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Soil Nematodes as Indicators of Heavy Metal Pollution: A Meta-Analysis

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Abstract

Nematodes have been used as bioindicators of soil quality for more than 20 years, and have been shown to have good potential for assessing the impact of heavy metal pollution on soil. They provide information about the biological condition of soil and can reveal dysfunctions linked to the presence of contaminants. In the case of contamination by multiple pollutants, bioindicators can reveal synergistic toxic effects (or "cocktail effects") on organisms living in soil. These impacts are not revealed by the individual measurement of each pollutant. As the effects of heavy metals on nematode communities are not fully known, identifying reliable nematode-based parameters is not straightforward. Currently, knowledge gaps limit the operational use of these types of indices by soil managers. In this study, we performed a meta-analysis on the results of 37 studies from different countries to reveal general trends regarding the effect of multiple types of heavy metal pollution on soil nematode communities and indices. Based on the contamination level of each metal and using known toxicological threshold values, we defined four contamination classes to categorize soil polluted by heavy metals: normal concentration (c0), low contamination (c1), high contamination (c2), and very high (c3) contamination. The most sensitive nematode parameters, showing a strong relationship with the level of soil pollution, were the structure footprint, community footprint, abundance per trophic group (plant feeders, bacterial feeders and omnivores/predators) and taxonomic richness: all these parameters decreased with increased contamination. Our findings showed that fungal-feeding nematodes were relatively insensitive to metal contamination of soil and actually had a higher abundance in the very high contamination class (c3).

Keywords

Bioindicator, Soil Pollution, Heavy Metal, Meta-Analysis, Soil Quality

1. Introduction

Anthropogenic pollution, including heavy metal pollution, can pose a severe threat to humans and the environment ([1] [2]). As soil has been identified as a primary biodiversity resource (both as food and habitat) that is particularly exposed to pollution, soil is now the subject of EU policy in the form of a Thematic Strategy for Soil Protection. Soils provide habitats for a multitude of organisms involved in various ecological processes (e.g. the water cycle, the terrestrial carbon cycle and nutrient cycles), and are crucial for both human well-being and ecosystem sustainability [3] [4]. Identifying the effects of soil pollution on soil biodiversity is essential to better understand the impact of human activity on ecosystem functioning [5] [6] [7], allowing the development of effective management strategies for polluted soils [8] [9] and ensuring a more sustainable use of this important resource. Several methods have been proposed to assess the environmental quality of soil using chemical and/or biological approaches [10] [11] [12]. Of the biological approaches, nematode communities have been shown to be relevant bioindicators for assessing soil disturbance in terrestrial systems [13] [14] [15], in particular in soils polluted by heavy metals [16] [17]. Indeed, nematodes are ubiquitous, abundant and highly diverse, and play a key role in soil functioning [18] [19]. Five major trophic groups of nematodes have been defined: bacterial feeders, fungal feeders, plant feeders, omnivores and predators [20]. Nematode taxa can also be classified into five groups according to a colonizer-persister (cp) scale based on their lifecycle characteristics and sensitivity to perturbation [21]: from cp1 (opportunistic feeders with a short generation time and a high reproduction rate) to cp5 (persisters with a long lifespan, a low reproduction rate and greater sensitivity to soil disturbance). A combination of the cp scale and feeding habits are used to define functional guilds as proposed by Bongers and Bongers [13]. Indices based on these characteristics can be used to analyse nematode community structure [21] [22] and any changes due to environmental disturbance [23] [24].

The effect of heavy metals on soil-inhabiting nematode communities has been the subject of many experiments and metallurgical, industrial, mining and agroecosystem studies [25] [26] [27] [28] [29]. These studies have reported a greater sensitivity to heavy metal pollution in cp4 and cp5 nematodes than in cp1, cp2 and cp3 nematodes, leading to profound changes in nematode assemblage, diversity and community structure in the polluted area. These changes imply certain trophic web alterations that could disrupt soil functions such as respiration and nutrient cycling [7] [30] [31]. However, the effects of heavy metal pollution on trophic groups such as fungal-feeding or bacterial-feeding ne-

matodes seem to differ according to the site characteristics and to the type of pollution involved [17] [25] [27] [32]. There is currently no consensus in the scientific literature concerning the most appropriate indices to monitor the effect of soil pollution by heavy metals on soil biological functioning [21] [24] [27] [33]. In fact, soils are rarely polluted by a single heavy metal, but typically by multiple heavy metals at different levels of concentration. The specific combination depends primarily on the history of the site and the source of the pollution. Consequently, it is difficult to compare the results of different studies in order to select the most sensitive nematode index or to analyse the global effect of heavy metals on nematode communities.

To address this complexity, we conducted a meta-analysis that evaluated the effect of soil pollution by multiple heavy metals on nematode communities. The global effects of pollution were measured by taking into account the heterogeneity of the investigated sites. In the meta-analysis, we summarized the currently available literature on soil polluted by heavy metals and the responses of soil nematodes with the aim of: 1) measuring the responses of nematode communities to heavy metal pollution, 2) testing if the proposed classification of four levels of contamination by multiple heavy metals is relevant to the global response of nematodes to soil pollution, and 3) identifying the most sensitive nematode parameters for use by soil managers. We extracted nematodes parameters and classified soil contamination for each of the 37 studies selected in order to measure the effect of metal contamination on soil nematofauna.

2. Material and Methods

2.1. Data Collection

We reviewed literature published in peer-reviewed journals up to January 2016 that focused on nematode communities in soil contaminated by metals. Google Scholar and the ISI Web of Knowledge were used to collect relevant publications. We selected only studies that presented, for a given soil, both nematode community indices and the soil's total metal contents of arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), mercury (hg), molybdenum (Mo), nickel (Ni), lead (Pb), thallium (Tl) or zinc (Zn). Eco-toxicological studies on water nematode communities or at nematode species level (e.g. *Caenorhabditis elegans*) were excluded. Thirty-seven studies, published between 1991 and 2015, were selected (**Table 1**). Of these, 28 were published in international journals and 9 as institutional reports. The 37 studies were conducted in 13 countries: France (10 sites), China (9 sites), the Netherlands (5 sites), Spain (1 site), Germany (1 site), the United States (2 sites), Hungary (1 site), Scotland (1 site), Uzbekistan (1 site), Britain (1 site), New Zealand (1 site), Slovakia (1 site) and South Korea (1 site). Most investigated sites were contaminated with heavy metals and/or metalloids following mining or industrial activities or accidents. In 11 studies, the heavy metal contamination was performed experimentally in pots or in field experiments.

Table 1. List of the 37 studies used in the meta-analysis with descriptions of the studied sites.

Reference	Year	Country	Near to	Number of modalities	Number of replicates/ modality	Land use	Contamination type	Metals studied
Bakonyi *et al.*	2003 [34]	Hungary	Nagyhorcsok	5	2	arable land	experimental	Cd, Cr, Se, Zn
Bardgett *et al.*	1994 [35]	New Zealand	Levin	8	3	grassland	accidental	Cu, Cr, As
Chen *et al.*	2009 [36]	China	Lanzhou	5	5	arable land	mine	Cd, Cr, Cu, Pb, Zn
Ellis *et al.*	2001 [37]	Scotland	(not precised)	6	1	industrial area	industrial	As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn
Georgieva *et al.*	2002 [17]	England	Gleadthorpe RC	22	2	arable land	experimental	Ni, Zn + Ni, Cu, Zn + Cu, Zn
Korthals *et al.*	1996 [28]	Netherlands	Wageningen	16	8	arable land	experimental	Cu
Korthals *et al.*	1998 [38]	Netherlands	Wageningen	24	6	arable land	experimental	Cu, Zn
Korthals *et al.*	2000 [39]	Netherlands	Wageningen	5	6	arable land	experimental	Cu, Zn
Korthals *et al.*	1996 [40]	Netherlands	Wageningen	27	3	arable land	experimental	Cd, Cu, Ni, Zn
Le Guedard *et al.*	2016 [41]	France	Saint Etienne	9	3	industrial area	mine	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Le Guedard *et al.*	2016 [41]	France	St Cyprien	4	4	grassland	industrial	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Le Guedard *et al.*	2016 [41]	France	Carcasonne	3	3	moors and heathland	mine	As, Cu, Pb
Li *et al.*	2006 [42]	China	Shenyang	13	6	arable land	industrial	Cu, Zn
Li *et al.*	2011 [43]	China	Shenyang	9	4	moors and heathland	urban	Cu, Zn
Liang *et al.*	2006 [44]	China	Shenyang	18	3	arable land	industrial	Cu, Zn, Cd
Nagy	1999 [45]	Hungary	Nagyhorcsok	17	2	arable land	experimental	Al, As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Sr, Zn
Nagy *et al.*	2004 [33]	Hungary	Nagyhorcsok	14	2	arable land	experimental	Cd, Cr, Cu, Se, Zn
Park *et al.*	2011 [46]	Sud Corea	Gijang	2	7	moors and heathland	industrial	Cd, Cr, Cu, Ni, Pb, Zn
Pen-Mouratov *et al.*	2008 [25]	Uzbekistan	Almalyk mine	8	10	grassland	mine	As, Cd, Cu, Pb, Zn
Peres *et al.*	2015 [47]	France	Rennes	8	3	grassland	industrial	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Salamun *et al.*	2012 [24]	Slovakia	Kovohuty	4	4	grassland	industrial	As, Cd, Cr, Cu, Pb, Zn
Sanchez *et al.*	2007 [27]	Spain	Guadiamar river bassin	8	17	intertidal zones	accidental	Pb, Ni, Cu, Zn
Shao *et al.*	2008 [48]	China	Baoshan mine	6	3	moors and heathland	mine	Pb, Zn
Sharma *et al.*	2014 [49]	USA	Cleveland and Colombus	2	22	urban green space	urban	As, Cd, Cr, Pb, Zn
Smit *et al.*	2002 [50]	Netherlands	Heel	30	5	grassland	experimental	Zn
Tomar *et al.*	2009 [51]	China	Shenyang	6	5	arable land	industrial	Pb

