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NEMATODE COLONIZATION OF PYRITE CINDER-POLLUTED SOIL (1)

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The nematofauna was studied in the early stages of a remediation trial at an industrial site where pyrite cinders had accumulated for 40 years in a large area and were then covered with mineral soil. The cinders were contaminated with several metals and metalloids. The soil of the experimental plots was derived from the mixture of cinders with the covering soil in a 1:1 ratio. Plots were amended with manure and sown with 3 metal resistant plants: *Sorghum bicolor* L., *Helianthus annuus* L., and *Arundo donax* L. Samples were taken : a) at the beginning of the trial on the pyrite cinders and covering soil, separately; b) from the mixture before the application of manure; c) from cultivated plots. Nematode communities were compared by using general composition, trophic structure, biodiversity and ecology indices. Nematofauna and other soil fauna were not detected in the pyrite cinders. Nematodes were recorded in the covering soil and after mixing with the cinders. In these early stages of the remediation process, amending and cropping increased nematode abundance and biodiversity compared to the initial situation of the pyrite cinders. The nematode community structures and all calculated indices showed an increase in the quality of the soil after the remediation process. Our results showed that phytoremediation brought about the repopulation of an extremely compromised area. Moreover, the analysis of nematofauna could be a useful tool for assessing the degree of soil disturbance and soil remediation.

KEY WORDS: Recovery, Remediation, Biodiversity, Maturity Index, Ecological Indices, Nematofauna.

INTRODUCTION

The industrial production of sulfuric acid from pyrite results in a large quantity of cinders which are extremely contaminated by heavy metals and arsenic. Because of the specific chemical and physical properties of pyrite cinders, the natural colonization of pyrite cinder disposal sites is a very slow process. For this reason, various methods of reclamation are applied. One of these methods is phytoremediation, which consists of the combined use of plants, soil amendments, and agronomic practices to remove pollutants from the environment or to decrease their environmental toxicity (SALT *et al.*, 1998).

The main objective of soil remediation is to reduce pollutants and heavy metals, support fauna and flora colonization, and increase soil health. Consequently, chemical analysis for a substance-based approach is not sufficient because soil is a very complex living matrix including soil fauna (MANACHINI *et al.*, 2009; EFSA, 2017). Thus, it is necessary to also consider ecotoxicological analysis for a matrix-based approach (PIVATO *et al.*, 2017; PIVATO *et al.*, 2018).

Nematodes are considered useful bioindicators for many different situations. They can be used in ecotoxicological bioassays investigating the effects of a substance or a specific soil directly on a focal species in the laboratory and/or in microcosms (BEYREM *et al.*, 2007; PIVATO *et al.*, 2017; PIVATO *et al.*, 2018; GARBO *et al.*, 2019), or the changes of their communities and biodiversity due to different stresses can be studied directly in the field (WILLIAMSON *et al.*, 2005; ZHANG *et al.*, 2006; EFSA, 2017; MANACHINI *et al.*, 2009). Following the natural colonization of a polluted ecosystem, the effectiveness of land reclamation may be evaluated using nematodes as bioindicators (BONGERS and FERRIS, 1999; FERRIS and MATUTE, 2003).

Although changes in the structure of nematode communities have been widely used to assess the ecotoxicological effects of different soil disturbances including several pollutants, e.g. herbicides and heavy metals (GEORGIEVA *et al.*, 2002; KORTHALS *et al.*, 1996a; KORTHALS *et al.*, 1996b; PEN-MOURATOV *et al.*, 2008), there are few studies of nematodes associated with waste dumps (ŠÁLY, 1983; URZELAI *et al.*, 2000; DMOWSKA, 2001). Wastes from industrial and agricultural activities are complex substrates in which individual harmful substances can combine to produce a variety of effects (HÁNĚL, 2004). Analyses of successive changes in nematode assemblages in both reclaimed and unreclaimed power plant ash dumps have been done in order to provide information about the sensitivity of functional groups of nematodes (DMOWSKA, 2001; DMOWSKA and ILIEVA-MAKULEC, 2006). Other studies have focused on nematofauna associated with coal-mining dumps undergoing different reclamation practices (HÁNĚL, 2002).

