EFFECTS OF HEAVY METAL SOIL POLLUTION ON NEMATODE COMMUNITIES AFTER THE AZNALCOLLAR MINING SPILL

A. Navas[1], P. Flores-Romero[1], S. Sánchez-Moreno[2], J. A. Camargo[3], and E. C. McGawley*[,4]

[1]Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales. CSIC. C/ José Gutierrez Abascal, 2 Madrid 28006, Spain; [2]Unidad de Fitosanitarios, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid 28040, Spain; [3]Departamento de Ecología, Universidad de Alcalá. Alcalá de Henares 28871, Madrid, Spain; and [4]Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803 USA. *Corresponding author: emcgawley@agctr.lsu.edu.

ABSTRACT

Navas, A. P. Flores-Romero, S. Sanchez-Moreno, J. A. Camargo, and E. C. McGawley. 2010. Effects of heavy metal soil pollution on nematode communities after the Aznalcollar mining spill. Nematropica 40:13-29.

In 1998, the rupture of a mining reservoir containing mineral residues polluted a large section of Guadiamar River Basin in southern Spain with heavy metals. To determine the effect of the spill on soil nematodes, soil samples were collected in 24 locations in a non-polluted area (48 samples) and in 57 locations (89 samples) in the polluted area. Soil content of lead (Pb), cadmium (Cd), nickel (Ni), copper (Cu), titanium (Ti), vanadium (V), and zinc (Zn) was analyzed. Maturity Indices (MI, MINO, PPI, ∑MI) and Diversity Indices (Trophic Diversity (T), Shannon-Weaver (H'), Simpson (D), Margalef and Camargo) were calculated to assess the effects of soil pollution on nematode communities in the polluted area. Differences in heavy metal content were observed between the non-polluted and polluted sites. Forty-five nematode genera were identified, with decreasing numbers in the polluted area five months after the spill. Nematode taxa abundance and frequency, as well as diversity and maturity indices, were all negatively affected by the toxic sediments. Factorial Analysis of Correspondence, used to infer nematode sensitivity to metal soil pollution, showed six different associations of nematode genera associated to different metal content ranges. The effect of soil covering by the toxic mud on total number of nematodes was demonstrated experimentally. The diversity and maturity of the nematode community was significantly lower in the polluted than in the non-polluted area five months after the spillage. Ni and Cu seemed to be the metals most toxic to the nematode community.

Key words: bioindicator, diversity index, ecology, heavy metals, nematode, soil pollution.

RESUMEN

Navas, A. P. Flores-Romero, S. Sanchez-Moreno, J. A. Camargo, and E. C. McGawley. 2010. Efectos de la contaminación con metales pesados en las comunidades de nematodos después del derrame de Aznalcóllar. Nematropica 40:13-29.

En 1998, la ruptura de una reserva de minería que contenía residuos minerales contaminó con metales pesados una sección grande de la Cuenca del Río Guadiamar en el sur de España. Para determinar el efecto del derrame sobre los nematodos del suelo, se colectaron muestras de suelo en 24 lugares no contaminados (48 muestras) y en 57 lugares contaminados (89 muestras). Se analizó el contenido de plomo (Pb), cadmio (Cd), níquel (Ni), cobre (Cu), titanio (Ti), vanadio (V) y zinc (Zn). Se calcularon los Indices de Madurez (MI, MINO, PPI, ∑MI) y de Diversidad (Diversidad Trófica (T), Shannon-Weaver (H'), Simpson (D), Margalef y Camargo) para medir los efectos de la contaminación sobre las comunidades de nematodos. Se observaron diferencias en el contenido de metales pesados entre los lugares contaminados y los no contaminados. Se identificaron 45 géneros, con menores densidades en las zonas contaminadas cinco meses después del derrame. Los sedimentos

tóxicos afectaron negativamente la abundancia y la frecuencia de taxones de nematodos, así como los índices de diversidad y de madurez. El Análisis Factorial de Correspondencia, utilizado para inferir la sensibilidad de los nematodos a la contaminación por metales, indicó seis asociaciones de géneros de nematodos con rangos de contenido de metales. El efecto del cubrimiento con el lodo tóxico se demostró experimentalmente. La diversidad y la madurez de la comunidad de nematodos fueron significativemente más bajas en las áreas contaminadas que en las no contaminadas cinco meses después del derrame. El nitrógeno y el cobre fueron los metales más tóxicos para la comunidad de nematodos.

Palabras clave: bioindicador, contaminación del suelo, ecología, índice de diversidad, metales pesados, nematodo.

INTRODUCTION

In April 1998, the breakage of a reservoir containing mineral residues at the Aznalcollar Mine in Southern Spain discharged more than 5 hm^3 of mud and acid water loaded with high levels of heavy metals, producing one of the worst ecological disasters ever recorded in Spanish history. Though spillage was mechanically controlled, a 60 km stretch of the Guadiamar River basin, the main water source of one of the most important European wetlands (Doñana National Park), was thoroughly covered by a fine toxic sludge. This became a 20 cm thick layer of dry toxic silt within a few days. Ten days after rupture of the reservoir, the concentrations of metals in the soil was up to 50 times higher in the polluted than in the non-polluted area that was situated upstream from the spill point. In subsequent years, much scientific effort focused on the fate of visible metazoa and flora but little attention was given to the pollution effects of heavy metals on microscopic soil fauna including nematodes.

Nematodes are the most abundant metazoans that inhabit soils. Their great abundance and diversity play important roles in the soil food web, affecting soil nitrogen mineralization, organic matter decomposition, microbial growth and carbon distribution and allocation in the rhizosphere (Chen, 1999; DeRuiter, 1995; Djigal, 2004; Poll, 2007; Sohlenius, 1988; Yeates, 1987). The structure of the nematode community may be used as one indicator of physical disruption of soil or pollution and has been proposed as a tool useful in the overall evaluation of soil health (Bongers, 1990; De Goede, 1993; Ekschmitt, 2001; Ettema, 1993; Freckman, 1993). Several maturity indices (Bongers, 1990; Yeates, 1994), based on the relative abundance of nematode families classified along a colonizer-persister (c-p) scale, have been shown, under laboratory and field conditions, to accurately reflect soil pollution with metals (Georgieva, 2002; Korthals, 1996a-d). The c-p scale classifies nematodes into five groups, from c-p 1 (microbial feeding, enrichment opportunistic nematodes with short life cycles, r-strategists) to c-p 5 (sensitive predatory and omnivore nematodes with long life cycles, K-strategists) (Bongers, 1995). The abundance of such c-p groups reflects the nematode assemblage recovery after disturbance and the process of ecological succession (Hanel, 2003). In general, nematode density and diversity decrease with heavy metal pollution and acidification (Gyedu-Ababio, 1999; Parmelee, 1997; Ruess, 1992; Yeates, 1994).

