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Entomopathogenic Nematode *Steinernema feltiae* as an Indicator of Soil Pollution with Oil Derivatives in Bioremediation Process

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Abstract: Petroleum-derived substances (PDSs) pollutants negatively affect agricultural soil biocenosis, disturbing the biological properties of soils. This paper aimed to test the effect of oil derivatives during soil bioremediation processes on *Steinernema feltiae* nematode and determine their potential use as an indicator of the degree of soil pollution. Two test series were applied (with bioremediation and without bioremediation) in which the soil was contaminated with diesel fuel, petrol and engine oil. A preparation was designed for the bioremediation of soils polluted with oil derivatives and used in the experiments. Soil pollution with oil derivatives has a negative effect on *S. feltiae* nematode ability to penetrate *Tenebrio mollitor* larvae. The most negative and long-lasting effect of soil pollution with oil derivatives was registered when engine oil and diesel fuel were used. The application of biopreparation accelerated the bioremediation process and diminished a negative effect of soil pollution with engine oil on virulence of *S. feltiae* nematode. The mortality of test insects caused by the nematode *S. feltiae* was a sufficient indicator of the progress of bioremediation of petroleum pollutants, such as diesel oil and engine oil.

Keywords: nematodes; bioremediation; oil derivatives; bioindication; virulence

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1. Introduction

The problem of environmental pollution by oil derivatives has become more severe over recent years [1,2]. Catastrophes which happen during fuel transport by sea or road pose a particularly serious hazard, since they can result in vast areas becoming contaminated for many years. Agricultural soils may also be polluted with PDSs, which has a disastrous effect on their properties [3,4]. The toxic effect of pollution with oil derivatives on living organisms is mainly associated with polycyclic aromatic hydrocarbons [5]. Oil derivative pollutants penetrate into the bodies of living organisms through their body covers or through food [6], which may lead to bioaccumulation. As a result of natural processes taking place in the environment, the degree of environmental pollution by oil derivatives usually diminishes over time; moreover, the process may be also supported by the cultivation of appropriate vegetation [7]. Some of the compounds evaporate from the soil; others with a greater mass remain for longer in the soil environment, providing a source of energy for some soil microorganisms.

An important process is bioremediation, using microorganisms or microorganisms in combination with phytoremediation [8–12]. Degradation of oil derivatives in contaminated soil naturally occurs, as well as through the application of living organisms [13,14]. Hydrocarbon-degrading microorganisms are used when native microbial communities are unable to efficiently carry out the bioremediation process [15,16]. In order to accelerate natural bioremediation, specially-selected microorganism strains are used: *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Oligella*, *Acinetobacter*, *Methylobacterium*, *Stenotrophomonas*, *Morganella*, *Bacillus* and *Corynebacterium* bacteria [17]. Moreover, bacteria and nematodes are used for the biodegradation of oil derivatives polluting water environments [18]. An important issue during the remediation process is monitoring its progress. Chemical and physical methods are used to check the content of PDSs in soil. However, soil invertebrates have been regarded by some authors as better indicators of soil pollution with PDS than chemical tests [19,20]. Soil macrofauna such as Nematoda, Collembola, Arachnida or Insecta are often used as bioindicators of soil pollution [21–23]. However, nematodes are usually more sensitive to soil contamination with PDSs than other groups of macrofauna [24]. Instances in the literature have pointed to a disadvantageous effect of oil derivatives on soil nematodes [25]. On the other hand, Remelli et al. [26] noted that nematodes are able to survive at higher PDSs concentrations than Collembola and Acarina. The effect of PDSs on soil nematodes is often investigated at the community level [27]. However, the effect of the biopreparations used for bioremediation of polluted soils on soil organisms, including soil nematodes, has not been fully explored.

Enthomopathogenic species from the *Steirnematidae* family are a relatively well-known group of soil nematodes. These nematodes are natural enemies of many harmful insects. Therefore, they have been increasingly used for biological plant protection [28–30]. The *S. feltiae* nematode naturally occurs in many soil habitants and is used in plant protection to control soil pests and foliage damaging pests, such as Sciaridae, Bradysia spp. or western thrips (*Frankliniella occidentalis*) [31,32]. The invasive larvae of *S. feltiae* have the ability to survive and actively search for a potential host, but they prefer a host-waiting strategy due to lower energy costs [33]. To locate the host, nematodes use chemicals secreted by insects, e.g., carbon dioxide [32]. In recent years, research has focused on the potential application of nematodes as indicators of environmental pollution by heavy metals [34]. Enthomopathogenic nematodes are also sensitive to oil derivatives, which affect their death rate and ability to penetrate the bodies of host insects [35,36], making them candidates as indicators for this kind of pollution. Bioindication has become an important tool in assessing the quality of soil environments in recent years. To uncover the real impact of pollutants on the environment, biological, not only physico-chemical, methods should be used [37].