To date, information on nematodes in soil conta-

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minated by pyrite cinders is limited to one study, conducted in the Czech Republic by HÁNĚL (2002), which investigated the natural plant colonization process of iron pyrite dumps and other materials deposited into the soil over 40 years ago. The author found that nematode colonization of waste dumps was slow, with a prolonged initial stage dominated by bacterial feeders of Rhabditida with a short generation time. Moreover, even though nematode diversity increased as the humus content increased and moss and grass invaded the dumps, the communities exhibited many differences from the nearby semi-natural environment.

Our study focused on a remediation project that started in 2005 in an industrial area in north-eastern Italy where pyrite cinders were buried in the soil and covered by mineral soil (MARCHIOL *et al.*, 2007). We examined the influence of this pyrite contamination (that ended in the 1970s) and the recent phytoremediation on the soil nematode fauna, assessing the bioindicative power of nematode analysis. This article reports the changes in nematode communities during the early stages of the phytoremediation process, considering: (i) the natural colonization of the nematofauna and (ii) the effects of plant species on nematode communities.

MATERIALS AND METHODS

STUDY AREA

The polluted site is located in north-eastern Italy (Torviscosa, UD, 45° 49' N, 13° 16' E, 14 m above sea level). The climate is temperate, with a mean annual temperature of 13.5 °C and an average annual precipitation of 1200 mm.

The study was conducted in an experimental field located in a chemical factory that produced primary basic as well as fine chemicals. Decree n. 468/2001 of the Italian Ministry for the Environment, Land, and Sea includes this area and its surroundings in the national priority list of polluted sites, under the name "Laguna di Grado e di Marano".

The whole area, approximately 110 ha, is polluted with heavy metals and/or organic compounds, and the soil of the experimental site is contaminated with pyrite cinders, a by-product of sulfuric acid manufacturing operations (800 °C roasting temperature); it contains a large amount of iron (about 50%) and other pollutants, such as arsenic, cadmium, copper and zinc, which are commonly associated with pyrite. The cinders were deposited on the soil for 40 years until the late 1970s. In the site, a layer of cinders about 1 m deep was covered by a thick layer (about 0.2 m) of less polluted, gravelly soil; beneath the cinders, deep impermeable clay meant that metal leaching was almost negligible.

A selection of analytical characteristics of the cinders and the covering soil and mixture, before manure amendment, is reported in Table 1. Particle size analyses, determined by sedimentation, indicated that the pyrite cinders were sandy loam (USDA classification), with a prevalence in the silty fraction of the finest sized that implies a susceptibility to cementation and low permeability. This disadvantage may cause serious problems to plant roots and soil fauna. The cinders showed a neutral pH (1:2.5 soil:water), an absence of organic matter, a negligible amount of carbonate, and low conductivity (saturated paste extraction). Total metal concentration in the cinders was determined by ICP-AES after microwave-assisted digestion. Furthermore, the cinders were extracted by the DTPA/TEA, LINDSAY and NORWELL (1978).

The covering soil was loamy sand, moderately alkaline, with a low content of organic matter and a small amount of carbonate.

In March 2005, the experimental field was prepared in an area of 200 m^2. The covering soil was dislodged and an equal amount of cinders was excavated from the cinder layer; they were then mechanically mixed and scattered over the area. The two soil components (ratio 1:1) had chemical and physical characteristics intermediate to those of the pyrite cinders and the covering soil (Table 1).

EXPERIMENTAL PLAN

The experiment was arranged into 9 plots of 15.75 m^2 (4.5 m × 3.5 m) each. In order to increase the organic matter and nutrient content of the mixture, all plots were amended with a dose equivalent to 90 t/ha of well-rotted cow manure 15 days before sowing. Chemical characterization of the manure is reported in Table 2. On 4 May 2005, the plots were sown with metal resistant plant species (3 plots per species): sorghum (*Sorghum bicolor* L.), sunflowers (*Helianthus annuus* L.), and giant reeds (*Arundo donax* L).

The species were chosen for their ability to accumulate several heavy metals (MURILLO *et al.*, 1999; KAMEL and KHATER, 2004; PAPAZOGLOU *et al.*, 2005) and for their adaptability to different ecological conditions. Indeed, a preliminary study by MARCHIOL *et al.* (2007) in the same area demonstrated the tolerance of these plant species to different levels of pyrite cinders. The field trial was arranged in a randomized block design, with one factor (species) and three replications. Due to legal issues, the experimental trial was interrupted in July 2005 and, consequently, it was not possible to continue the study after this date.