In this paper, we present historical data to evaluate short and medium-term effects of heavy metal pollution of soil on the nematode community. The objectives of this

research were to: a) evaluate acute effects of spillage and heavy metal pollution on the nematode community composition, trophic groups, diversity, and maturity; b) determine which descriptors of the nematode community best reflect the effects of soil contamination; and c) study the responses of individual nematode taxa to heavy metal contamination.

MATERIALS AND METHODS

Preliminary experiment: influence of coverage with non-polluted soil silt on nematode communities

In a non-polluted area along the banks of the Guadiamar River, two different blocks (each block 10 × 4 m) were used to establish eight plots (5 × 2 m; four plots per block). Plots were either covered with "clean" silt (non polluted and biologically inert river silt) or left uncovered, comprising two treatments, each replicated four times. Covered plots received a 20 cm depth layer of silt (8 m^3 of semi-dry river silt). After seventy days (December 17 to February 25), the silt covering was removed and five subsamples taken to a depth of 15 cm deep were collected from each of the 8 plots. Nematodes were processed, extracted and compared using the procedures and indices described below.

Study area and soil sampling

The study area was located along the banks of the Guadiamar River, which constitutes the main hydrographic system feeding the mars of Doñana National Park. This area is the end of the ecological "corridor" that connects two of the most iconic Mediterranean ecosystems in Southern Spain, the high mountains of Sierra Morena and the highly diverse coastal sand dunes. The study area has a Mediterranean sub-humid climate, with a yearly average temperature of 18-19°C, and is a homogenous physiographic, biological and ecological unit of about 50 km in length.

Soil samples were collected at each sampling site along the Guadiamar River banks, both above and below the spill point (Fig. 1). Vegetation was composed of herbaceous/grassland, reeds (*Juncus* spp.), oleander (*Nerium oleander*), ash (*Fraxinus excelsior*) and poplar (*Populus alba*).

In June 1998, one month after the rupture, soil samples were collected at 32 one square meter sites down-stream from the spill point after physically removing the

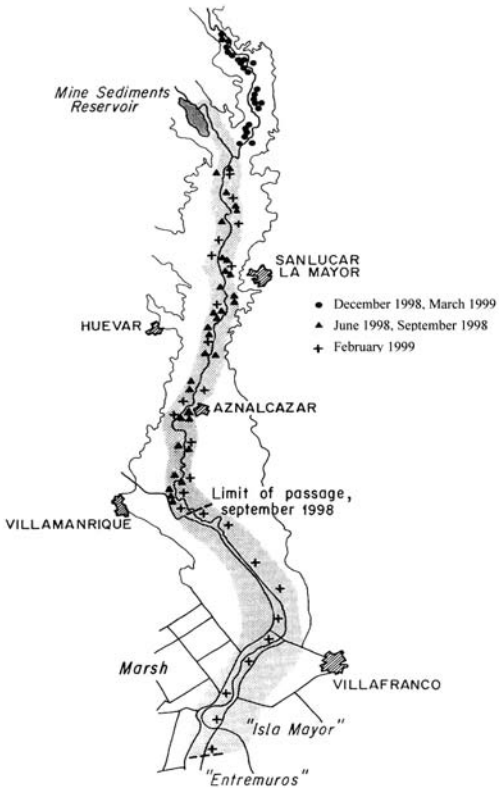

Fig. 1. Distribution of sampling localities in the area along the Guadiamar River banks affected by the Doñana Park mining spill. Shaded area was covered by the toxic mud. Dotted line indicates the limit of passage of the toxic spill. Samples taken at different dates are indicated with different symbols (● control samples; ▲ and +, affected samples).

overlying layer of toxic mud. These sampling sites, situated in the riverbank between two and five meters away from the main water channel, were positioned at regular intervals from the spill point to the edge of the Doñana marshes. Locations of each site were recorded and re-sampled in September 1998. An additional 25 sites were established and the toxic mud was removed in July of 1998 (2 months after the spill). Soil at these sites along the river bank was re-sampled in February of 1999. As a non-polluted control, twenty-four samples from non-affected localities, situated above the affected area, were collected in December 1998 and March 1999. None of the sampling localities were buried by the toxic mud more than 68 days. Four subsamples collected at 0-15 cm depth were collected with a hoe at each sampling site and mixed together by hand, composing the final one-kg sample of homogenized soil that was used for nematode and metal analyses. Samples were maintained at 5°C until processing. Metal content of the sludge ranged between 15.8-31.4 mg/kg for Ni, 34.8-78.9 for V, 1100.7-2175 mg/kg for Cu, 6246.6-8063.4 mg/kg for Zn, and 4352.9-9635.9 mg/kg for Pb. No data on Ti soil content are available (Simon, 1999).

Nematode extraction and identification

Nematodes were extracted from a of 100 cm^3 subsample of soil using the sugar centrifugal flotation method (Barker, 1985) and enumerated using a dissecting microscope at 40X. At least 100 nematodes from each sample were identified to the genus and family levels based on the system of Bongers and Tarjan (Bongers, 1989; Tarjan, 1977). If less than 100 nematodes were present in a sample, all of nematodes were identified. When necessary, further identifications were performed using a light microscope. Nematode taxa were then classified into five trophic groups: bacterial feeders, fungal feeders, plant parasites/herbivores, predators and omnivores. Nematode diversity and maturity indices data were then calculated for each sample. Shannon (H'; [Pielou, 1977]) and Simpson's (D; [Pielou, 1977]) diversity indices assess the relation between the richness and abundance of taxa in a community. The Trophic diversity index (T; [Heip, 1998]) measures the relative contribution of each trophic group to the community. Margalef's (Margalef, 1977) and Camargo's diversity indices (Camargo, 1992 and 1995] were included in this study to evaluate their usefulness in assessing the effect of pollution on nematode communities in soil. Maturity Indices were also calculated: MI (for free living nematodes, [Bongers, 1989]), PPI (for plant parasitic nematodes, [Bongers, 1990]), $\sum$MI (free living and plant parasitic nematodes, [Yeates, 1994]) and MINO (or $MI_{2\text{-}5}$; free living nematodes excluding opportunistic nematodes with c-p value = 1, (Popovici, 1992). Samples containing no nematodes were included in the analyses with a diversity or maturity value = 0.

Soil analyses

Soils were luvisols/cambisols classified as sandy loam (92%) or sandy clay loam (8%). No significant differences ($P \geq 0.05$, data not shown) were detected among sampling sites for pH values (the average for all samples was 6.9). For each soil sample, Pb, Cd, Ni, Cu, Ti, V and Zn contents were analyzed in a graphite chamber connected to an atomic absorption spectrometer (Perkin Elmer, Mod. HGA500). Soil texture was determined by the granulometrical density procedure and pH was measured in saturated paste 1:1, water to soil.

