Cah. Biol. Mar. (2015) 56 : 39-48

Experimental evidence of the effects of an antimitotic agent, Colchicine, on a nematode community through a microcosm approach

Fehmi BOUFAHJA, Salma ISMAÏLY and Hamouda BEYREM
Laboratory of Biomonitoring of the Environment,Coastal Ecology and Ecotoxicology Unit, Carthage University, Faculty of Sciences of Bizerte, Zarzouna 7021, Tunisia
E-mail: fehmiboufahja@yahoo.fr

Abstract: An experiment was conducted to make out if an antimitotic agent, the Colchicine, may have negative effects on a nematode community from the "Rimel" beach (Bizerte bay, NE Tunisia). Three sedimentary doses of Colchicine (in Dry Weight) (1.5428 ppm, 15.428 ppm and 154.28 ppm) were tested during 30 days of exposure. Univariate analyses showed a clear decrease in terms of quantity and quality of nematodes present in Colchicine treatments compared to the control. Multivariate analyses revealed a discernible reorganization of the community evidently due to the different species sensitivities against Colchicine. The nematode species *Ptycholaimellus ponticus*, *Theristus modicus* and *Kraspedonema reflectans* were identified as Colchicine-sensitive, due to population reduction after treatment. The Colchicine seems acting on these species by direct epidermic contact, by internalization through cuticular pores or by ingestion of contaminated sediment particles. Our results appeared rejecting the eutely for cells of the cuticular epidermis and those of the digestive tube of *Ptycholaimellus ponticus*, *Theristus modicus* and *Kraspedonema reflectans*.

Résumé : *Evidence expérimentale des effets d'un agent antimitotique, la colchicine, sur une communauté de nématodes, par une étude en microcosme.* Une étude expérimentale a été menée afin de savoir si un agent antimitotique, la colchicine, peut avoir des effets négatifs sur une communauté de nématodes de la plage 'Rimel' (Baie de Bizerte, NE Tunisie). Trois doses sédimentaires de colchicine (en Poids Sec) (1,5428 ppm, 15,428 ppm et 154,28 ppm) ont été testées au cours de 30 jours d'exposition. Les analyses univariées ont montré une nette diminution sur les plans quantitatif et qualitatif des nématodes présents dans les microcosmes traités par la colchicine par rapport à ceux témoins. Les analyses multivariées ont révélé une réorganisation de la communauté évidemment en raison de l'élimination d'espèces sensibles vis-à-vis de la colchicine (essentiellement *Ptycholaimellus ponticus*, *Theristus Modicus* et *Kraspedonema reflectans*). La colchicine semble agir sur ces espèces *via* un contact épidermique direct par internalisation à travers les pores cuticulaires ou par ingestion de particules sédimentaires contaminées. A l'issue, nos résultats paraissent rejeter le concept eutélique pour les cellules de l'épiderme cuticulaire et celles du tube digestif de *Ptycholaimellus ponticus*, *Theristus modicus* et *Kraspedonema reflectans*.

Keywords: Eutely ● Colchicine ● Microcosms ● Marine nematodes ● *Ptycholaimellus ponticus* ● *Theristus modicus* ● *Kraspedonema reflectans*

Reçu le 19 juin 2014 ; accepté après révision le 4 novembre 2014.
Received 19 June 2014; accepted in revised form 4 November 2014.

Introduction

Nematodes are widely considered to be eutelic (Van Cleave, 1932). According to this author, eutely, or cell constantly, refers to a specific number of cells in the adult form of an animal, consistent across the species. This view of development results essentially from the extrapolation of the remarkable constancy of somatic cell lineages of *Caenorhabditis elegans* Maupas, 1900 to the rest of the phylum. However, several studies on soil and parasitic nematodes have described a variation in cell or nuclear number in intestine, epidermis, ventral nerve chord and vulva. This was the case of the parasitic nematode *Oxyuris curvula* Rudolphi, 1803 (Martini, 1923) and the soil-dwelling nematodes such as *Rhabditis anomala* Hertwig, 1922 (Wessing, 1953) and *Panagrellus redivivus* (Goodey, 1945) (Sternberg & Horvitz, 1982). Similar results were also reported in case of few marine nematode species by Rusin & Malakhov (1998) and Voronov & Panchin (1998) and the researchers showed that the total number of epidermal cells of adults for the same species varied. In spite of the above examples suggesting that nematodes are not eutelic in at least one organ, especially in the intestine and epidermis, yet the view that absolute eutely still is the rule in the phyla Nematoda (Malakov, 1994; Wiggers et al., 2002).

The original idea of this study was to expose a reference nematode community to an antimitotic agent which will simply permit to retain (no effect) or reject (discernible effect) the eutelic concept for nematodes. One such antimiotic agent is the Colchicine which is extracted from *Colchicum autumnale* (Linneaus, 1753) (Colchicaceae) (Krzaczkowski, 2008). According to this author, Colchicine is an alkaloid known to arrest mitotic cell division at metaphase by interfering with the structure of the mitotic spindle. Interest in the suppressive effects of such plant extracts on parasitic nematodes and the identification of nematicidal principles in the extracts them has been increasing (Krzaczkowski, 2008). For example, development of *Meloidogyne javanica* (Treub, 1885), was inhibited in roots treated with Colchicine three days after inoculation of the nematode (Wiggers et al., 2002). These authors suggest that this chemical affect nematode growth *via* its effects on giant cell development. Similar results were previously recorded for the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White, 1919) by Nidiry et al. (1993). This work aims to assess the effects of the Colchicine on the taxonomic and feeding structure of a free-living marine nematode community. The principle of interpretation of the results is simple; if our experiment shows that there are no sensitive species, this will consolidate the eutely concept for our reference nematodes. If appropriate, the eutely will be rejected since Colchicine acts by following one way only, stopping cell multiplication.

Material and Methods

Collection site and environmental measurements

The collection of surface sediment to a depth of 5 cm was carried out on 9th March 2012 in an intertidal beach in Bizerte Bay (Tunisia), Rimel (37°15.18'N-9°55.34'E) (Fig. 1). The dissolved oxygen of the water-sediment interface was evaluated with an oxymeter (WTW OXI 330/SET, WTW, Weilheim, Germany). Temperature and salinity were measured using a thermo-salinity meter (WTW LF 196, Weilheim, Germany) and pH with a pH meter (WTW pH 330/SET-1, Germany).