NEMATODE SAMPLING AND EXTRACTION

Three bulk samples consisting of 9 cores (core diameter 50 mm) were taken from removed pyrite cinders as well as from the dislodged covering soil (March 2005). All the experimental plots were sampled before manure amendment (May 2005) and 2 months (July 2005) after sowing (3 replicates for each crop). In each plot, nine cores (core diameter 50 mm) from 0-20 cm soil depth were taken randomly and combined into one bulk-sample per plot. Nematodes were extracted from 2 sub-samples each of 150 g of soil using modified Baermann

Table 1 - Physical and chemical characteristics of pyrite cinders, covering soil, and mixture before manure amendment

		Pyrite cinders	**Covering soil**	**Mixture**
pH (H_2O)		6.90	8.22	7.75
Organic carbon (g kg^{-1})		0.00	14.60	9.14
Electric conductivity (mS cm^{-1})		2.39	0.57	n.d.
Total carbonate (g kg^{-1})		0.14	64.00	n.d.
Gravel (%)		0.00	72.00	33.00
Sand (%)	2 - 0.05 mm	n.d	n.d.	72.40
	2 - 0.2 mm	23.4	58.60	n.d.
	0.2 -0.1-mm	21.1	10.20	n.d.
	0.1 - 0.05 mm	26.5	9.00	n.d.
Loam (%)	0.05-0.002 mm	n.d.	n.d.	25.60
	0.05-0.02 mm	7.60	6.70	n.d.
	0.02 - 0.002 mm	19.9	12.5	n.d.
Clay (%)	< 0.002 mm	1.50	3.00	2.00

n.d.: below detection limit

funnel. In the case of the cinders, we decided to increase the quantity analyzed up to 3000 g because no nematodes were recorded in 300 g of sample. Moreover, we decide to check for the presence of nematodes in the manure in order to understand if there was a contribution of manure to the soil nematofauna.

The extraction of nematodes was carried out for 24 hours. After counting the total number of nematodes, all were identified at genus level.

Table 2 - Chemical characterization of the manure used in the field experiment

Organic C (mg g^{-1} d.w.)	316.0
TKN (mg g^{-1} d.w.)	19.1
C/N	16.0
Total P (mg g^{-1} d.w.)	8.26

DATA TREATMENT AND STATISTICAL ANALYSIS

Nematode diversity was described using the Shannon-Wiener Index (H') calculated at the genus level (SHANNON, 1948). As a measure of functional diversity, the Maturity Index (MI) was calculated according to BONGERS (1990), considering the c-p (colonizers-persisters) indices assigned to every family. The c-p indices describe nematode life strategies and their sensitivity to environmental disturbances, with values ranging from 1 to 5 (from colonizers to persisters). We chose to use the original version of MI (BONGERS, 1990) because the very low abundance of plant feeders recorded in the present study did not affect the data. Moreover, following BONGERS (1990), we decided to consider the genus *Butlerius* as c-p 1 because it is strictly associated with organic matter, even though other authors (GROOTEART *et al.*, 1977) gave to the genera belonging to Diplogasteridae a high c-p equal to 3. However, Cp1 taxa can also indicate extremely nutrient enriched environments, and Cp2 taxa are considered by some authors to be the most tolerant group (FERRIS *et al.*, 2003).

The nematodes were classified into functional groups (FERRIS *et al.*, 2001) to calculate four indices: Enrichment Index (EI), Basal Index (BI), Structure Index (SI) and Channel Index (CI). The EI indicates fast-growing, bacteria-feeding and fungi-feeding nematodes with c-p values of 1 or 2. The BI contains bacteria-feeding and fungi-feeding nematodes with c-p values of 2. The SI measures the slow growing and reproducing predatory and omnivore nematodes with c-p values of 3, 4, and 5 (BERKELMANS *et al.*, 2003). The Channel Index (CI), a weighted ratio of opportunistic bacterial and fungal feeder nematodes, was calculated according to FERRIS and MATUTE (2003).

The total abundance was transformed into logarithms (x + 1) for the analysis of variance. Data on proportions of families and trophic groups underwent arcsine square root transformation. The combined effects of manure and plant species was tested by ANOVA using SPSS (version 14.0). We did not consider seasonal effects on nematofauna following RHAMAN *et al.* (2007), who reported that neither free-living nor plant-parasitic nematodes increased or decreased consistently between the sampling months.