Statistical analyses

One-way ANOVA and post hoc tests (Tukey for unequal N S/S test) were used

to assess significant differences in soil metal content, nematode community composition and abundance at different sampling times. Percentage of samples in which each taxa appeared in soil from the polluted and the non-polluted sampling sites was compared using contingency tables analyzed by chi-square tests.

Discriminate Analysis and Multiple Regression (calculation of intercept has been chosen as included in the model) were used to estimate the effects of heavy metals on nematode diversity and maturity indices. Backward Stepwise Discriminate Analysis was used to assess differences between the polluted and the non-polluted sites, taking into account all sampling dates and biological indices. The individual discrimination power of each index is expressed as the value of the F-statistic, determining whether the variable was retained in the model. Data were logarithmically transformed (log (x + 1)) in order to standardize the variables. Finally, Correspondence analysis was applied in order to detect an overall structure in nematode community taxa composition, diversity and maturity relative to heavy metal pollution. STATISTICA 6.0 software (Statsoft, 1996) was employed for analysis of data.

RESULTS

Preliminary experiment

Most of the nematode taxa present in soil in the non-covered plots of the preliminary experiment were also present in soil at the study site. Only seven genera (*Dolichodorus*, *Helicotylenchus*, *Hemicycliophora*, *Hoplolaimus*, *Monhystera, Prismatolaimus* and *Rotylenchus*) were absent in soil at the experimental site. Covering soil with a layer of inert silt increased the total number of nematodes in the community by 27% ($P \leq 0.05$), while all the other descriptors did not show significant differences in nematode parameters between covered and non-covered plots (Table 1).

Table 1. Mean (± standard error), nematode abundance (total nematodes/kg of soil), maturity and diversity indices in covered and non-covered plots.

	Non-covered plots (n = 20)	Covered plots (n = 20)	F-value
Total Nematodes	550 ± 153	750 ± 321	6.26***
MI	2.32 ± 0.1	2.17 ± 0.1	2.731 NS
MINO	2.57 ± 0.1	2.53 ± 0.1	0.092 NS
∑MI	2.43 ± 0.1	2.31 ± 0.4	1.498 NS
PPI	2.53 ± 0.1	2.72 ± 0.1	2.117 NS
Margalef	1.57 ± 0.6	1.49 ± 0.4	0.284 NS
Shannon (H´)	2.87 ± 0.7	2.77 ± 0.6	0.278 NS
Simpson (D)	7.24 ± 3.6	5.65 ± 2.2	2.792 NS
T.Diver.(T)	2.60 ± 0.6	2.38 ± 0.5	1.530 NS
Camargo	10.84 ± 4.1	10.75 ± 3.1	0.007 NS

F-value and level of significance for each model is indicated.
NS = not significant; *** $P \leq 0.001$.

Soil metal content

Concentrations of Pb, Ni, Cu, Ti, and Zn were generally significantly higher in the polluted than in the non-polluted area ($P \leq 0.05$) (Table 2). Cd soil concentrations were below detection levels (<0.3 mg/kg soil; data not shown). Pb soil content was approximately 10 times greater in the polluted site than in the non-polluted site. Ni, Cu, Ti, and Zn were, respectively, approximately 1.62, 100.00, 2.85, and 10.30 times greater in the polluted sites than in the non-polluted sites.

Nematode community composition, diversity and maturity

Forty-five different genera of soil-inhabiting nematodes were identified (Table 3). In both the non-polluted and polluted sites, the frequency of taxa (percentage of samples in which each taxon is present) was influenced significantly by both sampling date and site. Taxa appeared less frequently in the polluted site than in the non-polluted site up to February of 1999. According to Chi-square analysis, only *Nothotylenchus* appeared more frequently in the polluted than in the non-polluted site ($P \leq 0.05$). Twelve taxa (*Acrobeles*, *Alaimus*, *Aphelenchus*, *Boleodorus*, Dorylaiminae, *Monhystera*, *Mononchus*, *Panagroalimus*, *Pratylenchus*, *Pratylenchoides*, and *Trichodorus*) occurred in a significantly greater numbers ($P \leq 0.05$) of samples in the non-polluted site. Eight months after the initial toxic spill, 24 taxa of nematodes were not found in soil at the polluted site.

Results of the analysis of variance indicate that the toxic sediments present in soil at the polluted site affected nematode abundance and diversity. The MI, MINO, D, T, and Camargo diversity indices were significantly lower in the polluted than in the non-polluted site beginning with the September 1998 sampling. ∑MI, PPI, Margalef diversity index and H' presented significantly lowered values in February 1999. Heavy metals and diversity and maturity indices did not indicate significant differences between the two sampling dates in soil of the non-polluted site.

Discriminant analysis

Squared Mahalanobis distances among centroids of the five sampling dates confirmed (Table 4) that there were no significant differences in nematode assemblage diversity and maturity between December 1998 and March 1999 in the non-polluted site. Differences between the non-polluted site and the polluted site ($F = 71.07$; $p < 0.0001$) were more evident when samples from the non-polluted site were grouped together irrespective of sampling interval (Fig. 2). The Camargo diversity index, PPI, T, and MI, had the greatest discrimination power (Table 5), being able to distinguish among the five sampling dates. While the total number of nematodes was not significantly correlated with any other descriptor, the Camargo diversity index was strongly correlated to the other diversity indices: Margalef ($r = 0.93$, $P \leq 0.001$), H' ($r = 0.84$, $p < 0.001$), D ($r = 0.73$, $P \leq 0.001$), T ($r = 0.57$, $P \leq 0.05$), while the MI was correlated to PPI ($r = -0.76$, $P \leq 0.001$) and MINO ($r = 0.61$, $P \leq 0.001$).

Multiple regression analysis

Multiple regression was used to estimate the combined effects of heavy metals on nematode abundance, maturity and diversity. The number of indices that were significantly affected by Ni and Cu indicated that these heavy metals were the most relevant pollutants, followed by Ti and Zn (Table 6). However, the effects of the other pollutants (except V that shows low correlation values) may be masked by their strong correlation with Cu and Ni (data not shown). The pro-

Table 2. Mean (± standard error) heavy metal soil content (mg/kg soil), nematode abundance (total nematodes per kg of soil), and maturity and diversity indices at different sampling intervals.