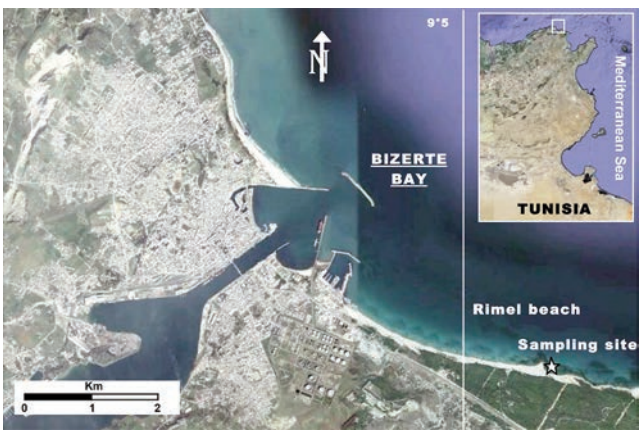


Figure 1. Aerial photograph of Bizerte bay (Tunisia) showing the location of Rimel beach and the position of the sampling site.

The sedimentary coarse fraction was obtained by a wet-sieving analysis through a 63 µm mesh (Mahmoudi et al., 2007). Other sediment sub-samples were dried at 45°C and used for quantifying total organic matter by ignition at 450°C for 6 hours (Fabiano & Danovaro, 1994). The sediment water content was evaluated by drying 100 g at 45°C for 96 hours (Mahmoudi et al., 2008). Three sedimentary aliquots were always used in sediment analyses.

Sampling and manipulation of sediment

Numerous Plexiglas hand-cores of 3.6 cm (internal diameter) were taken to a 5 cm depth within the sediment and temporarily preserved in a plastic container. Upon return to the laboratory, the sampled sediment was homogenized by gently stirring with a large spatula and three small spoons were checked for presence of moving nematodes.

Twelve glass bottles of 1.5 litre were used and each one was considered to be an independent experimental unit or 'microcosm'. The same amount of homogenized fresh sediment (200 g Wet Weight, water content = 20.06 ± 1.79%) was added to each bottle (Mahmoudi et al., 2005; Beyrem et al., 2007; Hedfi et al., 2013). The bottles were carefully filled with one liter 1 µm filtered seawater from the native site (i.e. Rimel beach) and left for one week for acclimatization before application of treatments. Care was taken to avoid the inclusion of large macrofaunal animals (e.g. polychaetes) into the microcosms. During this period, bottles were connected to a closed aeration circuit, and a rubber bung with two holes for inflow and outflow of air was placed into each bottle.

Treatments (Fig. 2)

In order to prepare the natural sediment collected on 9th March 2012 from Rimel for contamination, a sequence of freezing at -20°C and thawing at the ambient temperature was repeated three times to defaunate it (Beyrem et al., 2010). Then, it was sieved through a 0.3 mm mesh to separate coarser sand particles from the finer ones. The choice of the sieve mesh was based on the mean value of median grain size equal to 0.35 mm evaluated previously for sediments from Rimel by Boufahja et al. (2010). The sedimentary finer fraction was contaminated with appropriate amounts of solubilized Colchicine (VWR BDH Prolabo), shaken overnight and then mixed with the coarser fraction. The treated sediment was then left to equilibrate for one week at +5°C. On day of treatment with Colchicine, all microcosms were slowly emptied of seawater and aliquots of 100 g Wet Weight of treated sediment were added to every microcosm containing already 200 g Wet Weight of natural sediment in order to obtain four final sedimentary concentrations (in Dry Weight) corresponding to control (C, without addition of Colchicine), low (L, Colchicine dose of 1.5428 ppm), medium (M, Colchicine dose of 15.428 ppm) and high (H, Colchicine dose of 154.28 ppm). Three replicates were randomly assigned to each treatment. Thereafter, all microcosms were gently filled with filtered seawater from Rimel site, maintained in darkness to avoid light sensitivity of the Colchicine and primary production; and left for 30 days as part of the experiment (Carman et al., 1995; Millward et al., 2004).

Doses were chosen based on data of Nidiry et al. (1993) who indicated that 1% of pure Colchicine showed 18% nematicidal activity against the plant-parasitic nematode *Meloidogyne incognita* after 48 hours of treatment. The mean length of the nematodes were 1.6 mm in previous studies from the same location (Boufahja et al., 2010, 2011 & 2012) and similar to that of *M. incognita* (about 1.5 mm for adult worms; Brito et al., 2004). In the case of this species, 100% mortality occurs theoretically after 30 days of treatment with an aqueous solution of 0.37% Colchicine. To obtain this concentration, 3.7 g of Colchicine was dissolved in 1 litre of seawater. Since this chemical compound is known to be stable, incompatible with strong oxidizing agents and hydrosoluble (10 mg ml^{-1}), we assume that once