Continued

Villenave *et al.*	2012 [52]	France	Auzon	7	4	arable land	industrial	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Villenave *et al.*	2012 [52]	France	St Etienne	3	4	industrial area	industrial	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Villenave *et al.*	2012 [52]	France	Metz	2	4	arable land	mine	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Villenave *et al.*	2012 [52]	France	Douai	7	4	arable land and forest	industrial	Al, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, Zn
Villenave *et al.*	2012 [53]	France	St Laurent le minier	7	3	industrial area	mine	As, Cd, Pb, Tl, Zn
Villenave *et al.*	unpublished	France	Lille	10	1	industrial area	industrial	Cu, Zn
Wang *et al.*	2008 [54]	China	Shenyang	12	1	arable land	experimental	Cu
Weiss, Larink	1991 [55]	Germany	Braunschweig	3	4	arable land	sewage sludge	Ni,Cd, Cr, Cu, Pb, Zn
Yeates *et al.*	1994 [29]	New Zealand	Levin	6	3	grassland	accidental	As, Cr, Cu
Zhang *et al.*	2006 [56]	China	Shenyang	14	3	arable land	experimental	Zn
Zhang *et al.*	2007 [57]	China	Hongtoushan copper smelter	4	4	arable land	mine	Cu, Zn

2.2. Nematode Parameters

We selected 15 nematode parameters for this meta-analysis:

- The abundance of six nematode trophic groups: opportunistic bacterial feeders (cp1, *i.e.* value 1 on the coloniser-persister scale [21]), other bacterial feeders (cp2, cp3 and cp4), fungal feeders (cp2, cp3 and cp4), omnivores plus carnivores (cp2, cp3, cp4 and cp5), plant feeders (cp2, cp3, cp4 and cp5) and total soil nematodes [20].
- Four nematode indices: Maturity Index (MI) [21], Nematode Channel Ratio (NCR) [58], Structure Index (SI) and Enrichment Index (EI) [59],
- Two diversity indices calculated on genus level: Number of genera (*S*) and the Shannon diversity index (*H'*)
- Three metabolic footprints: enrichment footprint (EFOOT), structure footprint (SFOOT) and community footprint (COMFOOT) [22].

Table 2 presents the nematode indices used in this study and their corresponding calculations.

2.3. Data Extraction

The soil and nematode parameters were extracted directly from the tables, text or figures of each study using PlotDigitizer 2.6.4 software. The software ELIPTO© developed by the French company ELISOL environnement was used to calculate the nematode parameters that were not included in the studies (e.g. abundance of trophic groups, MI, EI, SI, NCR, Shannon diversity index and metabolic footprints) using data on nematode taxa abundance included in the studies. Depending on the available data, it was not always possible to calculate all 15 selected nematode parameters.

Table 2. Nematode parameters used in the meta-analysis and their corresponding calculations.

		Description	Calculation
Nematode community indices			
EI		Enrichment Index: This index indicates the level of nutrient availability and activity of primary detrital consumers in soil. EI is based on the weighed abundance of the opportunistic non-herbivorous guilds indicators of **enriched conditions** (Ba1 and Fu2). EI increases with nutrient availability in soil (nitrogen in particular).	$EI = e/(e + b) \times 100$ $e = \sum Ke \times n$ $b = \sum Kb \times n$ (a)
SI		Structure Index: SI provides indication on the complexity and the trophic connectance in soil food web, thus informing about the stability of the environment. SI is based on the weighed abundance of the nematode indicators of **undisturbed conditions** and structured food web (nematodes with long generation time and high sensibility to disturbance). The higher the Structure Index, the higher the complexity of the soil food web, indicating a more stable environment.	$SI = s/(s + b) \times 100$ $s = \sum Ks \times n$ (a)
NCR		Nematode Channel Ratio: It is calculated from bacterivores (*Ba*) and fungivores (*Fu*) abundances. As Channel Index, NCR provides information on decomposition channel of soil organic matter. High value indicates that bacterial channel is dominating in soil.	$NCR = Ba/(Ba + Fu)$ (b)
MI		Maturity Index: It informs about maturity of the soil food web and stability of the environment. It is calculated from free living nematodes abundances and their respective cp-class; the higher the Maturity Index, the higher the stability of the environment.	$\sum Vi \times Pi$ (c)
Diversity indices			
S		Taxonomic richness: Number of nematode genera identified in a sample. This parameter informs about the level of patrimonial diversity.	
H'		Shannon index of diversity: This index is calculated by taking number of genera and equitability among taxa identified in a sample. It informs about the level of patrimonial diversity. The higher the Shannon index, the higher the diversity of a sample.	$H' = \sum Pi \times \ln Pi$ $Pi = Ni/N$ (d)
Metabolic footprints			
EFOOT	Enrichment footprint	Footprints give a measure of the metabolic impact of the nematofauna concerned (enrichment fauna, structure fauna, herbivore fauna and total community). They are calculated by taking into account the production component (amount of carbon partitioned into growth and eggs) and respiration of the concerned nematodes. The higher the value of metabolic footprint, the higher the activity of the functional group.	$F = \sum[Ni \times (0.1 \times Wi/Vi) + (0.273 \times Wf^{0.75})]$ µg C. kg^{-1} de sol sec (e)
SFOOT	Structure footprint		
PLTFOOT	Herbivore footprint		
COMFOOT	Composite footprint		

a) *Ke*, *Ks*, *Kb*: coefficients relative respectively to the enrichment, struture and basal fauna; *n*: abundance of the considered familly; b) *Ba*: number of bacterial feeders; *Fu*: number of fungal-feeders; c) *Vi*: cp value; *Pi*: proportion of the nematode of cp-class considered; d) *N*: total nematodes abundance; *Ni*: abundance of nematodes within genera; e) *Ni*: abundance of nematodes within family *i*; *Wi*: average weight of a nematode from the family *i*; *Vi*: cp value of family *i*.

For each study, mean values were extracted for the different parameters; the number of replicates per treatment in each study was also recorded and used for the statistical analyses. If several nematode analyses were conducted on different dates, results from each date were included in our analysis. In most cases, nematofauna analyses were made in the top layer of soil; however, if several soil layers were investigated in the studies, results from each soil layer were included in our analysis.

2.4. Soil Contamination Classification

The source and type of soil contamination in each study included in the meta-analysis are presented in Table 1. In most cases, the soil was contaminated by

several heavy metals or metalloids. In some studies, only certain concentrations of heavy metals were measured.

2.4.1. Contamination Levels for Each Metal

To assign a contamination level to each metal, we used published threshold values based on the lower and upper whisker values [60], used in the France's Network for Soil Quality Monitoring (Réseau de Mesure de la Qualité des Sols: RMQS) (**Table 3**) and a few other sources for elements that were not included in the previously cited studies [57] [61].

For each metal, four contamination levels were then defined as follows: concentrations corresponding to the normal geochemical background (lower than the lower whisker value), low concentrations (between the lower and upper whisker values), high concentrations (between higher than the upper whisker and ten time higher than the upper whisker value), and very high concentrations (higher than ten times higher than the upper whisker value).

2.4.2. Contamination Classes for Soil

The soil contamination classes were calculated by combining the values of the lower and upper whiskers of the various metallic elements as proposed in the French ADEME Bioindicateur 2 programme [62]. The c3 class was created to differentiate between high contamination and very high contamination. The four soil contamination classes were defined as follows:

- c0 (no contamination): all concentrations of metals were lower than the low contamination level;
- c1 (low contamination): at least one metal had a concentration between a low and high contamination level;
- c2 (high contamination): at least one metal had a concentration between a high and very high contamination level;
- c3 (very high contamination): at least one metal had a concentration higher than a very high contamination level.

In classes c1 to c3, when only one metal was found at a higher concentration than the threshold value, the soil was assigned to the contamination class corresponding to the highest concentration. Thus, the mean value for a given metal obtained from the different studies could be lower than its threshold value indicated in **Table 3**. The mean and median values of soil contamination by metals in the different classes are presented in **Table 4**.

Table 3. Metal concentrations (mg·kg^{-1} dry soil) used to define the different classes of contamination.