	Non-polluted site		Polluted site			
	December 1998 n = 24	March 1999 n = 24	June 1998 n = 32	September 1998 n = 32	February 1999 n = 25	F-value
Pb (lead)	11.6 ± 15.1 a	11.7 ± 5.7 a	134.0 ± 105.0 b	120.0 ± 102.8 b	73.0 ± 139.1 ab	35.419***
Ni (nickel)	7.9 ± 4.1 a	7.9 ± 4.0 a	11.6 ± 5.7 ab	13.2 ± 4.1 b	13.5 ± 8.5 b	12.557***
Cu (copper)	6.7 ± 4.4 a	7.1 ± 4.1 a	106.6 ± 132.0 b	91.7 ± 87.6 b	102.7 ± 66.5 b	84.420***
Ti (titanium)	596.2 ± 316.0 a	646.4 ± 231.0 a	900.1 ± 280.0 b	873.1 ± 288.0 b	1065.4 ± 224.7 b	14.325***
V (vanadium)	25.3 ± 8.8	27.9 ± 6.1	29.2 ± 4.2	29.2 ± 4.0	30.8 ± 9.4	2.130 NS
Zn (zinc)	48.3 ± 67.2 a	48.3 ± 69.8 a	502.1 ± 743.3 ab	373.9 ± 454.0 ab	610.4 ± 1082.0 b	43.245***
Total Nematodes	593.4 ± 481.5	673.8 ± 759.7	654.1 ± 729.9	773.1 ± 1481.0	94.8 ± 181.4	2.089NS
MI	2.88 ± 0.2 a	2.43 ± 0.2 a	2.23 ± 0.2 ab	1.58 ± 0.2 b	1.54 ± 0.2 b	8.092***
MINO	3.09 ± 0.2 a	2.68 ± 0.2 a	2.45 ± 0.2 ab	1.75 ± 0.3 b	1.59 ± 0.3 b	8.424***
∑MI	2.56 ± 0.3 a	2.47 ± 0.4 a	2.40 ± 0.4 a	2.24 ± 0.3 a	1.79 ± 0.9 b	6.938***
PPI	2.29 ± 0.1 a	2.59 ± 0.1 a	2.44 ± 0.1 a	2.25 ± 0.1 a	1.47 ± 0.2 b	12.774***
Margalef	1.35 ± 0.6 a	1.30 ± 0.5 a	0.99 ± 0.4 a	0.59 ± 0.6 ab	0.41 ± 0.4 b	15.929***
Shannon (H´)	2.41 ± 0.7 a	2.53 ± 0.6 a	2.06 ± 0.6 a	1.33 ± 1.0 ab	1.04 ± 0.9 b	17.606***
Simpson (D)	4.64 ± 2.1 a	5.07 ± 1.8 a	3.61 ± 1.5 ab	2.66 ± 1.7 b	2.22 ± 1.8 b	10.406***
T.Diver.(T)	2.04 ± 0.8 a	2.18 ± 0.6 a	1.72 ± 0.7 ab	1.44 ± 0.5 b	1.63 ± 0.9 ab	5.114***
Camargo	8.85 ± 3.1 a	8.87 ± 3.2 a	7.10 ± 3.0 a	4.63 ± 3.7 b	2.60 ± 2.2 b	19.750***

F-value and level of significance for each model is indicated. Means within rows with the same or no letter are not significantly different according to Tukey's HSD test ($P \geq 0.05$). NS= not significant; *** $P \leq 0.001$.

Table 3. Frequency and average abundance of nematodes/kg of soil at different sampling intervals in the Guadiamar River basin.

		Non-polluted site				Polluted site					
		December 1998 N[x] = 24		March 1999 n = 24		June 1998 n = 32		September 1998 n = 32		February 1999 n = 25	
Plant Parasites	c-p[w] value	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.
Apratylenchoides (Apr)[y]	3	0.0	0 ± 0.0	0.0	0 ± 0.0	3.1	10 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0
Boleodorus (Bol)	2	29.2	35 ± 8.4	25.0	386 ± 246.2	9.4	30 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0
Cephalenchus (Ceph)	2	0.0	0 ± 0.0	8.3	85 ± 15.0	6.2	85 ± 15.0	12.5	55 ± 18.5	0.0	0 ± 0.0
Criconemoides (Cri)	3	25.0	24 ± 9.5	29.1	42 ± 13.5	21.9	285 ± 255.8	15.6	22 ± 8.0	0.0	0 ± 0.0
Dolichodorus (Dol)	3	4.1	10 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0
Helicotylenchus (Hel)	3	20.8	26 ± 16.0	8.3	115 ± 95	15.6	204 ± 160.0	6.2	1470 ± 1330.0	4.0	10 ± 0.0
Hemicycliophora (Hem)	3	8.3	195 ± 16.5	8.3	125 ± 75	18.7	33 ± 5.6	6.2	490 ± 470.0	4.0	10 ± 0.0
Heterodera (Het)	3	4.2	10 ± 0.0	4.1	450 ± 0.0	0.0	0 ± 0.0	3.1	300 ± 0.0	0.0	0 ± 0.0
Hoplolaimus (Hop)	3	4.2	10 ± 0-0	0.0	0 ± 0.0	0.0	0 ± 0.0	3.1	10 ± 0.0	0.0	0 ± 0.0
Paratylenchus (Par)	2	91.7	217 ± 83.8	66.6	54 ± 10.7	81.2	225 ± 79.8	81.2	260 ± 65.1	24.0	23 ± 5.6
Pratylenchus (Prat)	3	54.2	34 ± 5.6	41.0	92 ± 52.9	37.5	74 ± 21.2	31.2	35 ± 14.4	12.0	23 ± 8.8
Pratylenchoides (Praty)	3	4.1	30 ± 0.0	16.6	25 ± 2.8	0.0	0 ± 0.0	0.0	0 ± 0.0	8.0	10 ± 0.0
Psylenchus (Psy)	2	29.2	63 ± 19.6	20.8	40 ± 5.4	21.8	67 ± 21.5	3.1	180 ± 0.0	4.0	80 ± 0.0
Rotylenchus (Rot)	3	4.1	10 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0	3.1	10 ± 0.0	0.0	0 ± 0.0
Tetylenchus (Tet)	2	0.0	0 ± 0.0	12.5	267 ± 227.0	9.4	33 ± 23.3	0.0	0 ± 0.0	0.0	0 ± 0.0
Trichodorus (Tri)	4	25.0	56 ± 28.1	37.5	111 ± 21.2	25.0	33 ± 4.9	6.2	25 ± 5.1	0.0	0 ± 0.0
Tylenchorhynchus (Tyle)	2	50.0	49 ± 19.4	41.7	29 ± 5.8	43.7	116 ± 44.9	31.2	48 ± 10.6	16.0	27 ± 7.5

[w]See Bongers, 1990.
[x]n= number of samples.
Freq. = frequency.
Abund. = abundance.
[y]for genera in and references of Fig. 3.

Table 3. (Continued) Frequency and average abundance of nematodes/kg of soil at different sampling intervals in the Guadiamar River basin.