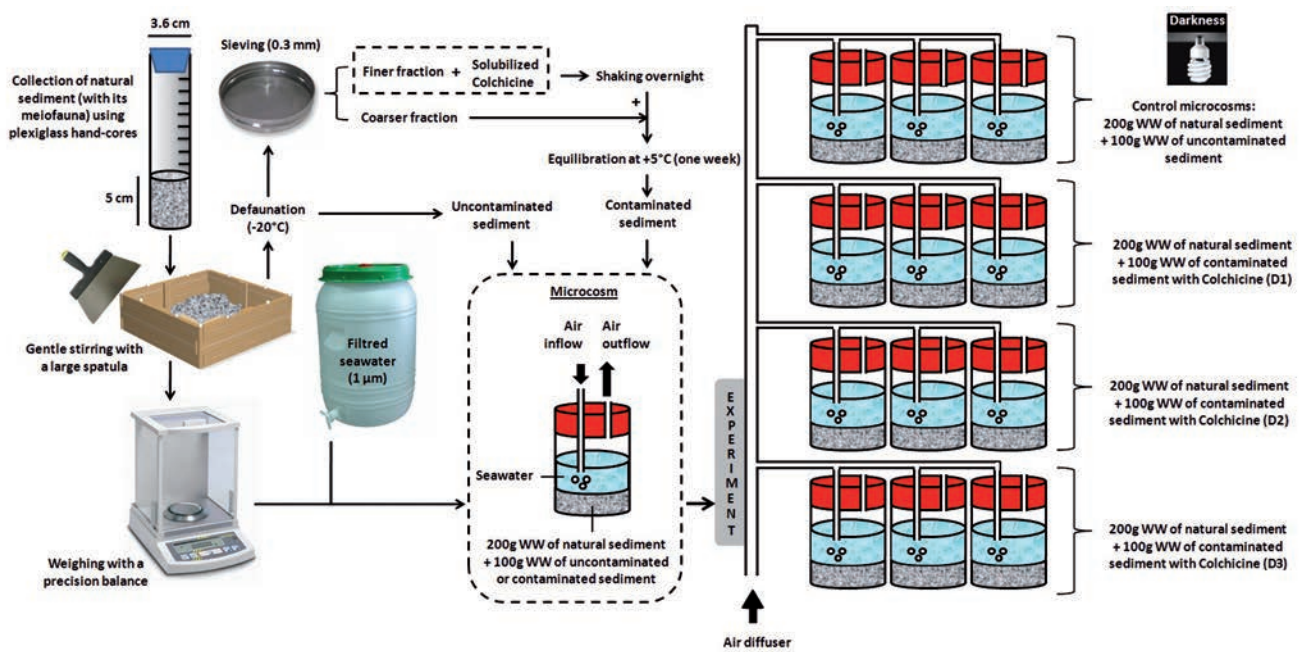


Figure 2. Scheme illustrating the steps of sediment treatment and the experimental design used in the microcosm experiment.

it is experimentally dissolved in the seawater, all the used quantity will arrive rapidly to the 300 g Wet Weight (= 239.82 g Dry Weight) sediment. As a result, an aqueous solution of 0.37% was considered to be equivalent to a sedimentary concentration of 15428.23 ppm (Dry Weight). In this work, we have chosen to use the following final sedimentary doses of Colchicine (in Dry Weight), 1.5428 ppm; 15.428 ppm; and 154.28 ppm, by considering a coefficient of 100 between the dose of Colchicine inducing 100% mortality (i.e. 15428 ppm) and our high dose (i.e. 154.28 ppm).

Sample processing

The content of each sample was first sieved on a 1 mm mesh to collect macrofauna and large detritus. Meiofauna was secondly extracted from the sediment using the classic resuspension-decantation method and sieving through a 40 µm mesh sieve (Mahmoudi et al., 2005; Beyrem et al., 2007). Subsequently, nematodes were enumerated under a 50x stereomicroscope (Model Wild Heerbrugg M5A) and the first 100 specimens encountered were placed in 10% glycerol, evaporated to anhydrous glycerol, and then mounted on permanent slides (Seinhorst, 1959). Microscopic observations of picked specimens were made by using: (1) a Nikon DS-Fi2 camera coupled to a Nikon microscope (Image Software NIS Elements Analysis Version 4.0 Nikon 4.00.07 - build 787- 64 bit); and (2) an Olympus XC50 camera coupled to an Olympus BX53 microscope (Image Software CellSens Standard Version 1.6). Nematodes were identified to genus or species where practicable using the pictorial keys by Platt & Warwick (1983 & 1988), Warwick et al. (1998) and to species level using the NeMys Database from the Marine Biology Section of the University of Gent (Deprez et al., 2005). They were also classified into four feeding groups, based on the structure of the buccal cavity (Wieser, 1953): 1A, selective deposit-feeders, nematodes with small or absent buccal cavity; 1B, non-selective deposit-feeders, nematodes with buccal cavity of moderate size; 2A, epistratum-feeders, species with small to medium sized buccal cavity, with small teeth and 2B, predators, nematodes with wide buccal cavity, armed with large teeth. Finally, we estimated the biovolume of specimens in nanolitre by using the equation of Warwick & Price (1979): $530 \times \text{Total Length} \times (\text{Maximum Width})^2$. These body measurements are determined in mm. Wet Weight (µg) of each nematode was obtained by using a specific gravity of 1.13 µg. nl^{-1} (Wieser, 1960) and converted to Dry Weight assuming a Dry/Wet Weight ratio of 0.25 (Vanaverbeke et al., 1997).

Data analysis

One-way ANOVA was used to test for differences in univariate measures: density, number of nematode species, Shannon-Wiener diversity index (H'), Margalef's species richness (d) and evenness (J'). The uptake data were first checked for fulfillment of normality by using Kolmogorov-Smirnov test. Bartlett's test was used to determine whether variances were non-homogeneous. Where Bartlett's test indicated non homogeneity of variances, data was *log*-transformed and Bartlett's test was repeated to determine if transformation improved their suitability to apply parametric assumptions. The test of Tukey HSD (equal series) and that of Spjotvoll-Stoline (series with different sizes), adjusted for multiple comparisons, were used to test for differences between treatments. ANOVA, Tukey's HSD and Spjotvoll-Stoline tests were carried out using STATISTICA 6.0 from StatSoft. A significance level of $P < 0.05$ was used in all tests.

Multivariate data analysis followed methods described by Clarke & Gorley (2006) using the PRIMER 6.0.2 (Plymouth Routines in Multivariate Ecological Research) software package. The data were subjected to non-metric multi-dimensional scaling ordination (nMDS) with the Bray-Curtis similarity measure. Non-metric multi-dimensional scaling is a good ordination method because it can use environmentally meaningful ways of measuring community dissimilarities (Clarke & Ainsworth, 1993). A good dissimilarity measure has a good rank order relation to distance along environmental gradients. Because nMDS only uses rank information and maps ranks non-linearly onto ordination space, it can handle non-linear species responses of any shape and effectively and robustly find the underlying gradients. Square root transformation was first applied on nematode species densities in order to make them more sensitive to changes in lower abundance and rarer species. Ordination is considered (1) reliable when stress value is less than 0.2, (2) fair when it's around 0.1 and (3) good to perfect when it's less than 0.05 (Clarke & Warwick, 2001). Non-parametric analysis of similarity (ANOSIM) was carried out to determine significant differences ($P < 0.05$) between nematode assemblages in different Colchicine treatments and the controls. The procedure SIMPER (similarity percentage) was also used to determine the contribution of individual species towards similarity/dissimilarity between treatments and controls.