The purpose of the present study was to assess the effect of soil pollution by petrol, diesel fuel and engine oil on virulence of *S. feltiae* nematodes. The investigations also aimed to assess the potential use of *S. feltiae* nematodes as bioindicators of the degree of soil pollution with oil derivatives during the running bioremediation process.

2. Material and Methods

2.1. Experimental Design

The research was conducted at the Experimental Station of the Soil Cultivation Department in Mydlniki, near Krakow. The agricultural land there is used as a hay meadow. Indigenous soil was placed in c.a. 1 m^3 containers and the natural arrangement of the soil layers was maintained. The containers were dug into the soil at ground level. A geomembrane was installed below each container to prevent environmental pollution with effluents from the contaminated soil. The detailed description of the containers and their arrangement in the experimental field has been presented in another article [38]. The soil in containers (loamy-sand, pH in H_2O = 7.12, $CaCO_3$ = 0.17%, K_2O = 17.17 mg 100 g^{-1}, P_2O_5 = 16.37 mg 100 g^{-1}, C_{total} = 1.04%, N = 0.09%) was artificially polluted with the following oil derivatives poured onto it: petrol, diesel fuel and used engine oil, in amounts equal to 6000 mg of fuel per 1 kg of soil dry mass, i.e., a typical concentration of oil derivatives occurring in medium polluted soils. The control was provided by unpolluted soil placed in an identical container in the same conditions of the environment. Two experimental variants were applied: in the first, ZB-01 preparation for bioremediation of polluted soils with oil derivatives was added; whereas, in the second variant, bioremediation process were run naturally without any preparation supplied. ZB-01 is a microbial preparation with confirmed usefulness in the decomposition of petroleum-derived substances. It has been used for cleaning soil polluted with refinery sludge and oil slurries [39], as well as for oil-containing wastewater from metal processing plants [40] and for highly contaminated spent-oil-based metalworking fluids [41]. ZB-01 contains a mixture of selected prokaryotic organisms, mainly bacteria (*Stenotrophomonas, Pseudomonas, Moraxella, Acinetobacter, Alcaligenes, Ochrobactrum, Comamonas, Burkholderia, Corynebacterium* and *Oligella*), which are isolated over time from sites heavily polluted with organic compounds [42].

The biopreparation was applied several days after the soil contamination with oil derivatives. The experiment was set up according to a randomized blocks design in four replications. The soil bioremediation process was controlled by means of an analysis of the sum of high-boiling organic substances extracted by light petroleum in Soxhlet's apparatus. The results were presented in the paper by Gospodarek et al. [43].

2.2. Nematode Analyses

Steinernema feltiae enthomopathogenic nematodes were used for an assessment of soil contamination with oil derivatives. Commercially available nematodes were used in the experiment-Nemasys®. Every month, soil samples were collected from a depth of 0–20 cm by means of Ebner's stick. The soil (50 g) was placed in 120 mL sterile containers. The nematodes, 10 thousand dosed infective juveniles (IJs) were introduced into the soil in 2 mL of distilled water. The containers were kept in a chamber at a temperature of 20 °C. After two weeks, the nematodes were isolated from the soil using the trap insects method. Test insects-*Tenebrio molitor* larvae were added to the soil in the amount of 10 specimens per container. In the experiment, mater larvae of similar weight (ca. 140 mg) were used. The death rate of trapped insects was checked every day. Dead larvae were transferred onto Petri dishes and placed in a chamber at a temperature of 25 °C. Two days after their death, *Tenebrio molitor* larvae were selected in order to count the number of enthomopathogenic nematode infective juveniles that infested their bodies [44]. Their activity in the contaminated soils was determined on the basis of the number of nematodes which penetrated the insects' bodies.