		Non-polluted site				Polluted site					
		December 1998 N[x] = 24		March 1999 n = 24		June 1998 n = 32		September 1998 n = 32		February 1999 n = 25	
Plant Parasites	c-p[w] value	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.	Freq.	Abund.
Tylenchus (Tyl)	2	66.6	133 ± 37.2	70.8	98 ± 24.5	59.3	138 ± 64.8	50.0	85 ± 27.8	36.0	116 ± 96.0
T. semipenetrans (Tsem)	2	0.0	0 ± 0.0	0.0	0 ± 0.0	3.1	80 ± 0.0	3.1	7920 ± 0.0	0.0	0 ± 0.0
Xenocriconemella (Xen)	3	0.0	0 ± 0.0	0.0	0 ± 0.0	0.0	0 ± 0.0	3.1	10 ± 0.0	0.0	0 ± 0.0
Xiphinema (Xip)	5	4.1	10 ± 0.0	12.5	10 ± 0.0	3.1	20 ± 0.0	3.1	10 ± 0.0	0.0	0 ± 0.0
Zigotylenchus (Zig)	3	0.0	0 ± 0.0	0.0	0 ± 0.0	6.2	190 ± 180.0	3.1	40 ± 0.0	0.0	0 ± 0.0

[w]See Bongers, 1990.
[x]n= number of samples.
Freq. = frequency.
Abund. = abundance.
[y]for genera in and references of Fig. 3.

Table 4. Squared Mahalanobis distances among the five sampling dates from non-polluted (December 1998 and March 1999) and polluted (June 1998, September 1998 and February 1999) areas resulted from the Stepwise Discriminant Analysis.

	Non-polluted site		Polluted site		
	Dec. 1998	March 1999	June 1998	Sept. 1998	Feb. 1999
Dec. 1998[w]	—	1.05 [NS]	1.49 *	3.32 ***	6.72 ***
March 1999		—	1.06 [NS]	2.63 **	7.22 ***
June 1998			—	1.34 *	5.81 ***
Sept. 1998				—	3.46 ***
Feb. 1999					—

[w]NS = not significant.
*P ≤ 0.05.
** P ≤ 0.01.
*** P ≤ 0.001.

portion of variance (R^2) explained by soil metal content was not significant for total nematode abundance.

Factorial analysis of correspondence

The ordination of the variables resulted from Factorial Analysis (60 variables × 136 cases; one sample in which *T. semipenetrans* was found in great abundance was removed to avoid ordination bias), showed that most of the variance was explained by the first three axes (36.25% of inertia). Variables scored along a main gradient of heavy metal soil content were represented by axis 1. The lowest values of axis 1 were associated with highest soil metal concentrations, while the highest values were associated with the lowest heavy metal soil content. Nematode taxa showed a disposition in the factorial space along axis 1 (Fig. 3), ranging from maximum to minimum values of heavy metal contents (negative to positive values of coordinates). Maturity and diversity index values scored at intermediate values of axis 1. Although nematode taxa scored in a continuum along axis 1, six groups were differentiated to facilitate the interpretation of the relationships between different nematode taxa and soil heavy metal content. Group 1 (*Alloionema, Amphidelus, Apratylenchoides, Microlaimus, Nothotylenchus, Rotylenchus, Tripyla, Xenocriconemella, Tylenchus, Zigotylenchus*) comprised genera associated with the polluted site, and except for *Rotylenchus*

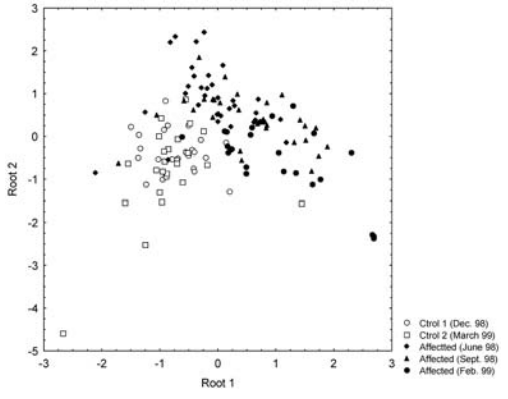

Fig. 2. Score of the variables in a bi-dimensional plot resulted from the Stepwise Discriminant Analysis; the position of the samples on the plane defined by canonical variables 1 and 2, extracted from total number of nematodes, maturity and diversity indices, are showed with open (not affected) and dark (affected) dots. Lines separate two significantly different groups (F = 4.62; P ≤ 0.0000).

Table 5. Power of discrimination of total nematode abundance, maturity and diversity indices considering five groups of samples from five sampling dates: non-polluted (December 1998 and March 1999) and polluted (June 1998, September 1998 and February 1999) resulted from the stepwise discriminant analysis.

Number of variables in the model	F-value	Variable removed
7[w]	4.87 ***	D (F = 1.70 [NS])
6	5.39 ***	H' (F = 1.01 [NS])
5	6.30 ***	Margalef (F = 1.70 [NS])
4	7.48 ***	MI (F = 2.02 [NS])
3	9.37 ***	T (F = 3.60 **)
2	12.42 ***	PPI (F = 7.17 ***)
1	18.51 ***	Camargo (F = 18.51 ***)

[w]NS = not significant.
*P ≤ 0.05.
***P ≤ 0.001.

and *Tripyla*, were never recorded from the non-polluted site. Group 2 (*Cephalenchus, Discolaimus, Monochromodora, Tylenchorhynchus, Plectus*) and Group 3 (*Acrobeloides, Alaimus, Aphelenchus, Cephalobus, Dolichodorus, Dorylaimus, Monhystera, Mononchus, Oionchus, Panagrolaimus, Paratylenchus, Pratylenchus, Pratylenchoides, Prismatolaimus, Psylenchus,* Rhabditidae, *Rhabditis, Xiphinema*) comprised nematodes recorded in samples from the polluted and the non-polluted sites (except *Oionchus* and *Dolichodorus*, which were absent from the polluted site) with intermediate heavy metal concentrations. Group 4 (*Acrobeles, Aphelenchoides, Criconemoides,* Dorylaiminae, *Heterodera, Hoplolaimus, Trichodorus*), Group 5 (*Helicotylenchus, Hemicycliophora*) and Group 6 (*Boleodorus, Tetylenchus*) comprised genera associated with low soil metal concentrations, generally present in the non-polluted site.

DISCUSSION

Nematodes have been used extensively to evaluate medium- and long-term effects of metal pollution of soil on soil biodiversity (Bakonyi, 2003; Georgieva, 2002; Korthals, 1996b). Our results show that nematode communities respond almost immediately to soil contamination by heavy metals. In the case of the Aznalcóllar accident, the polluted mud quickly dried after the spill, covering the soil with a cracked, porous, 20 cm thick crust of that probably allowed soil respiration (S. Sanchez-Moreno, 2006). It is likely that this dry layer of mud facilitated prolonged moisture retention by the underlying soil; thus reducing the summer effect (soil drying) on soil organisms. Experimental results indicated that although soil covering increased nematode abundance by approximately 27%, the abundance of nematode taxa did not change significantly, and consequently no significant effects of covering on maturity and diversity indices were observed.