Results

Environmental parameters

On the sampling day, environmental parameters in the study site were recorded as: 0.6 m depth, salinity of 38.3,

temperature of 14.9ºC and dissolved oxygen of 9.22 mg l^{-1}. The sediments collected were sandy (99.41 ± 0.15% of coarse size fraction) and contain 0.56 ± 0.09% of organic matter and 20.06 ± 1.79% of water content.

Abundance and individual weight

The graphical summary of univariate indices for nematode assemblages from each microcosm, and the results of significance testing using the one-way ANOVA (Fig. 3), illustrates significant differences between nematode assemblages from undisturbed controls and those from Colchicine treatments for most univariate indices measured. Nematode counts (25 ± 9 to 334 ± 56 individuals) showed significant changes, depending upon the dose of Colchicine ($P < 0.001$). Thus, a discernible decreasing trend, C-D1-D2-D3, characterized the progression from the control microcosms to those enriched by Colchicine ($P < 0.001$) (Fig. 3).

The variability of the mean individual weight (0.38 to 0.51 µg Dry Weight) was not likely discernible even after the exposure of nematodes to the highest doses of Colchicine D2 and D3, except when comparing C and D1 ($P = 0.002$).

Species inventory and biodiversity

Following the end of manipulation experiment, the nematode community was found to be divided into 18 families, 29 genera and 30 species (Table 1). The more diverse families were those of Desmodoridae (4 species), Thoracosthomopsidae (4), Xyalidae (4) and Chromadoridae (3).

Twenty seven species were present in controls with a predominance of species *Ptycholaimellus ponticus* (Filipjev, 1922) Gerlach, 1955, *Theristus modicus* Wieser, 1956 and *Kraspedonema reflectans* Gerlach, 1954. Replicates in the first dose of Colchicine were characterized by the dominance of three species: *P. ponticus*, *Sigmophoranema rufum* (Cobb, 1933) and *Lauratonema hospitum* Gerlach, 1956. Similarly species such as *S. rufum*, *Enoploides spiculohamatus* Schulz, 1932 and *P. ponticus* were characteristic of the nematofauna exposed to the second dose. Finally, for nematodes associated with the dose of Colchicine D3, three species constituted the top of the list: *L. hospitum*, *E. spiculohamatus* and *S. rufum* (see Table 1).

Two diversity indices were considered, the Shannon-Wiener index and species richness of Margaleff (Fig. 3). The one-way ANOVA did not reveal any significant difference between the Shannon-Wiener index of the four nematode groups (*log*-transformed data: df = 3, $F_{(3,8)} = 3.762$, $P = 0.059$). This was not the case for species richness which showed decreasing trend C-D1-D2-D3 with respect to its mean values (from 3.76 to 1.72). Only for C and D3, significant difference was recorded based on Tukey's HSD test (*log*-transformed data: $P = 0.040$). Pielou evenness showed a close trend among Shannon-Wiener index and only one discernible difference was found for C and D3 (in log x: $P = 0.022$) when compared with Tukey's HSD test.

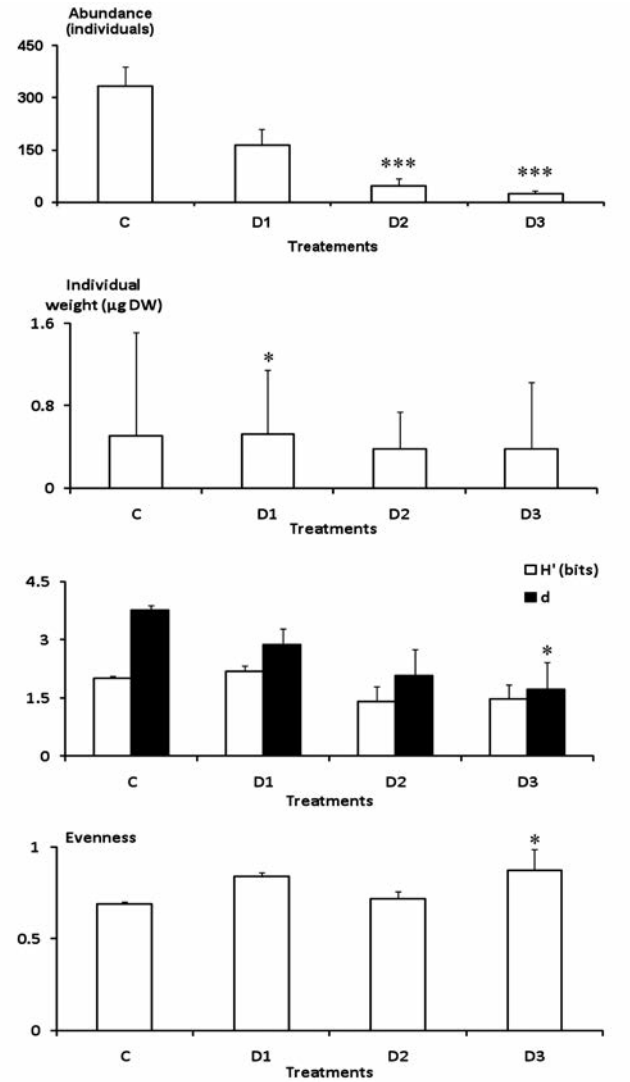


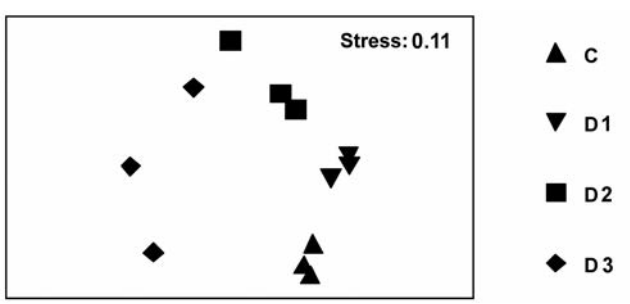
Figure 3. Mean values of univariate indices (± SD) for control nematode assemblage (C) and those exposed to Colchicine (D1, D2 and D3). Shannon-Wiener index (H'), Margalef's species richness (d). Multiple comparisons (log-transformed data): significant differences at $P < 0.05$ (*), highly significant differences at $P < 0.001$ (***) between C and treatment.