	As	Cd	Cr	Co	Cu	Hg	Mo	Ni	Pb	Tl	Zn
Normal concentration	<60*	<0.67	<116	<25.9	<42.7	<0.3*	<1.6	<61.5	<62.3	<1.37	<160.9
Low contamination	60 - 284	0.67 - 0.99	116 - 200*	25.9 - 38.1	42.7 - 100*	0.3* - 1.0	1.6 - 2.31	61.5 - 91.6	62.3 - 100*	1.37 - 1.96	160.9 - 231.5
High contamination	284 - 2840	0.99 - 9.9	200 - 2000	38.1 - 381	100 - 1000	1.0 - 10	2.31 - 23.1	91.6 - 916	100 - 1000	1.96 - 20.0	231.5 - 2315
Very high contamination	>2840	>9.9	>2000	>381	>1000	>10.0	>23.1	>916	>1000	>20.0	>2315

Values from Villaneau (2008) and * Baize (2000) and Zhang (2007).

Table 4. Median and mean values of metal concentration (mg·kg^{-1} dry soil) in the four soil contamination classes (c0 = uncontaminated, or normal concentration of metals, c1 = low contamination, c2 = high contamination, c3 = very high contamination).

	c0		c1		c2		c3	
	median	mean	median	mean	median	mean	median	mean
As	9.3	8.9	29.5	54.9	20	169	88	738
Cd	0.3	0.3	0.8	0.8	2.1	2.9	10.9	84.8
Co	19.3	19.3	12.2	11.8	19.3	16.8	50.0	46.5
Cr	45	38	57	56	85	152	1081	2445
Cu	11	14	49	50	107	185	513	1097
Hg	0.13	0.13	0.07	0.17	0.24	0.67	6.5	80.3
Mo	nd	nd	0.6	0.8	1.0	1.5	489	488
Ni	6.1	8.7	24.8	22.3	14.1	55.3	176	755
Pb	29	27	56	64	203	221	1855	3635
Tl	nd	nd	0.54	0.72	0.68	0.84	3.69	15.17
Zn	58	66	114	126	273	417	901	11329

2.5. Statistical Analysis

We used generalized linear mixed models (GLMMs) to estimate the nematode community responses to soil contaminated with metals; more specifically, we selected a Poisson distribution with a logarithmic link. These models were chosen because this model is appropriate for analyzing count data and for handling the variability of the observations both within and between studies [63]. The fixed effect measured the average effect (across the 37 studies) of metal contamination; the random effect measured the variation in responses across the studies. Model validity was verified by visually examining the residuals and by testing the significance of the estimated parameters. The GLMMs were fitted by restricted maximum likelihood assuming a Laplace approximation to the restricted maximum likelihood function. The appropriate linear contrasts between contamination classes were estimated and their significance statistically tested, checking multiple comparisons over metal contamination classes using the glht() function in R language. To visually illustrate the fitted results, we created box plots of nematode community parameters; outliers were not depicted. Finally, we assessed the size of the effect of high contamination (c2 vs c0) on all the nematode indices. This was calculated as the median (+/− 95% confidence interval) of (c2 − c0)/c0 values measured on each site. We used lme4, multcomp and ggplot2 libraries for the statistical analyses and creation of the graphics.

3. Results

3.1. General Characteristics of the Selected Studies

From the 37 studies used for this meta-analysis, 355 situations (defined as a ne-

matofaunal community in a specific place on a specific date, with a certain level of contamination) were extracted. These 355 situations corresponded to 1475 individual nematological analyses, with an average of four replicates per situation. The dataset available per nematode parameter (averaged over replicates) was variable since the different studies did not allow the calculation of all 15 selected nematological variables in every case. As a result, the number of values in the dataset per nematode parameter ranged from 37 to 150 depending on the parameter considered. On average, the datasets for c0, c1, c2 and c3 respectively contained 54, 53, 107 and 41 units of data, meaning that c0, c1 and c3 had a similar quantity of data, whereas about twice as much data was available for c2.

3.2. Nematode Responses to Increased Levels of Contamination

Overall, the GLMMs showed significantly negative effects of metal contamination of soil on nematode parameters. Fitted total nematode abundance decreased in line with increasing soil contamination (**Figure 1**). Of the nematode trophic groups, bacterial feeders cp1, other bacterial feeders (cp2 to 4), omnivores/carnivores and plant feeders also showed a significant decrease in abundance in line with an increase in the level of contamination from c0 to c3.

In contrast, the abundance of fungal feeders showed more erratic variation. In this trophic group, compared to the c0 level of contamination, abundance was significantly lower at c1 and c2, but significantly higher at c3. Moreover, the abundance of fungal feeders was lower in c1 (low contamination) than in c2 (high contamination). However, differences in the median abundance of fungal feeders between contamination classes was weak (<40 nematodes 100 g^{-1} dry soil) and, overall, abundance was low (100 - 200 nematodes/100g dry soil) compared to that of bacterial feeders.

The effects of metal contamination on selected nematode indices are presented in **Figure 2**. The Enrichment Index (EI) did not decrease in line with an increasing level of contamination: c1, c2 and c3 showed similar EI values; however, these values were lower than EI values for c0. The Structure Index (SI) decreased significantly with an increase in the level of soil contamination; differences were significant between c0, c1 and c2, but not between c2 and c3. The Maturity Index (MI) followed the same pattern as the EI: this index did not decrease significantly with an increasing level of contamination—c1, c2 and c3 had equivalent MI values, but these were lower than the value for c0. The Nematode Channel Ratio (NCR) was not affected by metal contamination: whatever the contamination level, the decomposition pathway was dominated by bacterial activity. The Shannon diversity index (H') decreased in line with an increase in soil contamination. Differences in the H' value were significant between c0, c1 and c2, but not between c2 and c3. Taxonomic richness also decreased in line with the level of soil contamination. It reached the lowest value in the c3 class (<15 taxa).

The metabolic footprint of nematode community was strongly impacted by metal contamination (**Figure 3**). The global nematode community footprint

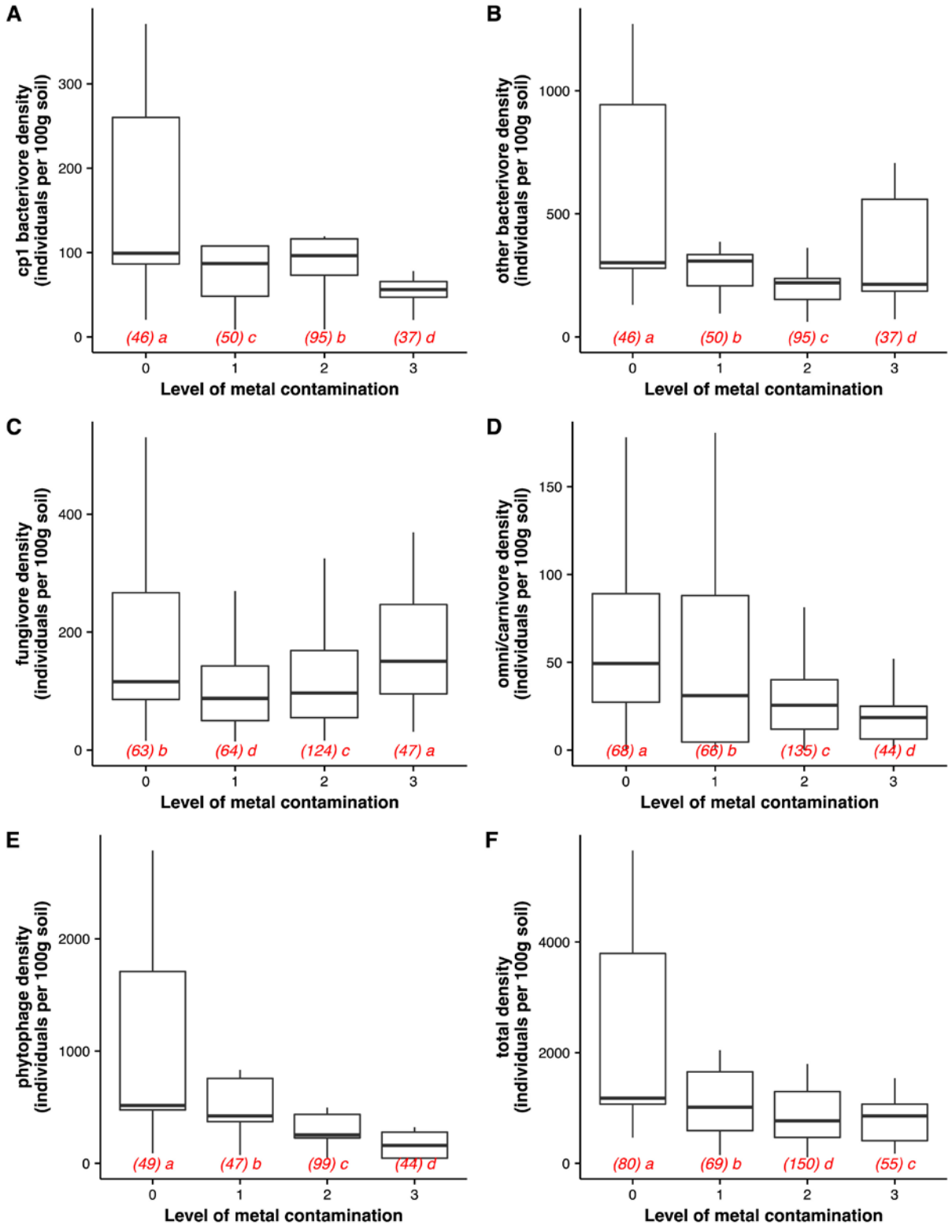

Figure 1. Fitted box plots of nematode abundance (nematodes per 100g dry soil) in relation to the level of soil contamination (c0 = uncontaminated, or normal concentration of metals), c1 = low contamination, c2 = high contamination, c3 = very high contamination) for opportunistic bacterial feeders (cp1), other bacterial feeders, fungal feeders, omnivore/carnivores, plant feeders and total nematode abundance. Different letters indicate significant differences between treatments. The numbers in parentheses are the numbers of observations.