Heavy metals have been demonstrated to affect both the diversity and abundance of fungi, bacteria (Bisessar, 1982), nematodes (Georgieva, 2002; Korthals, 1996a] and other soil invertebrates (Camargo, 1995). The concentrations of Pb, Cu, Zn,

Table 6. Results of the multiple regression analyses performed on nematode community descriptors.

	Total Nematodes	MI	MINO	∑MI	PPI	Margalef	H´	D	T	Camargo
Pb[w]	0.22 NS	-0.05 NS	0.09 NS	0.03 NS	0.06 NS	-0.11 NS	-0.12 NS	0.18 NS	-0.15 NS	0.04 NS
Ni	-0.09 NS	-0.22 **	-0.30 *	-0.48 ***	-0.33 ***	-0.34 **	-0.44 ***	-0.21 *	-0.49 ***	-0.37 ***
Cu	-0.14 NS	-0.07 NS	-0.11 NS	0.08 NS	-0.09 NS	-0.23 *	-0.19 *	-0.71***	-0.06 NS	-0.31 ***
Ti	0.07 NS	-0.24 **	-0.20 *	-0.01 NS	-0.16 NS	-0.02 NS	-0.02 NS	0.14 NS	0.10 NS	0.01 NS
V	0.004 NS	-0.02 NS	0.04 NS	-0.03 NS	-0.08 NS	-0.01 NS	-0.03 NS	-0.13 NS	-0.14 NS	-0.03 NS
Zn	-0.04 NS	0.12 NS	0.16 NS	0.17 NS	-0.18 *	0.19 NS	0.24 NS	0.22 NS	0.27 NS	0.25 NS
Intercept	-93.514	3.27	3.670	2.71	2.687	1.46	2.96	6.00	2.70	10.34
R^2	0.03	0.140	0.180	0.137	0.120	0.196	0.216	0.270	0.182	0.217
F	0.75 NS	11.17 ***	7.29 ***	10.49 ***	9.38 **	5.30 ***	5.99 ***	8.02 ***	4.81 ***	6.01 ***

[w]Significant variables in the partial regressions are indicated (**).

R^2, F and P value are indicated for the whole model. See Table 2 for abbreviations

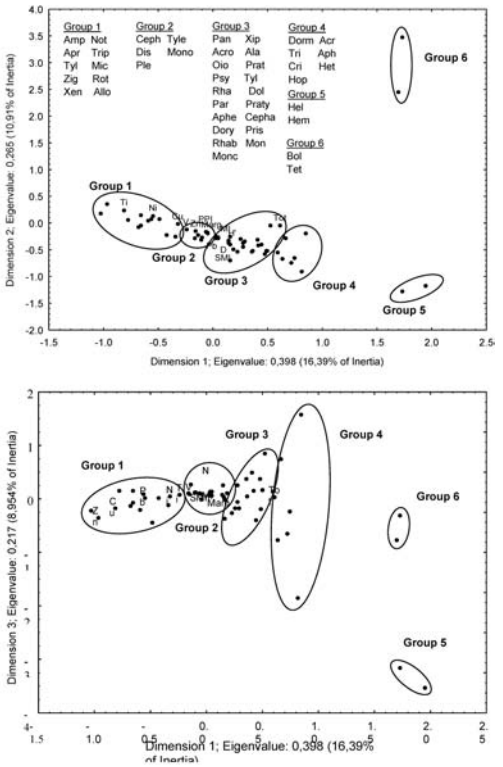

Fig. 3. Results of the Correspondence Analysis. Ordination of variables on the two-dimensional space represents axis 1-2 (Fig. 3a) and 1-3 (Fig. 3b). Six resulting nematode groups are marked with ellipses. Numbers are referred to nematodes on Table 1.

Ti and Ni in soil at the polluted site were at toxic levels during all sampling periods according to Spanish environmental guidelines (legal background references in [V. Conesa, 1997]). The total numbers of nematode taxa remained similar in the non-polluted and polluted sites until September 1998. After that date, the number of taxa declined numerically, but the frequency in which each taxa appeared was significantly different between the two sites. The high concentrations of Pb, Cu, Zn, Ti, and Ni present in the soil were likely responsible for the changes detected in the nematode community, which have been recommended as a bioindicator for the assessment of chemical impacts on soil ecosystems (Edwards, 1996).

Freckman and Ettema (1993) suggested that nematode abundance is a general indicator of soil disturbance, and Nombela *et al.* (1999) considered it to be a recovery indicator. However, statistical differences in total nematode abundance were not observed between the non-polluted and polluted areas monitored in this study. Total nematode abundance in the Guadiamar River banks did not respond measurably to heavy metal pollution until at least nine months after the accident occurred. This suggests, however, that nematode abundance may be used as an indicator of medium to long term effects of heavy metal soil pollution of soil.

Eight months after the accident (February 1999), the number of genera of plant parasitic nematodes was reduced from sixteen to eight, while taxa in the other trophic groups persisted. The number of samples in which non-plant parasitic nematodes appeared, however, declined in February 1999. Extreme colonizers such as Rhabditidae, *Alloionema* and *Panagrolaimus*, with short life cycles and high reproductive rates (c - p value = 1; Table 1) were more tolerant to the pollution-related disruption. Additionally, the persistence of these taxa was probably also influenced by food availability in addition to the resistance to chemical stress (Bongers, 1993; Korthals, 1996c). Few extreme persisters (c - p value = 5: *Discolaimus* and *Xiphinema*) were found in September 1998 and February 1999, four and eight months after the accident occurred. Except for *Amphidelus*, all nematodes in c - p groups 4 and 5 presented reduced abundances at the last sampling date, confirming that nematodes with long life cycles and low colonization abilities are sensitive to soil disturbance (Bongers, 1990; Neher, 1995).

In agreement with previous studies (Bardgett, 1994; Bengtsson, 1989; Korthals, 1996a; Salminen, 1996; Smit, 2002), the suitability of maturity and diversity indices to assess the effects of soil pollution on the nematode community was confirmed statistically by data of this research. Results of the Discriminate Analysis reflected the ecological differences among samples based on numerical combinations of maturity and diversity indices. According to the variables retained in regression models, the Camargo's diversity index, PPI and T showed the most robust discrimination values. Correlation among indices showed, as expected, two groups of indices that measure similar attributes of the nematode community. Diversity indices, including Camargo's and Margalef's diversity indices, originally designed to assess biodiversity in limnoterrestrial systems, were significantly correlated. Maturity indices (MI, MINO, and PPI) measured functional aspects of the community, and were also significantly correlated among them.