In order to consider both the virulence of nematodes and their penetration into the insects, the virulence coefficient (P_{co}) was calculated by multiplying the mortality of the test insects with the number of nematode larvae isolated from the body of the dead insects.

2.3. Statistical Analyses

Obtained results were subjected to statistical analysis. Two-way ANOVA was conducted in a totally randomized design using Statistica 10.0 PL (StatSoft, Kraków, Poland). Newman-Keuls critical intervals were computed, whereas the value of the final step was used for differentiating means (least significant difference—LSD) at the significance level $p < 0.05$. The Pearson coefficient was calculated between insect mortality or insect penetration and the petroleum derivatives content in soil.

3. Results

3.1. The Effect of Oil Derivatives on the Virulence of S. feltiae

Contamination of the soil with oil derivatives affected *S. feltiae* ability to infect the test insects (Table 1). In the unpolluted soil test, insect mortality caused by the nematode was 95% on average for the whole period of the experiment, whereas in the soil contaminated with oil derivatives, it was between 42.9 and 71.7% (Figure 1). No significant effect of the applied biopreparation on the virulence of the investigated nematodes was observed in the control soil without the oil derivative supplement. In both cases, the death rate of test insects was similar. The soil contamination with engine oil resulted in no test insects becoming infected by IJs during the first three months after the soil contamination (Table 1). On the other hand, when petrol and diesel oil were used for the soil pollution, the mortality of test insects due to IJs was registered a month after soil contamination. An increased number of test insects killed by *S. feltiae* nematodes was registered on subsequent dates. It was particularly apparent in replications where petrol was used. In this case, test insect mortality was approximate to that observed in the control section in the seventh and eighth month. Diesel oil and used engine oil influenced nematode virulence to a greater extent, because the death rate of test insects, similar to that observed on the control section, was only registered around weeks 20 to 22. The application of biopreparation in the polluted sections resulted in a negative effect of petrol and diesel oil on *S. feltiae* nematodes which then diminished sooner. Test insect mortality caused by *S. feltiae* nematode was approximate to that observed in the control group in the seventh month in the case of petrol contamination, and in the fifteenth month for diesel oil contamination. Only in the soil polluted with used engine oil was there a slight difference between test insect mortality in the sections with or without the biopreparation supplement.

Table 1. The effect of oil derivatives on the virulence of *S. feltiae*—presented as the mortality of test insects (larvae of *T. molitor*).

Months from the Moment Soil Was Contaminated	Mortality of Test Insects [%]								*LSD (α = 0.05)
	Series without Bioremediation				Series with Bioremediation				
	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil	
1	92.5	15.0	17.5	0.0	90.0	15.0	12.5	0.0	13.82
2	77.5	15.0	12.5	0.0	87.5	15.0	17.5	0.0	12.73
3	80.0	22.5	17.5	0.0	82.5	25.0	25.0	10.0	15.40
4	85.0	57.5	65.0	17.5	97.5	72.5	77.5	20.0	17.84
5	92.5	70.0	70.0	7.5	92.5	72.5	77.5	27.5	17.00
6	95.0	75.0	60.0	15.0	90.0	72.5	67.5	25.0	17.68
7	95.0	80.0	60.0	22.5	95.0	90.0	70.0	42.5	17.68
8	100.0	77.5	55.0	25.0	100.0	92.5	67.5	45.0	14.63
9	97.5	80.0	52.5	25.0	97.5	85.0	65.0	37.5	15.21
10	95.0	85.0	50.0	27.5	95.0	80.0	60.0	35.0	20.83
11	92.5	87.5	55.0	30.0	90.0	85.0	67.5	42.5	25.00
12	95.0	82.5	57.5	35.0	92.5	90.0	70.0	52.5	24.05
13	97.5	87.5	57.5	40.0	97.5	82.5	75.0	57.5	16.31
14	100.0	87.5	62.5	62.5	100.0	87.5	80.0	62.5	17.84
15	97.5	85.0	72.5	67.5	97.5	92.5	85.0	67.5	20.69
16	95.0	85.0	72.5	65.0	95.0	90.0	85.0	60.0	20.27
17	100.0	77.5	75.0	65.0	97.5	87.5	85.0	67.5	15.21
18	100.0	75.0	77.5	70.0	100.0	80.0	87.5	72.5	14.63
19	100.0	77.5	75.0	67.5	100.0	85.0	82.5	67.5	15.96
20	100.0	85.0	77.5	70.0	100.0	92.5	85.0	70.0	13.18
21	100.0	92.5	80.0	77.5	100.0	97.5	87.5	75.0	16.67
22	100.0	100.0	82.5	82.5	100.0	100.0	92.5	77.5	14.43
23	100.0	100.0	82.5	80.0	100.0	100.0	87.5	77.5	13.82
24	100.0	100.0	85.0	77.5	100.0	97.5	92.5	77.5	13.61

*LSD (least significant difference) if the difference between means is higher than the value of LSD they do not differ significantly (α = 0.05).