Multivariate analyses

The nMDS ordination indicated a possible effect of Colchicine on the species composition of nematodes (stress = 0.11), especially in the case of microcosms associated with the D3 (Fig. 4). Indeed, replicates of this dose were placed to the extreme left of those remaining treatments.

Table 1. Relative abundance (± SD) of meiobenthic nematode species identified in the control (C) and Colchicine enriched microcosms (D1, D2 and D3). Feeding groups according to Wieser (1953) (FG): 1A, selective deposit-feeders; 1B, non-selective deposit-feeders; 2A, epistratum-feeders; 2B, predators. Species absent (-). Underlined values indicate relative abundances of the three first species for every treatment.

Species	FG	C	D1	D2	D3
Ceramonema sp.	1A	-	0 .46 ± 0.8	1.51 ± 2.62	-
Cyartonema germanicum Juario, 1972		0.33 ± 0.57	-	-	-
Filithonchus filiformis (Warwick, 1971)		0.33 ± 0.57	-	-	-
Rhabditis sp.		1 ± 1	-	3.82 ± 4.2	-
Desmodora longiseta (Schuurmans Stekhoven, 1950)	1B	0.67 ± 1.15	-	-	-
Lauratonema hospitum Gerlach, 1965		0.67 ± 0.57	12.85 ± 11.91	2.55 ± 2.32	30.02 ± 34.59
Monoposthia mirabilis Schulz, 1932		0.67 ± 0.57	-	-	-
Paradesmodora supplementatis Inglis, 1968		2 ± 1	3.17 ± 1.88	-	-
Promonhystera tricuspidata Wieser, 1956		1.67 ± 2.08	0.36 ± 0.62	-	3.7 ± 6.41
Sabatieria splendens (Hopper, 1967)		0.67 ± 1.15	-	-	2.78 ± 4.81
Theristus modicus Wieser, 1956		15.67 ± 4.04	-	-	11.11 ± 19.24
Trichotheristus mirabilis Schuurmans Stekhoven & De Coninck, 1933		0.33 ± 0.57	3.70 ± 2.8	1.51 ± 2.62	-
Calomicrolaimus honestus (De Man, 1922)	2A	1 ± 0	-	-	3.7 ± 6.41
Chromadora brevipapillata Micoletzky, 1924		2.67 ± 0.57	-	-	-
Chromaspirina pontica Flipjev, 1918		1.67 ± 2.08	0.33 ± 0.57	-	2.38 ± 4.12
Hypodontolaimus sp.		0.67 ± 0.57	0.36 ± 0.62	-	2.78 ± 4.81
Kraspedonema reflectans Gerlach ,1964		8 ± 1.73	1.18 ± 1.1	1.04 ± 1.8	7.4 ± 12.83
Microlaimus cyatholaimoïdes De Man, 1922		2 ± 1.73	0.67 ± 1.15	-	-
Odontophora villoti Luc & De Coninck, 1959		1.67 ± 1.15	-	-	6.48 ± 5.78
Ptycholaimellus ponticus (Filipjev, 1922)		45 ± 2	26.17 ± 5.34	11.93 ± 11.34	-
Xyala striata Cobb, 1920		0.67 ± 0.57	5.25 ± 3.17	1.04 ± 1.8	-
Bathylaimus tenuicaudatus (Allgén, 1933)	2B	0.33 ± 0.57	11.67 ± 4.02	-	-
Enoploides spiculohamatus Schulz, 1932		2 ± 1	8.64 ± 3.57	23.32 ± 13.46	15.34 ± 7.62
Enoplolaimus longicaudatus (Southern, 1914)		4 ± 2	7.15 ± 0.76	2.55 ± 2.32	-
Latronema orcinum (Gerlach, 1952)		0.33 ± 0.57	-	-	-
Mesacanthion hirsutum Gerlach, 1953		-	0.82 ± 0.73	3.59 ± 3.23	-
Mesacanthion monhystera Gerlach ,1967		4 ± 1	1.82 ± 1.02	-	-
Oncholaimus campylocercoïdes De Coninck & Schuurmans Stekhoven, 1933		0.67 ± 1.15	0.36 ± 0.62	-	-
Sigmophoranema rufum (Cobb, 1933)		1.33 ± 2.3	14.05 ± 3.55	46.05 ± 11.99	14.28 ± 12.87
Thoöchus inermis Gerlach, 1953		-	0.92 ± 1.6	1.04 ± 1.80	-
Species number		**18 ± 0.6**	**14 ± 1.5**	**7 ± 3.1**	**5. ± 1.2**



The ANOSIM analysis did not detect significant differences in terms of species composition ($P = 0.1$) (Table 2). However, it was very clear that values of the average dissimilarity between treatments increase with increasing of the Colchicine concentration (Table 2).

Figure 4. Non-metric MDS ordination of square-root transformed nematode species abundance from control (C) and enriched microcosms by Colchicine (D1, D2 and D3).

Table 2. ANOSIM results and average dissimilarity (AD) between control microcosms and those enriched microcosms by Colchicine. All pairewise comparisons between treatments showed non significant differences (P = 0.1).

Treatments	D1		D2		D3	
	R statistics	AD (%)	*R* statistics	AD (%)	*R* statistics	AD (%)
C	1	61.44	1	89.37	0.815	87.90
D1			0.852	67.65	0.852	82.08
D2					0.519	72.73

Table 3. Species responsible for differences between control and enriched microcosms by Colchicines based on similarity percentages (SIMPER) analysis of square-root transformed data. +, more abundant; -, less abundant; elim, elimination. Feeding groups according to Wieser (1953) (FG): 1A, selective deposit-feeders; 1B, non-selective deposit-feeders; 2A, epistratum-feeders; 2B, predators. Species accounting for about 70% of overall dissimilarity between treatment groups are ranked in order of importance of their contribution percentages to this dissimilarity.