Maturity indices have been employed previously to assess the effects of heavy metals on nematode communities (Pen-Mouratov, 2008) and have been described as especially useful in Mediterranean ecosystems (Liang, 2005). In the Mediterranean soils tested, high proportions of colonizers were present, which usually results in low MI values (Nombela, 1994). Using the MINO, that excludes colonizer nematodes from the calculation of maturity, may thus be more appropriate to assess the effects of soil disturbance on Mediterranean soils. Our results, however, did not show any difference between both indices and their ability to detect changes in the nematode community due to soil pollution.

The MI is able to reflect soil disturbance in the short-term (which explains the decrease on MI values in September 1998, three months after the spillage). Although its high degree of sensitivity makes it a good indicator of disturbance, the MI may not "quantify" the disturbance as well as other indices (Neher, 1995).

There is no general agreement on the value of the PPI as an index of soil maturity. Bongers (1990) did not consider it to be an index of disturbance, but Freckman and Ettema (1993) considered it potentially more useful than the MI. Our results show that both PPI and $\sum$MI were significantly different in the polluted and non-polluted sites, and, although they did not respond to metal pollution until February 1999 (8 months after the spillage), our results suggest that both are good indicators of global disturbance (Yeates, 1994). Such different responses are in agreement with those of Yeates and Van der Meulen (Yeates, 1996), who proposed developing a series of nematode indicators to assess "soil health" problems rather than seeking a global index (Yeates, 1999).

Applicability of any of these indices as ecological measurements of soil condition must take into account the inner/outer statistical variability in order to modify and calibrate them at the needed scales (Neher, 1998). Our results show that heavy metals present significant effects on nematode fauna. Associations among faunistic structure and soil metal content detected by Factorial Analysis showed opposite scores of heavy metals and maturity and diversity values. In agreement with our results, Ni and Cu are considered to be among the most toxic metals to soil fauna (Bengtsson, 1989; Pokarzhevskii, 1998), although their toxicity and interactions in soils are complex.

In summary, heavy metal pollution in the Guadiamar River basin significantly altered composition, maturity and diversity of the nematode community in the study area. Our results show that maturity and

diversity indices allowed the assessment of the short-term impact of toxic residues on taxonomical and functional diversity of the nematode community in the surroundings of Doñana National Park, which represents a reservoir of Mediterranean biodiversity. Although significant correlations appeared among diversity indices and among and maturity indices, Camargo's diversity index, the Plant Parasitic Index, and Trophic diversity discriminated significantly between polluted and non-polluted areas. Environmental monitoring activities should embrace soil diversity studies which provide a knowledge base for preserving biological diversity in soil ecosystems.

ACKNOWLEDGEMENTS

This study was financed by a collaboration project CSIC- Junta de Andalucía, Spain. Thanks are also due to Adelino Benito, Hugo Lefranc and José Juan Chans for their assistance with preliminary studies.

LITERATURE CITED

Bakonyi, G., P. Nagy, and I. Kadár. 2003. Long-term effects of heavy metals and microelements on nematode assemblage. Toxicology Letters 140:391-401.

Bardgett, R. D., T. W. Speir, D. J. Ross, G. W. Yeates, and H. A. Kettles. 1994. Impact of pasture contamination by copper, chromium, and arsenic timber preservative on soil microbial properties and nematodes. Biology and Fertility of Soils 18:71-79.

Barker, K. R. 1985. Nematode Extraction and Bioassays. Pp. 19-35 *in* Barker, K. R., C. C. Carter, and J. N. Sasser, Eds., An Advanced Treatise on *Meloidogyne*. Volume II, Methodology, North Carolina State University Press, Raleigh, NC, USA.

Bengtsson, G., and L. Tranvik. 1989. Critical metal concentrations for forest soil invertebrates. Water, Air and Soil Pollution 49:381-417.

Bisessar, S. 1982. Effects of heavy metals on microorganisms in soils near a secondary lead smelter. Water Air and Soil Pollution 17:305-308.

Bongers, T. 1989. De nematoden van Nederland. Pp. 83. British Museum (Natural History), London, England. Wageningen Agricultural University, The Netherlands.

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

Bongers, T., and G. W. Korthals. 1993. The maturity index, an instrument to monitor changes in the nematode community structure. Pp. 80 *in* Proceedings of the International Symposium on Crop Protection, Gent, Belgium.

Bongers, T., R. G. M. De Goede, G. W. Korthals, and G. W. Yeates, 1995. Proposed changes of c -p classification for nematodes. Russ. Journal of Nematology 3:61-62.

Camargo, J. A. 1992. Temporal and spatial variations in dominance, diversity and biotic indices along a limestone stream receiving a trout farm affluent. Water, Air and Soil Pollution 63:343-359.

Camargo, J. A. 1995. On measuring species evenness and other associated parameters of community structure. Oikos 74:538-542.

Chen, J., and H. Ferris. 1999. The effect of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. Soil Biology and Biochemistry 31:1265-1279.

Conesa Fernández-Vitoria, V. 1997. Auditorías Medioambientales: guía metodológica. Mundi Prensa, Madrid.

De Goede, R. G. M. and H. H. Dekker. 1993. Effects of liming and fertilization on nematode communities in coniferous forest soils. Pedobiologia 37:193-209.

De Ruiter, P. C., A. M. Neutel, and J. C. Moore. 1995. Energetics, patterns and interactions strengths and stability in real ecosystems. Science 26:1257-1260.

Djigal, D., A. Brauman, T. A. Diop, J. L. Chotte, and C. Villenave. 2004. Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. Soil Biology and Biochemistry 36:323-331.

Edwards, C. A., S. Subler, S. K, Chen, and D. M. Bogomolov. 1996. Essential criteria for selecting bioindicator species, processes, or systems to assess the environmental impact of chemicals on soil ecosystems. Pp. 67-84 *in* Van Straalen, N. M. and D. A. Krivolutsky, Eds., Bioindicator Systems for Soil Pollution, NATO ASI Series 2: Environment, Kluwer, Dordrecht.

Ekschmitt, K., G. Bakonyi, M. Bongers, T. Bongers, S. Boström, H. Dogan, A. Harrison, P. Nagy, A. G. O'Donnell, E. M. Papatheodorou, B. Sohlenius, G. P. Stamou, and V. Wolters. 2001. Nematode community structure as indicator of soil func-

tioning in European grassland. European Journal of Soil Biology 37:263-268.

Ettema, C. H., and T. Bongers. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biology and Fertility of Soils 16:79-85.

Freckman, D. W., and C. H. Ettema. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agriculture, Ecosystems and Environment 45:239-261.