Table 3 - Total and available (DTPA sol. and ammonium sulphate) concentrations of metals and arsenic (As) in pyrite cinders, covering soil, and mixture (mg kg^{-1}), along with the concentration thresholds fixed by Italian law (ILTL = Italian legal threshold level, Legislative Decree 152/06) for residential soil utilization

		Pyrite cinders	**Covering soil**	**Mixture**	**ILTL**
Cd	Total	9.61	3.93	5.53	2.00
	DTPA sol.	0.25	n.m.	0.11	
Cr	Total	13.90	29.90	18.60	150.00
	DTPA sol.	< 0.50	n.m.	n.d.	
Cu	Total	1971.00	69.80	1527.00	120.00
	DTPA sol.	121.00	n.m.	228.00	
Ni	Total	20.20	31.80	22.90	120.00
	DTPA sol.	< 0.20	n.m.	0.22	
Pb	Total	410.00	107.00	255.00	100.00
	DTPA sol.	71.80	n.m.	13.80	
Zn	Total	1876.00	100.00	980	150.00
	DTPA sol.	55.80	n.m.	23.64	
As	Total	750.00	18.00	446.00	20.00
	$(NH_4)2SO_4$ sol.	n.d.	n.m.	3.51	

n.d. – below detection limit
n.m. – not measured

RESULTS

HEAVY METAL CONTAMINATION

The degree to which the materials studied (cinders, soil, and their mixture) were polluted was investigated through an analysis of the total and bioavailable heavy metal content (Table 3). Our data confirm the results of MARCHIOL *et al.* (2007) for the same area.

To measure the bioavailable fraction of heavy metals, we used 0.01M DTPA/TEA; this extractant is currently the most widely used for evaluating plant-available micronutrients and contaminants, showing good correlations with plant Cu and Zn uptake (SIMMONS and PONGSAKUL, 2004). The strength of DTPA extraction, nevertheless, was higher than those of other frequently adopted extractants (e.g. $CaCl_2$, $NaNO_2$).

NEMATODE COMMUNITY COMPOSITION

Nematodes were not detected in the pyrite cinders, probably due to high trace element concentrations and unsuitable physical properties. Nematodes were introduced into the soil/pyrite mixture from the covering soil. In fact, nematode density in the mixture was about half of that found in the covering soil. Nematode abundance differed significantly among the mixture and giant reed and sunflower plots, but not for sorghum. Among the cultivated plots, nematode abundance in sorghum was significantly lower than in the giant reed and sunflower plots (Table 4).

In all samples, bacterial feeders were the dominant trophic group, ranging between 96% in the mixture and 63% in the sorghum plots (Fig. I).

Bacterial feeder abundance was increased almost 4-fold by mixture amendment and cropping with sunflowers and giant reeds, but not with sorghum (Table 4). A shift in the bacterial feeder composition was recorded in all the cultivated plots, with *Rhabditis* becoming the most abundant and dominant genus. Fungal feeders were abundant in the covering soil (33%) (Fig. I); their presence dramatically decreased in the mixture, reaching less than 5% of the total nematofauna. They began to grow again in the cultivated plots, particularly in sorghum plots, due to *Aphelenchoides* (Table 4).

Omnivores were absent in the covering soil and in the mixture, while they were detected in the cultivated plots; the number of omnivores was significantly higher in sorghum plots than in giant reed and sunflower plots, though it was associated with a great variability. The only genus recorded was *Butlerius*, which comes from manure (Table 5).

In these early stages of the colonization, plant feeders were present in low percentage (<1%) both in the covering soil and in the mixture, and no differences were observed among the systems.

