaminated microcosm when compared to the control

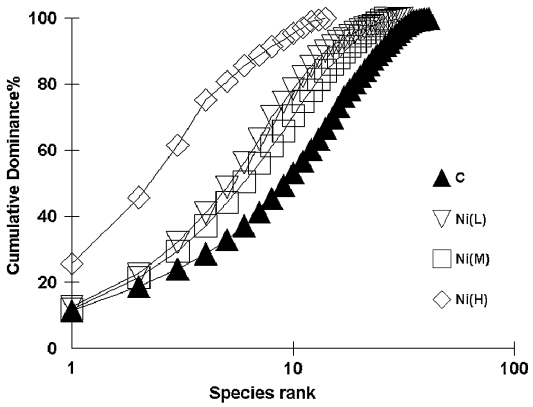

Fig. 2 *k*-dominance curves for uncontaminated sediment control microcosm (C) and nickel amended sediment treatments [Ni(L), Ni(M) and Ni(H)]

Table 1 Anosim results (*R* statistic and significance level) of pairwise tests for differences between k-dominance curves of treatments and control using square-root transformed nematode abundance data

Groups	*R* value	Significance level
C–Ni(L)	0.99	0.03*
C–Ni(M)	1.00	0.03*
C–Ni(H)	1.00	0.03*
Ni(L)–Ni(M)	0.21	0.20
Ni(L)–Ni(H)	1.00	0.03*
Ni(M)–Ni(H)	1.00	0.03*

* Denotes significant differences when $p < 0.05$

Results and Discussion

Figure 1 shows the graphical summary of univariate measures for nematode assemblages from controls and treatments. All indices were significantly reduced in comparison with the controls. The results of significance testing using the 1-way ANOVA for overall differences between univariate indices indicate that nickel contamination resulted in significant changes of univariate community attributes ($p < 0.05$). Results from multiple comparisons tests show significant differences between nematode assemblages from uncontaminated control microcosm and all those from nickel amended sediment treatments for most univariate indices (HSD test, $p < 0.05$). Total nematode abundance (I), Shannon–Weaver index H′, species richness (d), and number of species (S) decreased significantly with an increase of nickel contamination.

The k-dominance curves (Fig. 2) combined with ANOSIM results (Table 1) indicate that nematodes communities in all nickel amended sediment microcosms are less diverse than the control. In all treatments k-dominance curves were significantly different from the control and from each other except Ni(L)–Ni(M) (Table 1). In the MDS ordination (Fig. 3), all treated microcosm replicates are distinct from controls and the high nickel treatments are placed at the end of this ordination. This indicates a clear effect of nickel contamination on most nematode species.

ANOSIM results (Table 1) show a significant impact of nickel contamination on nematode assemblages. All treatments were significantly different ($p < 0.05$) from controls.

The control samples (C) were dominated by *Trichotheristus mirabilis* (12%), *Pomponema multipapillatum* (7%), *Ditlevsenella murmanica* (5%), *Leptonemella aphanothecae* (5%), *Oncholaimus campylocercoides* (4%), *Daptonema normandicum* (4%), *Pomponema elegans* (4%) and *Viscosia cobbi* (4%). In rank order of abundance there was a group of common species with abundances less then (3%): *Bathylaimus capacosus*, *Oncholaimellus mediterraneus*, *Xyala striata*, *Mesacanthion hirsutum*, *Gammanema conicauda*, *Viscosia glabra*, *Thersitus modicus*, *Symplocostoma* sp., *Sabatieria longisetosa*, *Microlaimus affinis* and then another group but even less abundant: *Lauratonema adriaticum* (2%), *Neochromadora trichophora* (2%), *Odontophora armata* (2%), and *Ptycholaimellus ponticus* (2%). The treatments Ni(L) were mainly dominated by

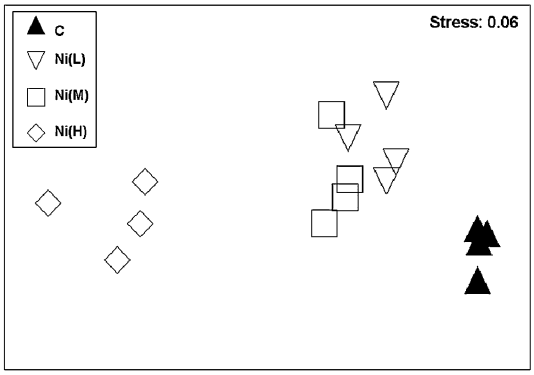

Fig. 3 Non-metric MDS ordination of square-root transformed nematode species abundance data from uncontaminated sediment control microcosm (C) and nickel amended sediment treatments [Ni(L), Ni(M) and Ni(H)]

O. campylocercoides (13%), *O. mediterraneus* (10%), *M. hirsutum* (10%), *B. capacosus* (9%), *Enoplolaimus propinquus* (8%) and *T. mirabilis* (8%). In the treatments Ni(M), *O. campylocercoides* (12%), *D. normandicum* (10%), *M. hirsutum* (8%), *Oncholaimellus mediterraneus* (8%) and *B. capacosus* (7%) were the most abundant species. The treatments Ni(H) were mainly dominated by *B. capacosus* (31%) and *O. campylocercoides* (25%).