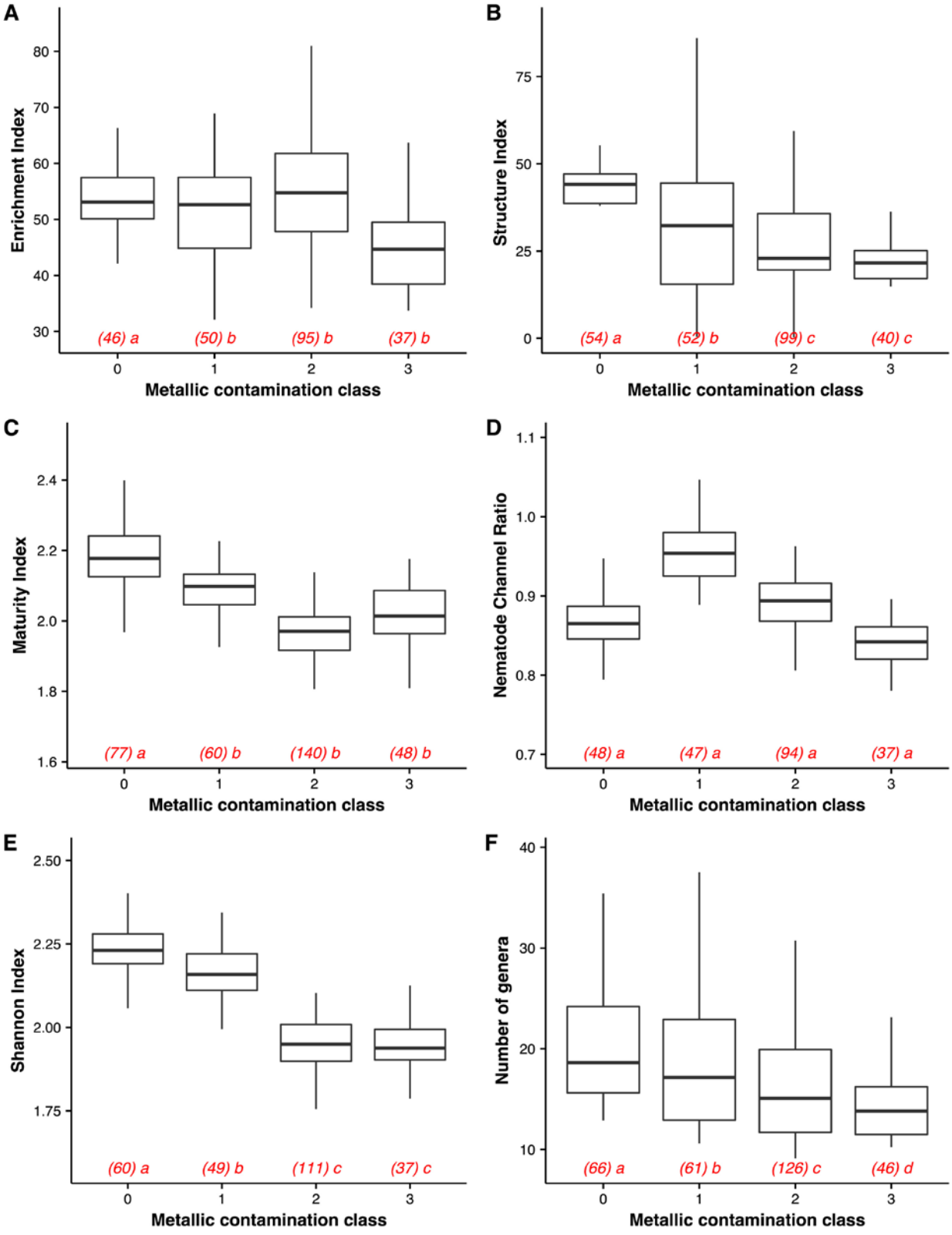

Figure 2. Fitted box plots of nematode indices in relation to the level of soil contamination: Enrichment Index (EI), Structure Index (SI), Maturity Index (MI), Nematode Channel Ratio (NCR), Shannon diversity index (*H*) and Number of genera (*S*). Different letters indicate significant differences between treatments. The numbers in parentheses are the numbers of observations.

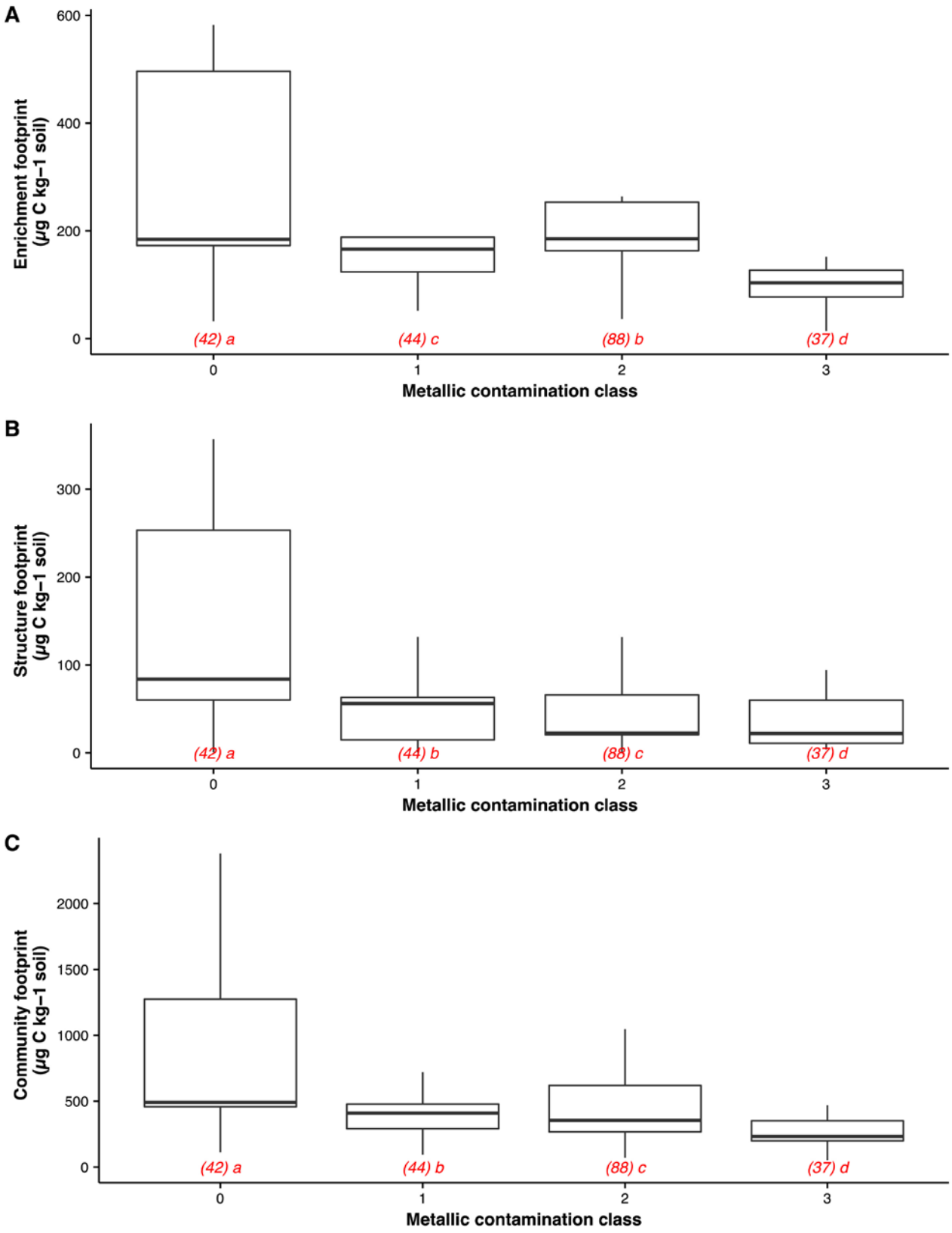

Figure 3. Fitted box plots of main nematode footprints in relation to the level of soil contamination: Enrichment footprint (EFOOT), Structure footprint (SFOOT) and Community footprint (COMFOOT). Different letters indicate significant differences between treatments. The numbers in parentheses are the numbers of observations.

(COMFOOT) decreased in line with an increase in contamination level; the median COMFOOT of the c3 class was two times lower than the median COMFOOT of the c0 class. The plant feeder footprint (PLTFOOT) and structure footprint (SFOOT) followed the same trend, reflecting a strong negative effect of soil contamination on the metabolic activity of plant-feeding nematodes and nematodes with long lifecycles (cp3, cp4 and cp5). The enrichment footprint (EFOOT) was smaller in contaminated areas, but the effects were dependent on the contamination level. Indeed, the c2 class showed a higher EFOOT than the c1 class; however, the lowest EFOOT was found for the c3 contamination class.