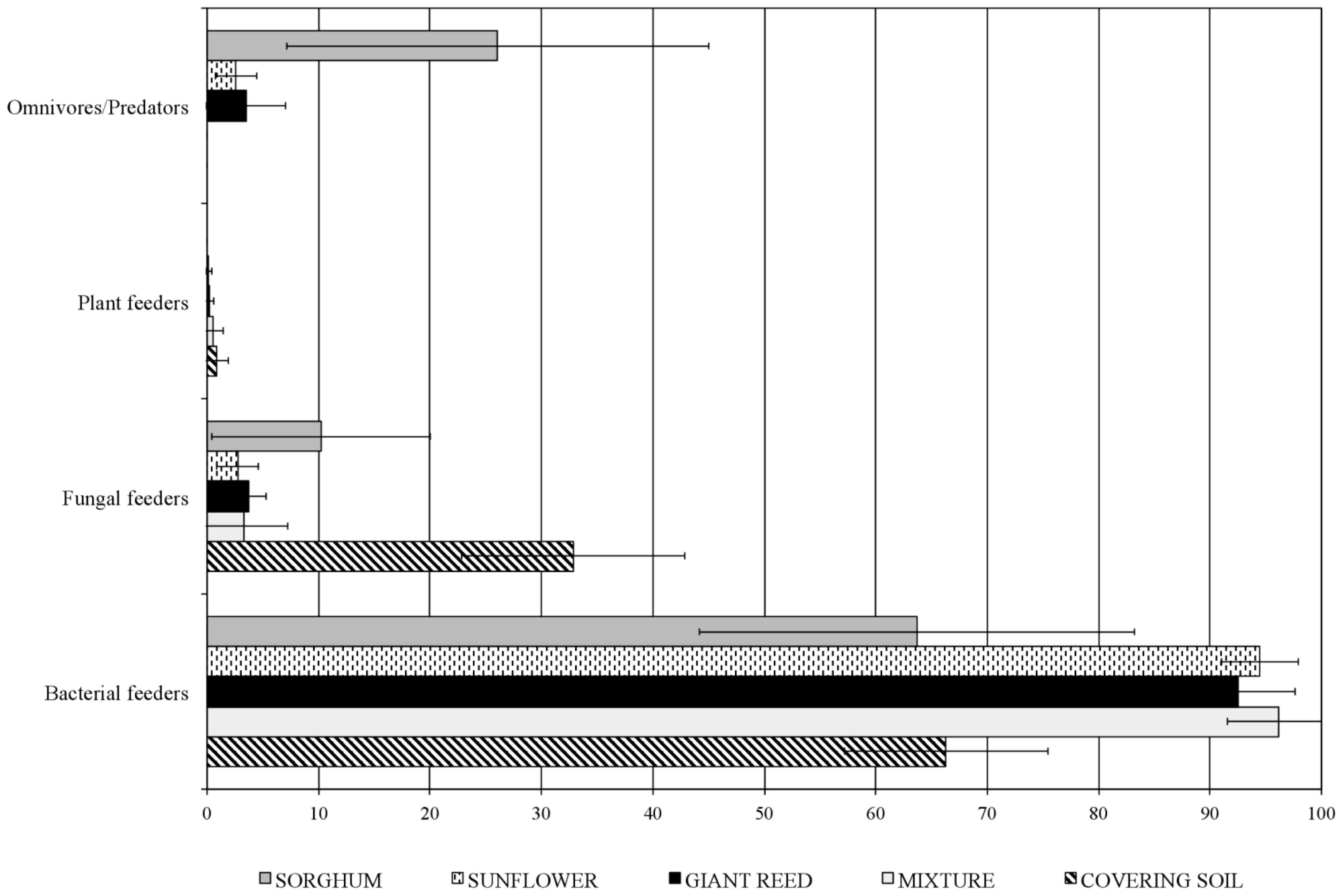

Fig. I - Trophic group composition (%) in covering soil and a 1:1 mixture of covering soil and pyrite cinders in May and two months after planting with giant reeds, sunflowers or sorghum following soil amendment with cow manure.

Table 4 - Total abundance, feeding groups, and abundance of individual taxa (150 g of sample)