D1	D2	D3
Ptycholaimellus ponticus (2A) 21.58 % -	*Ptycholaimellus ponticus* (2A) 40.62 % -	*Ptycholaimellus ponticus* (2A) 44.52 % elim
Theristus modicus (1B) 13.89 % elim	*Theristus modicus* (1B) 14.56 % elim	*Theristus modicus* (1B) 14.16 % -
Lauratonema hospitum (1B) 9.79 % +	*Sigmophoranema rufum* (2B) 7.32 % +	*Kraspedonema reflectans* (2A) 7.22 % -
Sigmophoranema rufum (2B) 8.90 % +	*Kraspedonema reflectans* (2A) 7.15 % -	*Lauratonema hospitum* (1B) 5.07 % +
Bathylaimus tenuicaudatus (2B) 8.53 % +		
Kraspedonema reflectans (2A) 6.18% -		

Based on results of the SIMPER analysis, the presence of Colchicine was led primarily by the decline or elimination of *P. ponticus* for all doses used (Table 3). Other changes with lower influence were also observed. The exposure to the lowest concentration D1 was secondly expressed by an elimination of *T. modicus* and a lower occurence of *K. reflectans*. Simultaneously, at D1, it was noted a proliferation of three species, *L. hospitum*, *S. rufum* and *Bathylaimus tenuicaudatus*. With the D2 treatment, results were secondly characterized by a disappearance of *T. modicus*, an increase of the presence of *S. rufum* and the reduction of that *K. reflectans*. Finally, the dissimilarity between the control treatment and those exposed to D3 was secondly explained by the elimination of *K. reflectans*, the decreased numbers of *T. modicus* and an increase of those of *L. hospitum*.

Discussion

The sampling beach, Rimel, was previously investigated with a focus on nematodes (Beyrem & Aïssa, 2000; Boufahja et al., 2010, 2011 & 2012). All of them clearly showed that it could be considered as a reference area. In particular, data from Boufahja et al. (2010) indicated that in March 2005, the abundance (1100-1500 ind 10 cm^{-2}) and Shannon-Wiener diversity (H' = 2.6-3.5 bits) of the nematode community taken from the exact sampling point were enough to start such microcosm experiment.

Generally, univariate indices very clearly showed the effect of Colchicine on marine nematodes. Indeed, compared to the control microcosms even the low dose (1.5428 ppm Dry Weight) caused a significant decrease in total nematode populations, species number and species richness (see Fig. 3). A comparable result was previously recorded for two plant-parasitic nematode species belonging to the genus *Meloidogyne*, *M. incognita* (Nidiry et al., 1993) and *M. javanica* (Wiggers et al., 2002).

Three species made up almost 70% of the reference nematode populations, namely the Chromadorid *P. ponticus* (45%), the Xyalid *T. modicus* (15.66%) and the Cyatholamid *K. reflectans* (8%). The generally uniform presence of coarse sandy sediment seems to be the major factor to explain the high presence of these steno-species. (Boufahja et al., 2014). Many published works seem to support these results including Tietjen (1977) and Rzeznic-Orignac (2004) for Chromadoridae, Heip et al. (1982) and Keller (1984) for Xyalidae and those of Lorenzen (1974) and Juario (1975) for Cyatholaimidae.

Multivariate analyses showed several community changes in the presence of Colchicine. The nMDS ordination distinguished control replicates from those enriched with Colchicine which indicates a gradual change in species composition as the concentration of the doses were increased. Based on the SIMPER analysis, *P. ponticus* (Chromadoridae), *T. modicus* (Xyalidae) and *K. reflectans* (Cyatholaimidae) appeared to be Colchicine-sensitive species. In contrast, other species tolerated the presence of Colchicine in their environment by expressing more

remarkable occurrence: *S. rufum* (Desmodoridae), *L. hospitum* (Lauratonematidae) and *B. tenuicaudatus* (Tripyloididae). This tolerance may be the result of genetic resistance, a possible adaptation (morphological, physiological or behavioural) and/or an opportunistic potential.

These differential responses of nematode species *versus* Colchicine could be explained in several independent or mutual ways:

● Direct effect: The negative effect of Colchicine on the marine nematode appears to be direct since significant numerical decline was noted for the entire community. This effect was maximal in the case of three species, namely, *P. ponticus* (2A), *T. modicus* (1B) and *K. reflectans* (2A). We can assume that Colchicine acts by direct contact with epidermis or through cuticular pores of the tiny fraction inevitably solubilized during the experiment (*P. ponticus*, *T. modicus* and *K. reflectans*) or by direct ingestion of contaminated sediment (*T. modicus*). In contrast, the presence of *S. rufum* (2B) and *B. tenuicaudatus* (2B), becoming more significant in the nematode communities under stress, could result from some avoidance strategies such as secretion of mucus to cover their cuticle, possession of thick cuticles or tightening cuticular pores due to the powerful muscles of predators (2B).

● Indirect effect: results of Lacaze (1993) support the idea that Colchicine may cause a reduction in photosynthetic productivity of sediments. These types of contaminated microhabitat will probably be poor in benthic diatoms. As a result, a decrease in presence of the epistratum-feeders such as *P. ponticus* and *K. reflectans* will inevitably occur. The proliferation of three opportunistic species, *S. rufum* (2B), *L. hospitum* (1B) and *B. tenuicaudatus* (2B) could be simply the result of their feeding regimes. Indeed, depending on the available food source, predators (2B) may occasionally become strict scavengers (Bastian, 1865; Moens & Vincx, 1997). It seems that these species followed such behaviour when Colchicine-sensitive species (*P. ponticus* and *K. reflectans*) died. This may also explain why the simultaneous occurrence of *S. rufum* (2B) and *L. hospitum* (1B) has been noted in the microcosms of the first Colchicine dose. The dissimilarities between the control community and those of the higher Colchicine doses were due to the proliferation of *S. rufum* for D2 and *L. hospitum* for D3. For nematodes of D2, the increase in abundance of *S. rufum* may reflect the availability of dead sensitive nematodes, but also that food sources remain enough diverse (e.g. bacteria, ciliates, cyanobacteria, green algae, nematodes, and oligochaetes. The high amount of Colchicine for D3 was expressed with high dead organic matter ready to be decomposed. That may explain easily why the non-selective deposit feeder species *L. hospitum* had a clear advantage.