3.3. The Size of the Effect of High Contamination (c2 versus c0)

The main goal of this study was to identify the nematode parameters most sensitive to soil contaminated by metals. To this end, we compared the size of the effect between c0 (no contamination) and c2 (high contamination) classes, which represented the majority of the contaminated soil situations.

Of the 15 nematode parameters analysed in this study, we observed three groups of responses to soil contaminated by metals (Figure 4). Group 1 included plant feeder abundance, non-opportunistic bacterial feeder abundance, Maturity Index (MI), diversity (*S*), Shannon diversity index (*H'*) and community footprint (COMFOOT). These parameters showed a significant decrease (10% to 20%) in c2 compared to c0.

Group 2 included omnivore/carnivore abundance, total nematode abundance, Structure Index (SI) and Nematode Channel Ratio (NCR). These parameters also showed a significant decrease (about 20% on average) in c2 compared to c0. Structure footprint (SFOOT) showed an even stronger decrease (about 30%). For most of these parameters, the confidence intervals were higher, indicating higher variability between studies.

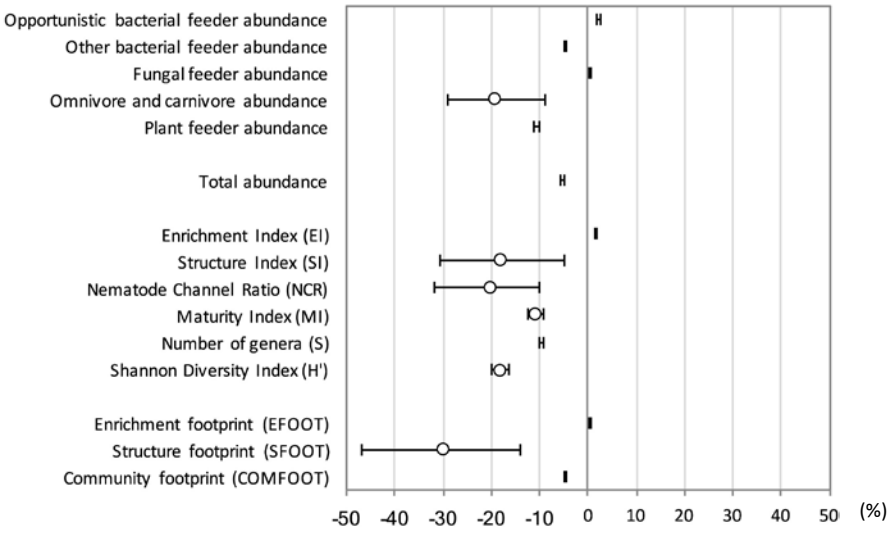

Figure 4. Fitted differences between two levels of contamination—no contamination (c0) and high contamination (c2)—for all studied nematode parameters with a 95% confidence interval.

Group 3 included opportunistic bacterial feeder abundance, fungal feeder abundance, Enrichment Index (EI) and enrichment footprint (EFOOT). These parameters showed no significant difference or low increased percentages on average in c2 compared to c0. In particular, opportunistic bacterial feeder abundance and EI showed an increase lower than 5%, while fungal feeder abundance and EFOOT showed no significant variation between c0 and c2.

4. Discussion

4.1. Soil Pollution by Heavy Metals

Nematodes have been used as bioindicators of soil quality for more than 20 years ([14] [18] [58] [64]). In the context of soil pollution, the advantage of bioindicators is that they can integrate the effects of all the contamination parameters (type, concentration, pollutant interactions, bioavailability of each pollutant, etc.), which are difficult to characterize comprehensively using physico-chemical analyses.

In this study, we analysed soil contamination based on measurements of the total content of heavy metals. Other analytical methods to quantify contaminants exist, such as extraction with $CaCl_2$ (which mimics the quantity extractible with rains [65]) or extraction with the chelating agent EDTA [66]. However, methods based on extractable elements cannot reflect with accuracy the bioavailability of metals for organisms in the soil or their potential impact on the environment. The advantage of bioindicators is that they allow the measurement of the combined effect of metallic contaminants (as well as organic pollutants) on ecosystem functioning and can also measure variation in biological activities by integrating all the accumulated individual effects [67]. In many polluted areas, multiple contaminations with heavy metals occur concurrently and could thus induce varying and complex nematode responses [17] [25] [44]. For example, a study by Georgieva *et al.* [17] has shown the additive effect of zinc and copper. It has also been demonstrated that acidifying compounds can create optimal conditions for the increased mobilization, bioavailability and thus toxicity of metals stored in soils ([9] [40] [68] [69]). The effect of metal pollution on nematodes can result from a number of different parameters, such as the concentration of the metal, the type of metal, its bioavailability, and the duration of contact with nematodes [70].

The contamination classes defined for this study and based on total metal content revealed general trends of nematode community responses to various levels of soil contamination, whatever the metallic nature. Although measurements of total content are not always pertinent, they are the most frequently available and, often, the only measurements reported in publications.

It should be noted that at low concentrations, some metals, such as copper (Cu), zinc (Zn) and molybdenum (Mo), are micronutrients that are essential to plant growth (at respective levels of about 2.5, 2.2 and 0.15 $mg \cdot kg^{-1}$ soil or less, depending on soil pH). But at higher concentrations, these elements have toxic

effects on living organisms and are considered contaminants, as are other metals such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), mercury (Hg) and arsenic (As) ([71] [72] [73]). If micronutrient concentration exceeds the binding capacity between a metal and the soil matrix, then metals can contaminate soil interstitial water and become bioavailable for the organisms living in the soil. As nematodes have a permeable cuticle, they are in direct contact with xenobiotics in interstitial water and may be adversely affected by these contaminants [21]. Trace metals have the potential to bind to proteins and to alter protein functionality [74]. This can cause metabolic disorders in nematodes, leading to a decrease in fitness, low motility, reduced juvenile body length, or even mortality. The effects depend on the type and concentration of metal [70]. For example, it has been shown that cadmium (Cd) has no acute effect on nematode communities up to 160 $mg{\cdot}kg^{-1}$ soil, while nickel (Ni), copper (Cu) and zinc (Zn) decreased the proportion of omnivores and predators at a level of 100 $mg{\cdot}kg^{-1}$ soil after 1 - 2 weeks exposure in experimental studies [40]. At this concentration, total nematodes abundance was also significantly decreased by nickel (Ni), while other metals did not significantly impact total abundance. Another crucial factor potentially impacting nematodes response to metal contamination in soil is the duration of contact, as some nematodes can develop tolerance and/or adapt to long-term soil pollution [9] [75].

Employing a meta-analysis method allowed us to combine the results of a number of studies that characterize the impact of both short-term and long-term effects of real or experimental metal pollution on soil nematode communities. We were able to integrate results obtained from diverse conditions: different soil types, climates (e.g. temperate or continental) and land uses (e.g. grassland, arable land, industrial, mining or urban areas). As nematode community composition is known to vary in response to such environmental factors [76] [77], aggregating this information permitted the statistical power to reveal robust trends of nematode responses to soil contamination. And this, independently of the duration of the pollution, the nature of the metal contamination (e.g. experimental, single or multiple metals, etc.), the climate and the land use.

4.2. Nematode Responses to Levels of Contamination

As reported in several studies, soil contamination by metals has an overall negative effect on nematodes abundance, diversity and community structure [17] [20] [28]. Our meta-analysis revealed that nematode parameters have different responses to the metal content of soil.

All nematode parameters apart from NCR showed sensitivity to metal pollution from the lowest level of contamination (c1). But certain did not reflect the level of soil contamination. These parameters should not be use as indicators of pollution impact.

In our meta-analysis, the abundance of plant feeders, non-opportunistic bacterial feeders (Ba2-4) and omnivores/carnivores showed an acute decrease in line

with an increase in soil contamination (across the four contamination classes). Shao *et al.* [48] also observed a decrease in abundance of plant-parasitic nematodes in line with increased soil contamination by lead and zinc. These authors highlighted that the abundance and proportion of these nematodes were linked with the recovery of the vegetation. As stated by Bongers and Ferris [14], the abundance of plant-parasitic nematodes is mostly determined by the community structure and biomass of plants. Thus, a decrease in plant-parasitic nematodes in a polluted area is probably related not only to the direct toxic effect of heavy metals, but also to reduced plant production [29] [35].

Some studies have reported no significant effect of Cu, Pb or Zn on cp2 bacterial-feeding nematodes, leading to doubts about using these nematodes to assess the effect of heavy metal contamination [17] [27]. However, our results clearly showed that the abundance of cp2-4 bacterial feeders (usually dominated by cp2) decreased in contaminated soil in the upper contamination classes (c2 and c3). The decrease was less notable between c0 and c1 (the fitted medians were similar). Studies have observed a high proportion of bacterial-feeding nematodes in contaminated areas, and some authors have suggested a higher resistance to metal contamination of some taxa in this functional guild, for example, *Acrobeloides* ([43] [57]). Georgieva *et al.* [43] also suggested that indirect long-term effects such as less competition and predation could favour bacterial feeders in contaminated areas.