	Pyrite cinders	Covering soil	Mixture	Giant reeds	Sunflowers	Sorghum	
Total abundance	–	64.0 ± 62.7	34.5 ± 13.9 a	132.0 ± 56.1 b	125.0 ± 52.4 b	46.8 ± 6.5 a	
Number of genera	–	10	9	9	10	11	
Genera							**c-p value**
Bacterial feeders (%)	–	45.7 ± 46.7	32.7 ±12.3 a	119.8 ± 43.9 b	116.8 ± 46.7 b	29.5 ± 8.3 a	
Rhabditis	–	5.3 ± 6.7	6.5 ± 3.4 a	99.3 ± 30.0 b	92.3 ± 30.9 b	20.8 ± 10.9 a	1
Diploscapter	–	1.0 ± 1.4	–	1.8 ± 2.9	2.8 ± 3.1	1.0 ± 1.2	1
Plectus	–	6.8 ± 7.9	3.0 ± 1.4	–	–	–	2
Cephalobus	–	26.0 ± 26.9	15.0 ± 5.6	14.8 ± 8.3	12.0 ± 8.8	4.8 ± 2.6	2
Eucephalobus	–	4.0 ± 4.5	4.5 ± 3.7	3.0 ± 2.2	7.3 ± 2.2	1.8 ± 2.1	2
Acrobeloides	–	2.3 ± 3.9	0.5 ± 0.6	–	0.5 ± 1.0	–	2
Teratocephalus	–	0.5 ± 1.00	3.3 ±4.0	1.0 ± 1.4	2.0 ± 1.8	1.3 ± 1.9	3
Fungal feeders (%)	–	17.3 ± 14.9	1.3 ± 1.9	5.5 ± 4.5	3.8± 3.6	5.3 ± 5.6	
Aphelenchus	–	8.8 ± 8.6	1.0 ± 1.4	1.8 ± 2.4	0.8 ± 1.0	1.0 ± 1.4	2
Aphelenchoides	–	8.5 ± 10.7	0.3 ± 0.5 a	3.8 ± 2.2 ab	3.0 ± 2.9 ab	4.3 ± 4.3 b	2
Plant feeders (%)	–	1.0 ± 1.4	0.3 ± 0.5	0.3 ± 0.5	0.3 ± 0.5	–	
Tylenchus	–	1.0 ± 1.4	0.3 ± 0.5	0.3 ± 0.5	0.3 ± 0.5	–	2
Omnivore/Predators (%)	–	–	–	6.0 ± 8.2 a	3.8 ± 3.3 a	12.0 ± 4.4 b	
Butlerius	–	–	–	6.0 ± 8.2 a	3.8 ± 3.3 a	12.0 ± 4.4 b	3

CONTRIBUTION OF MANURE TO THE SOIL NEMATOFAUNA

Rhabditis increased from 4- to 16-fold after the addition of manure to the mixture. *Aphelenchus* seemed to be unaffected by the manure application; *Butlerius* was introduced by manure (Table 5) and found suitable conditions to grow. *Diplogasteroides* and *Prorhabditis* did not survive in the new habitat.

Table 5 - Contribution of manure to nematofauna in soil

Genus	c-p value	Manure	Soil
Omnivore-Saprophytic		47.6	–
Diplogasteroides	1	47.5	–
Butlerius	1	0.1	–
Bacterial feeders		43.9	306.0
Rhabditis	1	39.2	35.3
Prorhabditis	1	4.7	–
Total abundance		91.8	428.0

BIODIVERSITY AND ECOLOGICAL INDICES

MI and H' values did not change after mixing the pyrite cinders and covering soil, whereas in the cultivated plots they decreased slightly after amendment and sowing (Table 6). The EI in all the cultivated plots was very high compared to the values recorded in the covering soil and mixture (Table 6), suggesting that the ecosystems were highly nutrient enriched because of the manure addition. The SI was lower in the covering soil and in the mixture than in the cultivated plots (Table 6). Moreover, the CI was higher in the covering soil compared to the values calculated for the other systems where fungal feeders were recorded in low abundance.

Table 6 - Maturity Index, Diversity Index and Ecological Indices for nematode communities sampled from covering soil, mixture and cultivated plots

Indices	Covering soil	Mixture	Giant reed	Sunflower	Sorghum
MI	1.9	1.8	1.3	1.3	1.2
H'	0.8	0.7	0.4	0.6	0.7
EI	50.5	54.1	95.0	94.7	88.6
BI	49.2	45.2	4.9	5.1	7.6
SI	1.2	3.5	14.0	14.5	21.3
CI	37.9	3.6	1.2	0.9	5.3

MI–Maturity Index; H'–Shannon-Wiener Diversity Index; EI–Enrichment Index; BI–Basal Index; SI–Structure Index; CI–Channel Index.

DISCUSSION

Probably due to their chemical and physical properties, the pyrite cinders were not inhabited by soil fauna. In the covering soil, nematodes were scarce and mainly represented by bacterial and fungal feeders. This could be due to the soil characteristic and/or sustained contact with the cinders. The presence of heavy metals could have negatively affected the potential colonization by the fauna, as reported by YEATES *et al.* (2003) who found a combined effect of Cu, Ni, and Zn on the nematode fauna in pasture plots.