3.2. The Effect of Oil Derivatives on the Intensity of Test Insect (T. molitor) Penetration by S. Feltiae IJs

In addition to test insect mortality, there was also a recorded increase in test insect penetration by IJs. In the unpolluted sections, the bodies of test insects were infested by about 9.0 IJs on average (Figure 2). The tested oil derivatives caused a considerable decrease in the intensity of infection in test insects from IJs. In the sections contaminated with petrol, the intensity of penetration in test insects was 6.6 IJs per insect on average. In the sections polluted with engine oil or diesel oil, the intensity of penetration in test insects was 1.4 and 2.6 IJs per insect, respectively. Application of the biopreparation did not have any marked influence on the intensity of penetration in test insects in the unpolluted soil. On the other hand, the bioremediation process used for the biopreparation caused the juvenile test insects in the sections polluted with engine oil and diesel oil to be more intensively penetrated by *S. feltiae* than in the sections where the bioremediation process naturally occurred. In the sections polluted with diesel oil and petrol, the number of nematode larvae infesting test insect bodies in the first months after pollution was very low (Table 2).

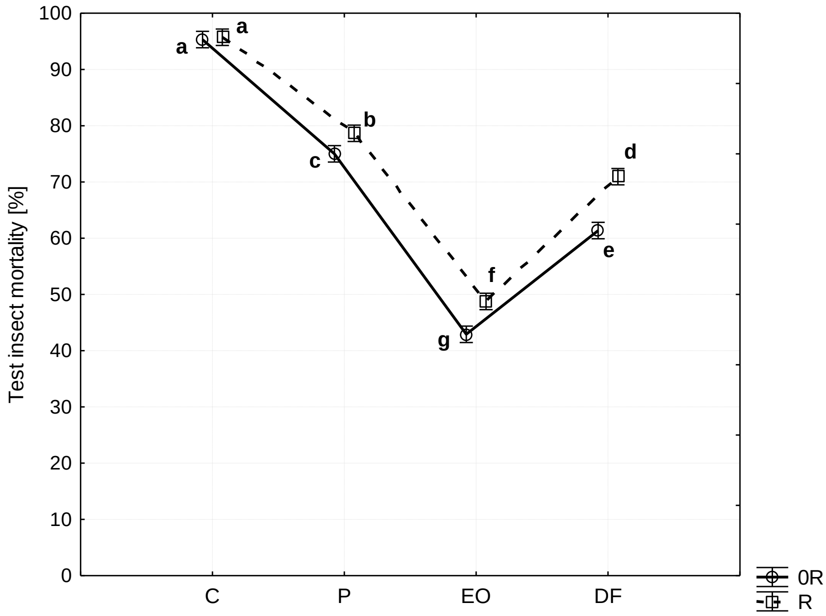

Figure 1. Average virulence of *S. feltiae* for the investigated period. C—unpolluted soil, P—soil contaminated with petrol, EO—soil contaminated with used engine oil, DF—soil contaminated with diesel fuel, 0R—series without bioremediation, R—series with bioremediation. Means marked with the same letters do not significantly differ according to NIR test at $\alpha = 0.05$; factors contamination x remediation.