Georgieva, S. S., S. P. McGrath, D. J. Hooper, and B. S. Chambers. 2002. Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. Applied Soil Ecology 20:27-42.

Gyedu-Ababio, T. K., J. P. Furtenberg, D. Baird, and A. Vanreusel. 1999. Nematodes as indicators of pollution: a case study from the Swartkops river system, South Africa. Hydrobiologia 397:155-169.

Hánel, L. 2003. Recovery of soil nematode populations from cropping stress by natural secondary succession to meadowland. Applied Soil Ecology 22:255-270.

Heip, C., P. M. J. Herman, and K. Soetaert. 1998. Data processing, evaluation and analysis. Pp. 197-231 *in* Higgins, R. P. and H. Thiel (Eds.), Introduction to the study of Meiofauna, Smithsonian Institution Press, Washington.

Korthals, G. W. 1996a. Pollutant - induced changes in terrestrial nematode communities. Thesis, Landbouwuniversiteit Wageningen, Wageningen Agricultural University, The Netherlands.

Korthals, G. W., R. G. M. De Goede, J. E. Kammenga, and T. Bongers. 1996b. The Maturity Index as an instrument for risk assessment of soil pollution. Pp. 85-94 *in* Van Straalen, N. M. and D. A. Krivolutsky, Eds., Bioindicator Systems for Soil Pollution. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Korthals, G. W., A. D. Aexiev, T. M. Lexmond, J. E. Kamenga,and T. Bongers. 1996c. Long-term effects of copper and pH on the nematode community and agroecosystem, Environmental Toxicology and Chemistry 15:979-985.

Korthals, G. W., T. A. Dueck, T. M. Lexmond, T. Bongers, and A. Fokkema, 1996d. Joint toxicity of copper and zinc to a terrestrial nematode community in relation to their bioavailability in an acid sandy soil. Pp. 49-61 *in* Pollutant-induced changes in terrestrial nematode communities, *in*: Korthals, G.W. Thesis Landbouwuniversiteit Wageningen, Wageningen Agricultural University, The Netherlands.

Liang, W. J., I. Lavian, S. Pen-Mouratov, and Y. Steinberger. 2005. Diversity and dynamics of soil free-living nematode populations in a Mediterranean agroecosystem. Pedosphere 15:204-215.

Margalef, R. 1977. Ecología, Ediciones Omega S.A., Barcelona, Spain.

Neher, D. A., S. L. Peck, J. O. Rawlings, and C. L. Campbell. 1995. Measures of nematode community structure and sources of variability among and within agricultural fields. Plant and Soil 170:167-181.

Neher, D. A., K. N. Easterling, D. Fiscus, and C. L. Campbell. 1998. Comparison of nematode communities in agricultural soils of North Carolina and Nebraska. Journal of Applied Ecology 8:213-223.

Nombela, G., A. Navas, and A. Bello. 1994. Structure of the nematofauna in Spanish Mediterranean continental soils. Biology and Fertility of Soils 18:183-192.

Nombela, G., A. Navas, and A. Bello. 1999. Nematodes as bioindicators of dry pasture recovery after temporary rye cultivation. Soil Biology and Biochemistry 31:535-541.

Parmelee, R. W., C. T. Phillips, R. T. Checkai, and P. J. Bolhen. 1997. Determining the effects of pollutants on soil faunal communities and trophic structure using a refined microcosm experiment. Environmental Toxicology and Chemistry 16:1212-1217.

Pen-Mouratov, S., N. Shukurov, and Y. Steinberger. 2008. Influence of industrial heavy metal pollution on soil free-living nematode population. Environmental Pollution 152:172-183.

Pielou, E. C. 1977. Mathematical ecology. Wiley, New York, USA.

Pokarzhevskii, A. D., N. M. Van Straalen, R. O. Butovsky, S. C. Verhoef, and Z. V. Filimonova. 1998. The use of detrital food-webs to predict ecotoxicological effects of heavy metals. *in*: Butovsky, R. O. and N. M. Van Straalen, Eds., Pollution - induced changes in soil invertebrate food-webs, Vrije Universiteit, Amsterdam, The Netherlands.

Poll, J., S. Marhan, S. Haase, J. Hallmann, E. Kandeler, and L. Ruess. 2007. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. FEMS Microbiological Ecology 62:268-279.

Popovici, I. 1992. Nematodes as indicators of ecosystem disturbance due to pollution. Studia Univ. Babes-Bolyai, Biologia 37:15-27.

Ruess, L., and W. Funke. 1992. Effects of experimental acidification on nematode populations in soil cultures. Pedobiologia 28:231-239.

Salminen, J., and P. Sulkava. 1996. Distribution of soil animals in patchily contaminated soil. Soil Biology and Biochemistry 28:1349-1355.

Sánchez-Moreno, S., J. A. Camargo, and A. Navas. 2006. Ecotoxicological assessment of the impact of residual heavy metals on soil nematodes in the Guadiamar River basin (Southern Spain), Environmental Monitoring and Assessment 116:245-262.

Simón, M., I. Ortiz, I. García, E. Fernández, J. Fernández, C. Dorronsoro, and J. Aguilar. 1999. Pollution of soils by the toxic spill of a pyrite mine (Aznalcollar, Spain). Science of the Total Environment 242:105-115.

Smit, C. E., A. J. Schouten, P. J. Van den Brink, M. L. P. van Esbroek, and L. Posthuma. 2002. Effects of zinc contamination on a natural nematode community in outdoor soil mesocosm. Archives of Environmental Contamination and Toxicology 42:205-216.

Sohlenius, B., S. Boström, and A. Sandor, 1988. Carbon and nitrogen budgets of nematodes in arable soil. Biology and Fertility of Soils 6:1-8.

Statsoft, I. STATISTICA for Windows (Computer Program Manual). 1996. Tulsa, OK, 74104, USA.

Tarjan, A. C., R. P. Esser, and L. C. Shih. 1977. An illustrated key to nematodes found in fresh water. Journal WPCF 49, 2318-2337, adapted by Allen Szalanski as Interactive Diagnostic Key to Plant Parasitic, Freeliving and Predaceous Nematodes, (http://nematode.unl.edu/key/nemakey.htm).

Yeates, G. W. 1987. How plants affects nematodes. Advanced Ecology Research 17:61-113.

Yeates, G. W. 1994. Modification and quantification of the nematode maturity index. Pedobiologia, 38:97-101.

Yeates, G. W. 1999. Effects of plants on nematode community structure. Annual Review of Phytopathology 37:127-149.

Yeates, G. W., and H. Van Der Meulen. 1996. Recolonization of methyl - bromide sterilized soils by plant and soil nematodes over 52 months. Biology and Fertility of Soils 21:1-6.

Received: *20/VII/2009*

Recibido:

Accepted for publication: *28/II/2010*

Aceptado para publicación