Although the two types of effects (direct and indirect) can, in absolute terms, occur simultaneously, it is known that abiotic environment acts on animals before biotic interactions especially when the stress period is short (Ramade & Papigny, 2000). Thus, since our experiment ended after only one month, Colchicine seems to act much more directly than through biotic interactions. Accordingly, Colchicine can take action directly (1) on or (2) through cuticles of nematodes and/or (3) after their ingestion of contaminated sediments. Our results suggest no eutely into the cuticular epidermis, pharynx and intestinal cells and evoke a mitotic arrest inside these tissues particularly for three Colchicine-sensitive species, namely, *P. ponticus*, *T. modicus* and *K. reflectans*. Similar findings were previously validated in the case of parasitic or soil nematodes: *O. curvula* (Martini, 1923), *R. anomala* (Wessing, 1953) and *P. redivivus* (Sternberg & Horvitz, 1982) and also for many free-living marine species belonging to the orders Enoplida (*Enoplus brevis* Bastian, 1865, *Pontonema vulgare* (Bastian, 1865), *Adoncholaimus thalassophygas* (de Man, 1876) and *Anoplostoma viviparum* (Bastian, 1865)), Chromadorida (*Paracanthonchus caecus* (Bastian, 1865)), Monhysterida (*Sphaerolaimus balticus* Schneider, 1906) and Araeolaimida (*Axonolaimus spinosus* (Bütschli, 1874)) where an absence of eutely in the cuticular epidermis was proved by Rusin & Malakhov (1998) and Voronov & Panchin (1998).

This work gave an experimental evidence of increased mortality of *P. ponticus*, *T. modicus* and *K. reflectans* after their exposure to an antimitotic (Colchicine). With respect to the community studied, it say simply ‘yes’ to answer the question ‘are there any possible mitoses in nematode tissues?’ and gave responses to the subsequent questions: (1) ‘which species could be non eutelic?’ and (2) ‘which tissues could be non eutelic?’. Now, further studies should be undertaken to verify the hypotheses related to this study by using more rigorous histological and molecular techniques (e.g. radioactive marking, DNA sequencing).

Acknowledgments

We thank Dr. Amor Hedfi for its support during the sampling and the setting up the experiment. Pr. John H. Tietjen and Dr. Punyasloke Bhadury are acknowledged for their critical revision of the English. The anonymous referees are also acknowledged for their constructive comments on an earlier version of the manuscript.

References

Bastian H.C. 1865. Monograph of the Anguillulidae, or free Nematoids, marine, land, and freshwater; with descriptions of 100 new species. *The Transactions of the Linnean Society of London*, **XXV**: 73-184.

Beyrem H. & Aïssa P. 2000. Les nématodes libres, organismes-sentinelles de l'évolution des concentrations d'hydrocarbures dans la baie de Bizerte (Tunisie). *Cahiers de Biologie Marine*, **41**: 329-342.

Beyrem H., Mahmoudi E., Essid N., Hedfi A., Boufahja F. & Aïssa P. 2007. Individual and combined effects of cadmium and diesel on a nematode community in a laboratory microcosm experiment. *Ecotoxicology and Environmental Safety*, **68**: 412-418.

Beyrem H., Louati H., Essid N., Aïssa P. & Mahmoudi E. 2010. Effects of two lubricant oils on marine nematode assemblages in a laboratory microcosm experiment. *Marine Environmental Research*, **69**: 248-253.

Boufahja F., Hedfi A., Amorri J., Aïssa P., Beyrem H. & Mahmoudi E. 2010. Approche dynamique de biosurveillance chez les nématodes libres de la baie de Bizerte (Tunisie). *Bulletin de la Société Zoologique de France*, **135**: 223-236.

Boufahja F., Hedfi A., Essid N., Aïssa P., Beyrem H. & Mahmoudi E. 2011. Morphometric and energetic differences between nematode feeding groups of Moens & Vincx (1997) and their suitability for the environmental biomonitoring. *Ekólogia (Bratislava)*, **30**: 91-109.

Boufahja F., Hedfi A., Essid N., Aïssa P., Mahmoudi E. & Beyrem H. 2012. An observational study on changes in biometry and generation time of *Odontophora villoti* (Nematoda, Axonolaimidae) related to petroleum pollution in Bizerte bay, Tunisia. *Environmental Science and Pollution Research***, 19**: 646-655**.**

Boufahja F., Vitiello P. & Aïssa P. 2014. More than 35 years of studies on marine nematodes from Tunisia: a checklist of species and their distribution. *Zootaxa*, **3786**: 269-300.

Brito M.A., Brites D., Butterfield D.A. 2004. A link between hyperbilirubinemia, oxidative stress andinjurytoneo-corticalsynaptosomes. *Brain Research*, **1026**: 33-43.

Carman K.R., Fleeger J.W., Means J.C., Pomarico S.M. & Mc Millin D.J. 1995. Experimental investigation of the effects of polynuclear aromatic hydrocarbons on an estuarine sediment food web. *Marine Environmental Research*, **40**: 289-318.

Clarke K.R. & Ainsworth M. 1993. Method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, **92**: 205-219.

Clarke K.R. & Gorley R.N. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, U.K.

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

Deprez T., Steyaert M., Vanaverbeke J., Speybroeck J., Raes M., Derycke S., De Smet G., Ingels J., Vanreusel A., Van Gaever S. & Vincx M. 2005. NeMys, World Wide Web electronic publication, www.nemys.ugent.be. Department of Marine Biology, Ghent University.

Fabiano M. & Danovaro R. 1994. Composition of organic matter in sediment facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. *Hydrobiologia*, **277**: 71-84.

Hedfi A., Boufahja F., Ben Ali M., Aïssa P., Mahmoudi E. & Beyrem H. 2013. Do trace metals (chromium, copper and nickel) influence toxicity of diesel fuel for free-living marine nematodes? *Environmental Science and Pollution Research***, 20**: 3760-3770.