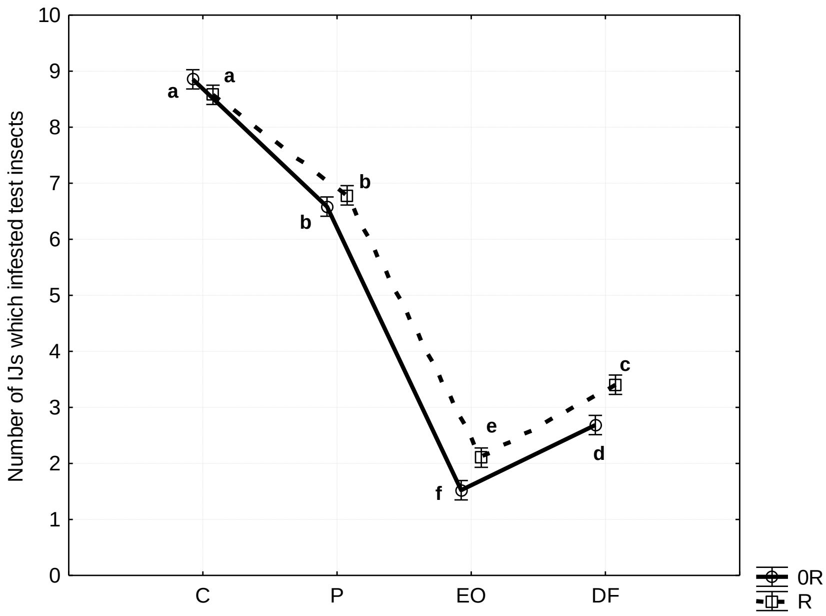

Figure 2. The intensity of penetration of test insects (*T. molitor*) by *S. feltiae* IJs, average for the investigated period. C—unpolluted soil, P—soil contaminated with petrol, EO—soil contaminated with used engine oil, DF—soil contaminated with diesel fuel, 0R—series without bioremediation, R—series with bioremediation. Means marked with the same letters do not significantly differ according to NIR test at $\alpha = 0.05$; factors contamination x remediation.

Table 2. The effect of oil derivatives on the intensity of penetration of test insects (*T. molitor*) by *S. feltiae* IJs.

Months from the Moment Soil Was Contaminated	Number of IJs Which Infested Test Insects								*LSD (α = 0.05)
	Series without Bioremediation				Series with Bioremediation				
	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil	
1	7.4	0.4	0.4	0.0	7.1	0.3	0.4	0.0	1.53
2	7.5	0.6	0.4	0.0	8.0	0.4	0.4	0.0	1.80
3	7.2	1.4	0.8	0.0	7.1	1.3	1.0	0.2	1.61
4	7.8	4.3	2.3	0.4	7.5	4.1	3.2	0.4	1.98
5	8.7	4.6	2.0	0.2	7.3	4.7	3.0	0.5	2.24
6	8.4	5.5	2.1	0.5	6.9	5.1	2.7	0.8	2.34
7	6.9	5.2	2.5	0.6	7.0	5.1	3.4	1.1	1.99
8	7.5	5.5	2.1	0.6	8.0	5.8	2.5	1.0	1.85
9	8.1	6.2	2.1	0.8	7.2	6.0	2.8	1.2	2.05
10	9.8	5.7	1.9	1.1	7.7	5.0	3.2	1.6	1.78
11	8.6	6.4	2.1	1.1	8.4	6.9	3.4	1.7	2.04
12	8.7	6.7	2.3	1.2	8.4	7.1	3.4	2.0	1.50
13	8.1	7.3	2.8	1.4	8.0	7.1	3.5	2.1	2.10
14	9.6	8.1	3.1	1.8	9.4	8.6	3.7	2.4	1.99
15	9.2	7.2	3.2	2.5	9.8	8.4	4.1	3.1	1.96
16	10.5	8.6	3.6	2.0	9.8	9.9	4.1	3.3	2.51
17	8.5	7.5	3.2	2.2	9.3	8.1	4.2	3.3	1.49
18	10.6	9.0	3.3	2.4	9.9	9.3	4.3	3.4	2.03
19	8.5	8.9	3.1	2.4	8.9	8.4	4.0	3.2	1.87
20	10.9	8.8	3.6	2.5	9.9	10.3	4.0	3.3	2.12
21	10.2	9.6	3.5	2.6	10.2	9.5	4.3	3.7	2.27
22	10.2	10.4	4.3	3.3	9.9	11.9	4.6	3.9	2.52
23	9.9	10.3	4.6	3.3	9.7	9.5	5.2	4.1	2.53
24	10.0	10.1	5.2	4.1	10.5	10.9	6.5	4.5	2.08

*LSD (least significant difference) if the difference between means is higher than the value of LSD they do not differ significantly (α = 0.05).