Our study confirmed what many others have described: that omnivore and carnivore nematodes are the most sensitive to contaminated soil, and their populations decrease in proximity to the pollution source ([21] [24] [25] [42]). The decrease of the number of genera in line with the increase in soil contamination in our results could reflect the loss of the most sensitive nematodes, mainly cp3, cp4 and cp5 nematodes and the omnivore/carnivore trophic group (Zhang *et al.*, 2007; Nagy *et al.*, 2004). The SI, whose calculation is based on the relative abundance of these nematodes, did not decrease between high (c2) and very high (c3) classes, whereas the community footprint (COMFOOT) and structure footprint (SFOOT) did. This indicates that despite a relatively similar nematode community composition in c2 and c3 contamination classes, the abundance and biomass of nematodes decreased in line with the increase in contamination between these two classes, differentiating them. Nematode biomass, used to calculate the nematode footprint, is considered a good indicator of soil pollution in several studies ([9] [25] [78]).

Few relationships were observed between the nematode parameter indicators of enrichment status and soil contamination levels. The EI was weaker in contaminated areas, but was not impacted by increased levels of contamination. Cp1 bacterial feeders were more abundant in the c2 contamination class than in c1 and showed the lowest abundance in c3. Enrichment footprint values showed the same patterns as the EI. The latter is a ratio of nematode abundance and does not take into account absolute abundance of nematodes, whereas EFOOT

does. Cp1 bacterial feeders are enrichment indicators and are linked with the nitrogen mineralization of organic matter in soils ([79] [80]). They are r-strategists with a tolerance to soil pollution ([17] [81]), and their development is strongly linked to the nutrient status and organic content of soil ([48] [79]). As a result, the abundance of cp1 bacterial feeders, the EI and the EFOOT cannot be considered good indicators of soil pollution and should not be the focus of investigation in contaminated conditions.

The MI showed lower values in polluted areas (c1, c2 and c3) compared to uncontaminated areas (c0), but did not vary between contamination levels. This index has been successfully used to characterize the effects of different levels of pollution on nematode communities ([21] [25] [36] [44]), but some authors have underlined the limitations of this indicator when nematode abundance is low [48]. Our results show that the MI is sensitive to pollution, as it has minimum values at the low contamination level (c1), indicating that the maturity of the nematode community was lower at any level of contamination. One interpretation might be that an increase in contamination level impacts nematode abundance, but not the proportion of cp3-5 nematodes in the community.

We found that fungal-feeding nematodes were the least affected by metal contamination. These nematodes actually showed higher abundance in the very high contamination class (c3), leading to an increase in total nematode abundance. The relative insensitivity of fungal-feeding nematodes to pollution has been reported in several other studies ([16] [27] [28] [45]). It seems that high levels of soil pollution promote fungal growth ([9] [82]). However, as bacterial feeder abundance is much higher than fungal feeder abundance in most soils, the NCR was not strongly modified with the increase in soil contamination, indicating that this index should not be used as an indicator of metal pollution in soil. This finding does not confirm the results from Zhang *et al.* [57].

5. Conclusions

In the 37 studies conducted in 13 different countries used for this meta-analysis, there was large heterogeneity in soil contamination between studied sites in terms of the type and concentration of metals. By classifying soil contamination based on the threshold values of each metal, we were able to compare the contamination level and its effect on the nematode community between studies in the context of multiple contaminations.

Nematodes are operational bioindicators of soil food web structure and our findings indicate that structure footprint, community footprint, abundance per trophic group (plant feeders, bacterial feeders and omnivores/predators) and taxonomic richness were the most sensitive nematode parameters in this context. Overall results confirmed that heavy metal contamination acts as a stress, forcing the nematode community and ecosystem into an early stage of development: dominance of the cp2-microbivorous (bacterial feeding) nematodes and an almost total absence of omnivores, carnivores and plant feeders in very polluted conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Duruibe, J.O., Ogwuegbu, M.O.C. and Egwurugwu, J.N. (2007) Heavy Metal Pollution and Human Biotoxic Effects. *International Journal of Physical Sciences*, **2**, 112-118.

[2] Rhind, S.M. (2009) Anthropogenic Pollutants: A Threat to Ecosystem Sustainability? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 3391-3401. https://doi.org/10.1098/rstb.2009.0122

[3] Brussaard, L., Behan Pelletier, V.M., Bignell, D.E., Brown, V.K., Didden, W., Folgarait, P., Fragoso, C., *et al.* (1997) Biodiversity and Ecosystem Functioning in Soil. *AMBIO*, **26**, 563-570.

[4] Wagg, C., Franz Bender, S., Widmer, F. and van der Heijden, M.G.A. (2014) Soil Biodiversity and Soil Community Composition Determine Ecosystem Multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 5266-5270. https://doi.org/10.1073/pnas.1320054111

[5] Boyd, R.S. (2010) Heavy Metal Pollutants and Chemical Ecology: Exploring New Frontiers. *Journal of Chemical Ecology*, **36**, 46-58. https://doi.org/10.1007/s10886-009-9730-5

[6] Nahmani, J. and Lavelle, P. (2002) Effects of Heavy Metal Pollution on Soil Macrofauna in a Grassland of Northern France. *European Journal of Soil Biology*, **38**, 297-300. https://doi.org/10.1016/S1164-5563(02)01169-X

[7] Kandeler, F., Kampichler, C. and Horak, O. (1996) Influence of Heavy Metals on the Functional Diversity of Soil Microbial Communities. *Biology and Fertility of Soils*, **23**, 299-306. https://doi.org/10.1007/BF00335958

[8] Sharma, H.D. and Reddy, K.R. (2004) Geoenvironmental Engineering: Site Remediation, Waste Containment, and Emerging Waste Management Technologies. John Wiley & Sons, Hoboken.

[9] Rodriguez Martín, J.A., Gutiérrez, C., Escuer, M., García-González, M.T., Campos-Herrera, R. and Águila, N. (2014) Effect of Mine Tailing on the Spatial Variability of Soil Nematodes from Lead Pollution in La Union (Spain). *Science of The Total Environment*, **473-474**, 518-529. https://doi.org/10.1016/j.scitotenv.2013.12.075

[10] Caldwell, B. (2005) Enzyme Activities as a Component of Soil Biodiversity: A Review. *Pedobiologia*, **49**, 637-644. https://doi.org/10.1016/j.pedobi.2005.06.003

[11] Cortet, J., GomotDeVauflery, A., PoinsotBalaguer, N., Gomot, L., Texier, C. and Cluzeau, D. (1999) The Use of Invertebrate Soil Fauna in Monitoring Pollutant Effects. *European Journal of Soil Biology*, **35**, 115-134. https://doi.org/10.1016/S1164-5563(00)00116-3

[12] Edwards, C.A. (2002) Assessing the Effects of Environmental Pollutants on Soil Organisms, Communities, Processes and Ecosystems. *European Journal of Soil Biology*, **38**, 225-231. https://doi.org/10.1016/S1164-5563(02)01150-0

[13] Bongers, T.M.B. (1998) Functional Diversity of Nematodes. *Applied Soil Ecology*, **10**, 239-251. https://doi.org/10.1016/S0929-1393(98)00123-1

[14] Bongers, T. and Ferris, H. (1999) Nematode Community Structure as a Bioindicator in Environmental Monitoring. *Trends in Ecology & Evolution*, **14**, 224-228. https://doi.org/10.1016/S0169-5347(98)01583-3

[15] Neher, D.A. (2001) Role of Nematodes in Soil Health and Their Use as Indicators. *Journal of Nematology*, **33**, 161-168.

[16] Bongers, T., Llieva-Makulec, K. and Ekschmitt, K. (2001) Acute Sensitivity of Nematode Taxa to $CuSO_4$ and Relationships with Feeding Type and Life-History Classifycation. *Environmental Toxicology and Chemistry*, **20**, 1511-1511.

[17] Georgieva, S.S., McGrath, S.P., Hooper, D.J. and Chambers, B.S. (2002) Nematode Communities under Stress: The Long-Term Effects of Heavy Metals in Soil Treated with Sewage Sludge. *Applied Soil Ecology*, **20**, 27-42. https://doi.org/10.1016/S0929-1393(02)00005-7

[18] Bardgett, R.D., Cook, R., Yeates, G.W. and Denton, C.S. (1999) The Influence of Nematodes on Below-Ground Processes in Grassland Ecosystems. *Plant and Soil*, **212**, 23-33. https://doi.org/10.1023/A:1004642218792

[19] Van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., De Goede, R.G.N., *et al.* (2019) Soil Nematode Abundance and Functional Group Composition at a Global Scale. *Nature*, **572**, 194-198. https://doi.org/10.1038/s41586-019-1418-6

[20] Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. (1993) Feeding Habits in Soil Nematode Families and Genera—An Outline for Soil Ecologists. *Journal of Nematology*, **25**, 315-331.