Heip C., Vincx M., Smol N. & Vranken G. 1982. The systematics and ecology of free-living marine nematodes. *Helminthological Abstracts (B)*, **51**: 1-31.

Juario J.V. 1975. Nematode species composition and seasonal fluctuation of asublittoral meiofauna community in the German Bight. *Veröentlichungen des Instituts fur Meeresforschschung in Bremerhaven*, **15**: 283-337.

Keller M. 1984. Influence du rejet en mer de l'égout de Marseille sur les peuplements du méiobenthos. Thèse de doctorat, Université d'Aix-Marseille II. 131 pp.

Krzaczkowski L. 2008. Recherche exploratoire de substances actives en pharmacologie antitumorale à partir des Bryophytes. Thèse de Doctorat en Pharmacologie. Université de Toulouse III, 199 pp.

Lacaze J.C. 1993. *La dégradation de l'environnement côtier - conséquences écologiques.* Masson : Paris. 149 pp.

Lorenzen S. 1974. Die Nematode fauna der sublittoralrn region der deutschenBucht, inbesondere im Titan-Abwassergebiet bei Helogoland. *Verffentlichungen des Instituts Meeresforschung in Bremerhaven,* **Suppl. 14**: 305-327.

Mahmoudi E., Essid N., Beyrem H., Hedfi A., Boufahja F., Vitiello P. & Aïssa P. 2005. Effects of hydrocarbon contamination on a free living marine nematode community: results from microcosm experiments. *Marine Pollution Bulletin*, **50**: 1197-1204.

Mahmoudi E., Essid E., Beyrem H., Hedfi A., Boufahja F., Vitiello P., Aïssa P. 2007. Individual and combined effects of lead and zinc of a free living marine nematode community: results from microcosm experiments. *Journal of Experimental Marine Biology and Ecology*, **343**: 217-226.

Mahmoudi E., Essid E., Beyrem H., Hedfi A., Boufahja F., Aïssa P., Vitiello P. 2008. Mussel-farming effects on Mediterranean benthic nematode communities. *Nematology*, **10**: 323-333.

Malakhov V.V. 1994. *Nematodes. Structure, development, classification and phylogeny.* Smithsonian Institution Press: Washington, DC. 286 pp.

Martini E. 1923. Die Zellkonstanz und ihre Beziehungen zu anderen zoologischen Vorwürfen. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, **70**: 179-259.

Millward R.N., Carman K.R., Fleeger J.W., Gambrell R.P. & Portier R.J. 2004. Mixtures of metals and hydrocarbons elicit complex responses by a benthic invertebrate community. *Journal of Experimental Marine Biology and Ecology*, **310**: 115-130.

Moens T. & Vincx M. 1997. Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, **77**: 211-227.

Nidiry E.S.J., Khan R.M. & Reddy P.P. 1993. *In vitro* nematicidal activity of *Gloriosa superba* seed extract against *Meloidogyne incognita*. *Nematologia Mediterranea*, **21**: 127-12

Platt H.M. & Warwick R.M. 1983. *Free-living marine nematodes. Part I. British Enoplids.* Synopses of the British Fauna no. 28. Cambridge University Press: Cambridge: 314 pp.

Platt H.M. & Warwick R.M. 1988. *Free-living marine nematodes. Part II. British Chromadorids.* Synopsis of the

British fauna (New Series) No. 38, E.J. Brill/W. Backhuys: Leiden. 502 pp.

Ramade F. & Papigny A-G. 2000. *Dictionnaire encyclopédique des pollutions: les polluants de l'environnement à l'homme.* Ediscience international: Paris. 690 pp.

Rusin L.Y. & Malakhov V.V. 1998. Free-living nematodes possess no eutely. *Doklady Biological Sciences*, **361**: 331-333.

Rzeznic-Orignac J. 2004. Contribution de la méiofaune aux transferts des métaux dans le réseau trophique de la Baie de Gdansk (Baltique) et de la baie de Marennes Oléron (Atlantique). PhD Thesis, Universities of La Rochelle and Gdansk, 266 pp.

Seinhort J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, **4**: 67-69.

Sternberg P.W. & Horvitz H.R. 1982. Post-embryonic non-gonadal cell lineages of the nematode *Panagrellus redivivus*: description and comparison with those of *Caenorhabditis elegans*. *Developmental Biology*, **93**: 181-205.

Tietjen J.H. 1977. Population distribution and structure of the free-living nematodes of Lang Islands. *Marine Biology*, **43**: 123-136.

Vanaverbeke J., Martinez Arbizu P., Dahms H.U. & Schminke H.K. 1997. The metazoan meiobenthos along a depth gradient in the Arctic Laptev Sea with special attentions to nematode communities. *Polar Biology*, **18**: 391-401.

Van Cleave H.J. 1932. Eutely or cell constancy in its relation to body size. *Quarterly Review of Biology*, **7**: 59-67.

Voronov D.A. & Panchin Y.V. 1998. Cell lineage in marine nematode *Enoplus brevis*. *Development*, **125**: 143-150.

Warwick R.M. & Price R. 1979. Ecological and metabolic studies on free-living nematodes from an estuarine sand flat. *Estuarine and Coastal Marine Science*, **9**: 257-271.

Warwick R.M., Platt H.M. & Somerfield P.J. 1998. *Free-living marine nematodes. Part III. British monohysterids.* Synopsis of British fauna (new series) No. 53. Field Studies Council: Shrewsbury. 296 pp.

Wessing A. 1953. Histologische Studien zu den Problemder Zellkonstanz: Untersuchungen an Rhabditis anomala P. Hertwig. *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere*, **73**: 69-102.

Wieser W. 1953. Die Beziehung zwischen Mundhoehlengestalt, Ernaehrugsweise und Vorkommen bei freilebenden marinen nematoden. *Arkiv für zoologie*, **4**: 439-484.

Wieser W. 1960. Benthic studies in Buzzards Bay. II. The meiofauna. *Limnology and Oceanography*, **5**: 121-137.

Wiggers R.J., Thornton N.T. & Starr J.L. 2002. The effects of Colchicine on number of giant cell nuclei and nematode development in *Pisum sativum* infected by *Meloidogyne incognita*. *Nematology*, **4**: 107-109.