No infective juveniles of these kinds of nematodes were re-isolated from the soil contaminated with engine oil for the first three months. This indicated a strong negative influence of engine oil on this nematode. In the subsequent months, the intensity of penetration of test insects by IJs was clearly increased in the petrol-contaminated sections, whereas in the sections polluted with diesel fuel and engine oil, an unfavorable effect of these pollutants on the tested nematode persisted for longer. In the section where the soil was contaminated with petrol, the effect of the applied biopreparation was less apparent than in the sections polluted with diesel fuel or engine oil. In the case of diesel fuel and engine oil, after 24 months from the moment of pollution, the intensity of penetration of the test insects by the nematode infective juveniles was significantly lower than in the control, despite biopreparation application. This indicated that the bioremediation process of polluted soil had not yet finished, despite such a long period.

3.3. Virulence Coefficient

The calculated virulence coefficient has a value from 0.0 to 1090.0 (Table 3). Lower values of the coefficient were observed in sections polluted with PDSs, especially during the first months of the experiment. Generally, in the control sections, the P_{co} value ranged from 576.0 to 1090.0. Similar values for P_{co} in the petrol-contaminated soil were observed after 14 (0R) or 12 months (R) after contamination. When diesel oil or engine oil was used, the value of P_{co} was still lower than in control even after 24 months.

Table 3. The virulence coefficient of *S. feltiae*.

Months from the Moment Soil Was Contaminated	Mortality of Test Insects [%]							
	Series without Bioremediation				Series with Bioremediation			
	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil	Unpolluted Soil	Petrol	Diesel Fuel	Used Engine Oil
1	684.5	6.0	7.0	0.0	639.0	4.5	5.0	0.0
2	581.3	9.0	5.0	0.0	700.0	6.0	7.0	0.0
3	576.0	18.0	14.0	0.0	585.8	20.0	25.0	2.0
4	663.0	120.8	149.5	7.0	731.3	166.8	248.0	8.0
5	804.8	161.0	140.0	1.5	675.3	217.5	232.5	13.8
6	798.0	210.0	126.0	7.5	621.0	239.3	182.3	20.0
7	655.5	175.5	150.0	13.5	665.0	270.0	238.0	46.8
8	750.0	231.0	115.5	15.0	800.0	397.8	168.8	45.0
9	789.8	189.0	110.3	20.0	702.0	314.5	182.0	45.0
10	931.0	193.8	95.0	30.3	864.5	352.0	192.0	56.0
11	795.5	286.0	115.5	33.0	756.0	425.0	229.5	72.3
12	893.0	495.0	132.3	42.0	777.0	549.0	238.0	105.0
13	789.8	490.0	161.0	56.0	780.0	511.5	262.5	120.8
14	990.0	621.3	193.8	112.5	940.0	682.5	296.0	150.0
15	1014.0	612.0	232.0	168.8	1023.8	869.5	348.5	209.3
16	997.5	731.0	261.0	130.0	931.0	891.0	348.5	198.0
17	850.0	581.3	240.0	143.0	906.8	708.8	357.0	222.8
18	1060.0	675.0	255.8	168.0	990.0	744.0	376.3	246.5
19	850.0	689.8	232.5	162.0	890.0	714.0	330.0	216.0
20	1090.0	748.0	279.0	175.0	990.0	952.8	340.0	231.0
21	1020.0	888.0	280.0	201.5	1020.0	926.3	376.3	277.5
22	1020.0	1040.0	354.8	272.3	990.0	1190.0	425.5	302.3
23	990.0	1030.0	379.5	264.0	970.0	950.0	455.0	317.8
24	1000.0	1010.0	442.0	317.8	1050.0	1062.8	601.3	348.8

3.4. The Relationships between PDSs Content in Soil and S. feltiae Virulence

A significant correlation was found between the mortality of the test insects and the content of petroleum substances in the soil contaminated with diesel oil and engine oil (Table 4). No such relationship was found in the case of the control sections. Furthermore, in the sections contaminated with petrol, the changes in the mortality of the test insects were not correlated with the content of petroleum substances. The strongest negative correlation was found in the case of soil contamination with diesel oil in sections without bioremediation. With the use of bioremediation, this relationship was smaller, but still significant. On the other hand, in the case of contamination with engine oil, the correlation coefficient was similar both in the sections with and without the biopreparation ZB-01. The correlation between the content of petroleum substances and the penetration rate of Ifs and the virulence coefficient was generally similar and did not considerably differ in comparison to the correlation between the mortality of the test insects and the content of PDSs. Generally, the highest correlation was between the content of oil derivatives in the soil and the mortality of test insects caused by *S. feltiae*.