[21] Bongers, T. (1990) The Maturity Index: An Ecological Measure of Environmental Disturbance Based on Nematode Species Composition. *Oecologia*, **83**, 14-19. https://doi.org/10.1007/BF00324627

[22] Ferris, H. (2010) Form and Function: Metabolic Footprints of Nematodes in the Soil Food Web. *European Journal of Soil Biology*, **46**, 97-104. https://doi.org/10.1016/j.ejsobi.2010.01.003

[23] Pen-Mouratov, S., He, X. and Steinberger, Y. (2004) Spatial Distribution and Trophic Diversity of Nematode Populations under *Acacia raddiana* along a Temperature Gradient in the Negev Desert Ecosystem. *Journal of Arid Environments*, **56**, 339-355. https://doi.org/10.1016/S0140-1963(03)00058-2

[24] Salamun, P., Renco, M., Kucanova, E., Brazova, T., Papajova, I., Miklisova, D. and Hanzelova, V. (2012) Nematodes as Bioindicators of Soil Degradation Due to Heavy Metals. *Ecotoxicology*, **21**, 2319-2330. https://doi.org/10.1007/s10646-012-0988-y

[25] Pen-Mouratov, S., Shukurov, N. and Steinberg, C. (2008) Influence of Industrial Heavy Metal Pollution on Soil Free-Living Nematode Population. *Environmental Pollution*, **152**, 172-183. https://doi.org/10.1016/j.envpol.2007.05.007

[26] Shukurov, N., Pen-Mouratov, S. and Steinberger, Y. (2005) The Impact of Almalyk Industrial Complex on Soil Chemical and Biological Properties. *Environmental Pollution*, **136**, 331-340. https://doi.org/10.1016/j.envpol.2004.12.007

[27] Sanchez Moreno, S. and Navas, A. (2007) Nematode Diversity and Food Web Condition in Heavy Metal Polluted Soils in a River Basin in Southern Spain. *European*

Journal of Soil Biology, **43**, 166-179. https://doi.org/10.1016/j.ejsobi.2007.01.002

[28] Korthals, G.W., Alexiev, A.D., Lexmond, T.M., Kammenga, J.E. and Bongers, T. (1996) Long-Term Effects of Copper and pH on the Nematode Commuinity in an Agroecosystem. *Environmental Toxicology and Chemistry*, **15**, 979-985. https://doi.org/10.1002/etc.5620150621

[29] Yeates, G.W., Orchard, V.A., Speir, T.W., Hunt, J.L. and Hermans, M.C.C. (1994) Impact of Pasture Contamination by Copper, Chromium, Arsenic Timber Preservative on Soil Biological Activity. *Biology and Fertility of Soils*, **18**, 200-208. https://doi.org/10.1007/BF00647667

[30] Bockl, M., Blay, K., Fischer, K., Mommertz, S. and Filser, J. (1998) Colonisation of a Copper-Decontaminated Soil by Micro- and Mesofauna. *Applied Soil Ecology*, **9**, 489-494. https://doi.org/10.1016/S0929-1393(98)00110-3

[31] Komulainen, M. and Mikola, J. (1993) Soil Processes as Influenced by Heavy Metals and the Composition of Soil Fauna. *Journal of Applied Ecology*, **32**, 234-241. https://doi.org/10.2307/2404432

[32] Lecomte-Pradines, C., Bonzom, J.-M., Della-Vedova, C., Beaugelin-Seiller, K., Villenave, C., Gaschak, S., Coppin, F., *et al.* (2014) First Use of Soil Nematode Communities as Bioindicator of Radiation Impact in the Chernobyl Exclusion Zone. *Science of the Total Environment*, **490**, 161-170. https://doi.org/10.1016/j.scitotenv.2014.04.115

[33] Nagy, P., Bakonyi, G., Bongers, T., Kadar, I., Fabian, M. and Kiss, I. (2004) Effects of Microelements on Soil Nematode Assemblages Seven Years after Contaminating an Agricultural Field. *Science of Total Environment*, **320**, 131-143. https://doi.org/10.1016/j.scitotenv.2003.08.006

[34] Bakonyi, G., Nagy, P. and Kadar, I. (2003) Long-Term Effects of Heavy Metals and Microelements on Nematode Assemblage. *Toxicology Letters*, **140**, 391-401. https://doi.org/10.1016/S0378-4274(03)00035-3

[35] Bardgett, R.D., Speir, T.W., Ross, D.J., Yeates, G.W. and Kettles, H.A. (1994) Impact of Pasture Contamination by Copper, Chromium and Arsenic Tomber Preservative on Soil Microbial Properties and Nematodes. *Biology and Fertility of Soils*, **18**, 71-79. https://doi.org/10.1007/BF00336448

[36] Chen, G., Qin, J., Shi, D.Z., Zhang, Y.M. and Ji, W.H. (2009) Diversity of Soil Nematodes in Areas Polluted with Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) in Lanzhou, China. *Environmental Management*, **44**, 163-172. https://doi.org/10.1007/s00267-008-9268-2

[37] Ellis, R.J., Neish, B., Trett, M.W., Best, J.G., Weightman, A.J., Morgan, P. and Fry, J.C. (2001) Comparison of Microbial and Meiofaunal Community Analyses for Determining Impact of Heavy Metal Contamination. *Journal of Microbiological Methods*, **45**, 171-185. https://doi.org/10.1016/S0167-7012(01)00245-7

[38] Korthals, G.W., Popovici, I., Iliev, I. and Lexmond, T.M. (1998) Influence of Perennial Ryegrass on a Copper and Zinc Affected Terrestrial Nematode Community. *Applied Soil Ecology*, **10**, 73-85. https://doi.org/10.1016/S0929-1393(98)00039-0

[39] Korthals, G.W., Bongers, M., Fokkema, A., Dueck, T. and Lexmond, T. (2000) Joint Toxicity of Copper and Zinc to a Terrastrial Nematode Community in an Acid Sandy Soil *Ecotoxicology*, **9**, 219-228. https://doi.org/10.1023/A:1008950905983

[40] Korthals, G.W., van de Ende, A., van Megen, H., Lexmond, T.M., Kammenga, J.E. and Bongers, T. (1996) Short-Term Effects of Cadmium, Copper, Nickel and Zinc on Soil Nematodes from Different Feeding and Life-History Strategy Groups. *Applied Soil Ecology*, **4**, 107-117. https://doi.org/10.1016/0929-1393(96)00113-8

[41] Le Guedard, M., Bessoule, J.-J., Cérémonie, H., Faure, A., Fayolle, G. and Nau, J.-F.C.V. (2016) Applicabilité à l'étude des sites Pollués du Biomarqueur Lipidique des Végétaux et du Bioindicateur Nématofaune. Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, 181 p.

[42] Li, Q., Jiang, Y. and Liang, W.J. (2006) Effects of Heavy Metals on Soil Nematode Communities in the Vicinity of Metallurgical Factory. *Journal of Environmental Sciences*, **18**, 323-328.

[43] Li, Q., Zhong, S., Li, F.P., Lou, Y.L. and Liang, W.J. (2011) Nematode Community Structure as Bioindicator of Soil Heavy Metal Pollution Along an Urban-Rural Gradient in Southern Shenyang, China. *International Journal of Environment and Pollution*, **45**, 297-309. https://doi.org/10.1504/IJEP.2011.040276

[44] Liang, W., Li, Q., Zhang, X., Jiang, S. and Jiang, Y. (2006) Effects of Heavy Metals on soil Nematode Community Structure un Shenyang Suburds. *American-Eurasian Journal of Agricultural & Environmental Sciences*, **1**, 14-18.

[45] Nagy, P. (1999) Effect of an Artificial Metal Pollution on Nematode Assemblage of a Calcareous Loamy Chernozem Soil. *Plant and Soil*, **212**, 35-43. https://doi.org/10.1023/A:1004657924496

[46] Park, B.-Y., Lee, J.-K., Ro, H.-M. and Kim, Y.H. (2011) Effects of Heavy Metal Contamination from an Abandoned Mine on Nematode Community Structure as an Indicator of Soil Ecosystem Health. *Applied Soil Ecology*, **51**, 17-24. https://doi.org/10.1016/j.apsoil.2011.08.006

[47] Pérès, G., Piron, D., Pauget, B., de Vaufleury, A., Le Guédard, M., Bessoule, J.J., Villenave, C., *et al.* (2015) Intérêt des Outils Biologiques pour Évaluer les Transferts et les Effets de Contaminants d'un sol Pollué dans le cadre d'un Projet D'Aménagement Urbain, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, 41 p.

[48] Shao, Y.H., Zhang, W.X., Shen, J.C., Zhou, L.X., Xia, H.P, Shu, W.S., Ferris, H. and Fu, S.L. (2008) Nematodes as Indicators of Soil Recovery in Tailings of Lead/Zinc Mine. *Soil Biology and Biochemistry*, **40**, 2040-2046. https://doi.org/10.1016/j.soilbio.2008.04.014

[49] Sharma, K., Cheng, Z. and Grewal, P.S. (2014.) Relationship between Soil Heavy Metal Contamination and Soil Food Web Health in Vacant Lots Slated for Urban Agriculture in Two Post-Industrial Cities. *Urban Ecosystem*, **18**, 835-855. https://doi.org/10.1007/s11252-014-0432-6

[50] Smit, C.E., Schouten, A.J., Van der Brick, P.J., van Esbroek, M.L. and Posthum, L. (2002) Effects of Zinc Contamination on a Natural Nematode Community in Outdoor Soil Mesocosmes. *Archives of Environmental Contamination and Toxicology*, **42**, 205-216. https://doi.org/10.1007/s00244-001-0029-y

[51] Tomar, V.V.S., Zhang, X.K., Li, Q., Jiang, Y. and Liang, W.J. (2009) Distribution of Soil Nematode Communities along a Section of Shen-Ha Highway. *Helminthologia*, **46**, 241-246. https://doi.org/10.2478/s11687-009-0044-5

[52] Villenave, C. (2012) Analyse de la Nématofaune Comme Bioindicateur de l'état du sol. Rapport Final du Projet Bioindicateur, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, 147 p.