As shown in Table 2, significant differences between control and treated microcosms mainly resulted from changes in the abundances of the dominant species. Elimination of *Leptonemella aphanothecae* and increasing number of *Oncholaimus campylocercoides*, *Oncholaimellus mediterraneus* and *Mesacanthion hirsutum* were responsible for significant difference between C and Ni(L).

Significant differences between control and the microcosms Ni(M) were mainly due to increasing abundances of *O. campylocercoides* and *Daptonema normandicum*, an elimination of *L. aphanothecae* and a significantly lower abundance of *Trichotheristus mirabilis*. Increasing levels of nickel contamination was accompanied with an elimination of two other species *Pomponema multipapillatum* and *Ditlevsenella murmanica*, a significantly higher number of *Bathylaimus capacosus* and *O. campylocercoides* and a lower abundance of *T. mirabilis*.

Results from multivariate data evaluation revealed significant differences between control and all treated microcosms ($p < 0.05$). This indicates that the responses of the free-living nematode community to nickel were dependent on the level of this metal contamination. In the MDS plot for the nematode assemblages, all treated microcosm replicates are distinct from controls but the distinction between low and medium doses is not marked (Fig. 3). The nickel high treatments appear to be more dissimilar from the control than medium and low treatments. *Leptonemella aphanothecae* was eliminated at all the nickel doses tested (Table 2) and seemed to be intolerant species to nickel contamination with effects observed even at the low concentration used (250 ppm). *Daptonema normandicum*, *Neochromadora trichophora* and *Odontophora armata* which significantly increased at 550 ppm nickel concentration appeared to be "opportunistic" species at this dose whereas *Oncholaimus campylocercoides* and *Bathylaimus capacosus* which increased at all doses tested (250, 550 and 900 ppm) seemed to be "nickel-resistant" species. Based on these observations, we can infer that species within the bay of Bizerte nematode community have a range of sensitivity to nickel.

The bioavailability of trace metals is strongly influenced by a suite of physical, chemical and biological factors in the sediment (Schratzberger et al. 2000; Austen and McEvoy 1997). The negative response of the bay of Bizerte nematofauna to experimental nickel contamination was

Table 2 Species responsible for differences between control and treated microcosms based on similarity percentages (SIMPER) analysis of square-root transformed data

Ni(L)	Ni(M)	Ni(H)
Oncholaimus campylocercoides (+)	*Oncholaimus campylocercoides* (+)	*Bathylaimus capacosus* (+)
Oncholaimellus mediterraneus (+)	*Trichotheristus mirabilis* (–)	*Oncholaimus campylocercoides* (+)
Mesacanthion hirsutum (+)	*Daptonema normandicum* (+)	*Trichotheristus mirabilis* (–)
Enoplolaimus propinquus (+)	*Leptonemella aphanothecae* (elim)	*Leptonemella aphanothecae* (elim)
Bathylaimus capacosus (+)	*Mesacanthion hirsutum* (+)	*Pomponema multipapillatum* (elim)
Leptonemella aphanothecae (elim)	*Neochromadora trichophora* (+)	*Ditlevsenella murmanica* (elim)
Trichotheristus mirabilis (–)	*Odontophora armata* (+)	
Ditlevsenella murmanica (–)	*Oncholaimellus mediterraneus* (+)	
Viscosia cobbi (–)	*Pomponema multipapillatum* (–)	
	Bathylaimus capacosus (+)	

+ More abundant, – less abundant, *elim* elimination of the species. Species accounting for ~50% of overall dissimilarity between treatment groups are ranked in order of importance of their contribution to this dissimilarity

consistent with a toxicological effect; most univariate indices decreased significantly in nickel treated microcosms (Fig. 1).

Effects of nickel treatment on nematodes species in our experiment could have occurred by the uptake of leached nickel from the sediment pore water through their permeable cuticle and the gut as well as direct ingestion of nickel particles with food (Nicolaidou et al. 1989; Schratzberger et al. 2002; Hédouin et al. 2007). By increasing mortality of the most sensitive species, nickel contamination was responsible for the decrease in nematode densities and diversity.

It is evident from our study that nickel has harmful effect on the diversity and structure of free-living nematode communities. Nematodes showed a clear species–specific response, depending on the level of nickel contamination. This finding supports the suggestion that these particular organisms could be used as biomonitors of heavy metals in coastal ecosystems.

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