After mixing the covering soil and the pyrite cinders in a 1:1 ratio, a "dilution effect" resulted in a reduction of about one half of the nematode density in the mixture soil and a change in the abundance of fungal feeders. Agronomic practices and cropping modified nematode abundance, in particular in the giant reed and sunflower plots.

In the covering soil, a very poorly developed nematode fauna was recorded, as demonstrated by the genera composition of the MI. In fact, the MI value was 1.9, which is low, indicating high levels of stress or the early stages of colonization and bad soil quality.

There was only one genus with cp-value >2 (*Teratocephalus*, cp3), and only in low numbers, indicating that a very poorly developed nematode fauna was present. In addition, the SI highlighted that the nematode community in the covering soil was not well structured, probably due to the absence of omnivores and predators.

After the pyrite cinders were mixed with the covering soil, the nematode populations seemed to be unaffected by pyrite cinders and agronomic treatments, as demonstrated by the MI and H' values which remained similar to those calculated for the covering soil. Moreover, regarding the composition of the communities, no differences were recorded before and after mixing, with the exception of a decrease of fungal feeders in the mixture.

The effect of the manure addition was evident in all the cultivated plots, as assessed by the EI, suggesting that the ecosystems were more nutrient en Riched in the cultivated plots than in the covering soil and the mixture. Our data confirm that EI tends to increase following organic soil amendment.

Moreover, from the data obtained for the genera composition, we can assume that manure could affect nematofauna not only by improving bacterial activity but also by introducing new nematode genera into the environment. In fact, a new genus was recorded in all the cultivated plots: *Butlerius*, which could have effects on the nematofauna and soil quality. Thus the use of manure could be strategic in managing extremely compromised or depleted soil. Further analyses would be useful for understanding the role of nematodes from the manure in the new soil ecosystems.

Our study revealed that all systems were dominated by bacterial feeders, though not belonging to the same c-p group. In fact, in the covering soil and the mixture, bacterial feeders mainly belonged to Cephalobidae, whereas in the cultivated plots Rhabditidae was prevalent. This Rhabditidae dominance was probably due to the short-term effects of the perturbation provided by the agronomic practices and, in particular, by the organic matter added at the beginning of the phytoremediation process. In fact, nutrient enrichment provided to a selection of the trophic groups increased opportunist nematodes, such as *Rhabditis*; this could be due to an increase of bacterial activity (WASILEWSKA and WEBSTER, 1975; FERRIS *et al.*, 2001).

The effect of different crops on the nematofauna was also demonstrated by the MI. In fact, the maturity of the communities in all the cultivated plots slightly decreased compared to the one in the mixture before amendment and sowing. Among the cultivated plots, the sorghum had a different genera distribution; indeed, *Rhabditis* abundance was quite low, and *Butlerius* was more abundant. Nematode communities inhabiting the covering soil, the mixture, and the cultivated plots consisted of a very small number of genera, which is in accordance with the results DMOWSKA (2001) found in waste dumps. The biodiversity of the communities in the mixture and in the covering soil was low, and it did not change in the cultivated plots. The H' values recorded in all the cultivated plots were very low compared to the ones calculated by DMOWSKA (2001). In fact, in a study on the effects of reclamation on nematode communities, this author found that H' ranged from 0 to 1.49 in the non-reclaimed ash, and it had higher values in both short (3 years) and long (11 years) reclamation trials (1.40−2.55 and 2.28−3.05, respectively). In our case, nematode collection was done only two months after sowing sorghum, sunflowers, and giant reeds, and the H' values were below 1, showing the early succession of the nematofauna colonisation.

In addition, our results confirm that nematode colonization of pyrite cinders was a rather slow process, with an initial step dominated by Rhabditidae, as hypothesized by HÁNĚL (2002, 2004). Moreover, amending and cropping induced an intermediate disturbance (MESTRE *et al.*, 2020) that provide an increase in the abundance of enrichment-opportunistic nematodes. Thus, in view of following up on the course of remediation through nematofauna analysis, it is essential to consider that the early stages of phytoremediation may be strongly affected by agronomic practices. In fact, besides the evaluation of the potential of plant phytoremediation in terms of metal removal, it is important to consider the soil quality and capacity to support the life and recovery of soil organisms. The variation of soil nematode communities as bioindicators reflected a gradual restoration process after the phytoremediation of pyrite-contaminated industrial soil.

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