[53] Villenave, C. (2012) Rapport d'étude sur la Nématofaune du sol à Saint Laurent le Minier sur d'Anciens sites d'Activités Minières. Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, 19 p.

[54] Wang, S.-B., Li, Q., Liang, W.-J., Jiang, Y. and Jiang, S.-W. (2008) PCR-DGGE Analysis of Nematode Diversity in Cu-Contaminated Soil. *Pedosphere*, **18**, 621-627.

https://doi.org/10.1016/S1002-0160(08)60056-9

[55] Weiss, B. and Larink, O. (1991) Influence of Sewage Sludge and Heavy Metals on Nematodes in an Arable Soil. *Biology and Fertility of Soils*, **12**, 5-9. https://doi.org/10.1007/BF00369381

[56] Zhang, X.K., Li, Q., Wang, S.B., Jiang, Y. and Liang, W.J. (2006) Effect of Zinc Addition in Soil on Nematode Community Structure. *Bulletin of Environmental Contamination and Toxicology*, **76**, 589-594. https://doi.org/10.1007/s00128-006-0960-8

[57] Zhang, W.D., Wang, X.F., Li, Q., Jiang, Y. and Liang, W.J. (2007) Soil Nematode Response to Heavy Metal stress. *Helminthologia*, **44**, 87-91. https://doi.org/10.2478/s11687-007-0009-5

[58] Yeates, G.W. (2003) Nematodes as Soil Indicators: Functional and Biodiversity Aspects. *Biology and Fertility of Soils*, **37**, 199-210. https://doi.org/10.1007/s00374-003-0586-5

[59] Ferris, H., Bongers, T. and de Goede, R. (2001) A Framework for Soil Food Web Diagnostics: Extension of the Nematode Faunal Analysis Concept. *Applied Soil Ecology*, **18**, 13-29. https://doi.org/10.1016/S0929-1393(01)00152-4

[60] Villanneau, E., Perry-Giraud, C., Saby, N., Jolivet, C., Marot, F., Maton, D., Floch-Barneaud, A., *et al.* (2008) Détection de Valeurs Anomaliques d'éléments Traces Métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. *Etudes et Gestion des Sols*, **15**, 183-200.

[61] Baize, D. (2000) Teneurs Totales en "Métaux Lourds" dans les sols Français. Résultats Généraux du Programme ASPITET. No. 39, Institut National de la Recherche Agronomique, Paris, 39-54.

[62] Taibi, S., Lepelletier, P., Dantan, J., Bodin, J., Dur, J.-C. and Rouge, L. (2012) Gestion et Traitement des données du Programme Bioindicateurs II: Approche Statistique de Sélection d'Indicateurs et de Biomarqueurs dans la Surveillance de la Qualité des sols et l'Évaluation des Risques. Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, 92 p.

[63] Michel, L., Brun, F. and Makowski, D. (2017) A Framework Based on Generalised Linear Mixed Models for Analysing Pest and Disease Surveys. *Crop Protection*, **94**, 1-12. https://doi.org/10.1016/j.cropro.2016.12.013

[64] Coll, P., Le Cadre, E. and Villenave, C. (2012) How Are Nematode Communities Affected during a Conversion from Conventional to Organic Farming in Southern French Vineyards? *Nematology*, **14**, 665-676. https://doi.org/10.1163/156854112X624195

[65] Novozamsky, I., Lexmond, T.M., Houba, V.J.G. (1993) A Single Extraction Procedure of Soil for Evaluation of Uptake of Some Heavy Metals in Plants. *International Journal of Environmental Analytical Chemistry*, **51**, 47-58. https://doi.org/10.1080/03067319308027610

[66] Lakanen, E. and Erviö, R. (1971) A Comparison of Eight Extractants for the Determination of Plant Available Micronutrients in Soils. *Acta Agralia Fennica*, **123**, 223-232.

[67] Grand, C., Faure, O., Harris-Hellal, J., Hedde, M., Le Guédard, M., Pauget, B., Péres, G., *et al.* (2012) Quels Bioindicateurs, Pour quels Besoins en sites Contaminés? *Compte rendu des Journées de Restitution du projet "Bioindicateur Pour la caractérisation des sols"*, Paris.

[68] Clemente, R., Walker, D.J., Roig, A.B. and Bernal, M.P. (2003) Heavy Metal Bioavailability in a Soil Affected by Mineral Sulphides Contamination following the Mine Spillage at Aznalcóllar (Spain). *Biodegradation*, **14**, 199-205.

https://doi.org/10.1023/A:1024288505979

[69] Cappuyns, V., Swennen, R. and Verhulst, J. (2004) Assessment of Acid Neutralizing Capacity and Potential Mobilisation of Trace Metals from Land-Disposed Dredged Sediments. *Science of the Total Environment*, **333**, 233-247. https://doi.org/10.1016/j.scitotenv.2004.05.007

[70] Sochova, I., Hofman, J. and Holoubek, I. (2006) Using Nematodes in Soil Ecotoxicology. *Environment International*, **32**, 374-383. https://doi.org/10.1016/j.envint.2005.08.031

[71] Zhao, J. and Neher, D. (2013) Soil Nematode Genera That Predict Specific Types of Disturbance. *Applied Soil Ecology*, **64**, 135-141. https://doi.org/10.1016/j.apsoil.2012.11.008

[72] Fageria, N.K., Baligar, V.C. and Clark, R.B. (2002) Micronutrients in Crop Production. *Advances in Agronomy*, **77**, 185-268. https://doi.org/10.1016/S0065-2113(02)77015-6

[73] Ekschmitt, K. and Korthals, G.W. (2006) Nematodes as Sentinels of Heavy Metals and Organic Toxicants in the Soil. *Journal of Nematology*, **38**, 13-19.

[74] Nieboer, E. and Richardson, D.H.S. (1980) The Replacement of the Nondescript Term 'Heavy Metals' by a Biologically and Chemically Significant Classification of Metal Ions. *Environmental Pollution Series B, Chemical and Physical*, **1**, 3-26. https://doi.org/10.1016/0143-148X(80)90017-8

[75] Millward, R.N. and Grant, A. (2000) Pollution-Induced Tolerance to Copper of Nematode Communities in the Severely Contaminated Restronguet Creek and Adjacent Estuaries, Cornwall, United Kingdom. *Environmental Toxicology and Chemistry*, **19**, 454-461. https://doi.org/10.1002/etc.5620190227

[76] Nielsen, U.N., Ayres, E., Wall, D.H., Li, G., Bardgett, R.D., Wu, T.H. and Garey, J.R. (2014) Global-Scale Patterns of Assemblage Structure of Soil Nematodes in Relation to Climate and Ecosystem Properties. *Global Ecology and Biogeography*, **23**, 968-978. https://doi.org/10.1111/geb.12177

[77] Villenave, C., Jimenez, A., Guernion, M., Pérès, G., Cluzeau, D., Mateille, T., Martiny, B., *et al.* (2013) Nematodes for Soil Quality Monitoring: Results from the RMQS BioDiv Programme. *Open Journal of Soil Science*, **3**, 30-45. http://dx.doi.org/10.4236/ojss.2013.31005

[78] Zullini, A. and Peretti, E. (1986) Lead Pollution and Moss-Inhabiting Nematodes of an Industrial Area. *Water, Air, and Soil Pollution*, **27**, 403-410. https://doi.org/10.1007/BF00649421

[79] Ferris, H. and Bongers, T. (2006) Nematode Indicators of Organic Enrichment. *Journal of Nematology*, **38**, 3-12.

[80] DuPont, S.T., Ferris, H. and Van Horn, M. (2009) Effects of Cover Crop Quality and Quantity on Nematode-Based Soil Food Webs and Nutrient Cycling. *Applied Soil Ecology*, **41**, 157-167. https://doi.org/10.1016/j.apsoil.2008.10.004

[81] Gutiérrez, C., Fernández, C., Escuer, M., Campos-Herrera, R., Rodríguez, M.E.B., Carbonell, G. and Martín, J.A.R. (2016) Effect of Soil Properties, Heavy Metals and Emerging Contaminants in the Soil Nematodes Diversity. *Environmental Pollution*, **213**, 184-194. https://doi.org/10.1016/j.envpol.2016.02.012

[82] Rajapaksha, R.M.C.P., Tobor-Kapłon, M.A. and Bååth, E. (2004) Metal Toxicity Affects Fungal and Bacterial Activities in Soil Differently. *Applied and Environmental Microbiology*, **70**, 2966-2973. https://doi.org/10.1128/AEM.70.5.2966-2973.2004