Table 4. The relationship between petroleum derivatives content in soil and nematode activity.

	Corelation Coefficient							
	Control		Petrol		Diesel Fuel		Engine Oil	
	0R	R	0R	R	0R	R	0R	R
Mortality of Test Insects	−0.042	0.003	−0.246	0.063	−0.783 *	−0.537 *	−0.761 *	−0.765 *
The Intensity of Test Insect Penetration by IJs	0.120	0.056	0.188	−0.054	−0.781 *	−0.587 *	−0.694 *	−0.732 *
P_{co}	0.079	0.059	0.223	−0.010	−0.716 *	−0.571 *	−0.577 *	−0.674 *

Note: 0R—series without ZB-01; R—series with ZB-01. * Significant corelation at $\alpha = 0.05$.

4. Discussion

4.1. The Effect of Oil Derivatives on S. feltiae Virulence

A clear negative effect of the investigated oil derivative substances on *S. feltiae* virulence was observed. The effect depended not only on the kind of PDS pollution, but also on the time which passed from the moment of the application of pollutants and biopreparation. Other authors have also noted that different oil derivatives differ in their toxicity to living organisms [45,46]. Considering the applied oil derivatives, the most disadvantageous and long-lasting effect on nematode virulence was observed for engine oil and diesel fuel. Unlike petrol, engine oil hardly evaporates from the soil and permanently contaminates it [47]. Furthermore, pollution with diesel fuel persists in the soil for longer than petrol pollution, which is probably due to its composition. Petrol usually pollutes the soil for a shorter time than other oil derivatives. This was confirmed by the present research, which demonstrated that a negative effect of soil contamination with petrol on the ability of *S. feltiae* to kill test insects and penetrate their bodies persisted for a relatively short period of time after initial contamination. In the sections contaminated with engine oil, diminished penetration of test insects by the nematodes IJs was observed during the entire period of investigation.

4.2. Oil-Derivatives Bioidication Potential of S. feltiae

Chemical analysis may not have revealed any significant soil contamination with oil derivatives, however, an unfavorable effect of soil contamination could last longer. However, the process can also be measured by the biological properties of contaminated soil. In this approach, the bioremediation process is finished when soils regain their original biological properties. A month after pollution, the content of oil derivatives in the sections contaminated with petrol was at a similar level as the control in which the soil was not artificially polluted [43]. Nevertheless, petroleum contamination caused significant changes in the soil which resulted in a reduction of the nematode's ability to kill and penetrate the test insects. Therefore, the use of living organisms to determine the degree of pollution may prove a more reliable method. This view has also been shared by other authors [48,49]. These investigations not only aimed to evaluate the effect of soil contamination with oil derivatives on *S. feltiae* enthomopathogenic nematodes, but also to determine whether enthomopathogenic nematodes can be used as bioindicators of the efficiency of the bioremediation process.

As both the mortality of the test insects and the content of oil derivatives in the soil changed during the experiment, a potential relationship between these two variables was investigated. In the case of contamination with petrol, there was a significant effect on the activity of nematodes, but due to the fact that the PDSs content quickly decreased (31), no significant correlation was found. On the other hand, a significant negative correlation was found between the mortality of the test insects and the content of petroleum substances in the soil contaminated with diesel oil and engine oil. Penetration rates and P_{co} were also negatively correlated with the content of PDSs in the diesel oil and engine oil sections.

As the correlation was significant, the progress of the natural remediation process, as initiated by the applied biopreparation, was determined on the basis of *S. feltiae* entomopathogenic activity; e.g., mortality of test insects, number of infective juveniles isolated from test insect bodies and P_{co} values on the subsequent dates from the moment of soil pollution with oil derivative. It also enabled us to approximately fix the moment when the process is completed—the content of oil derivatives was returned to original values and the number of nematodes isolated from the soil subjected to oil derivatives effect was approximate to the number of nematodes isolated from the control soil.

Based on the P_{co} values, the remediation process after the investigated period was completed for petrol-contaminated soil. However, it should be noted that other organisms have different susceptibility to oil derivatives [24,26], and for many of them, the process may not finish at the same point. The negative effect of pollution has been observed for some groups of invertebrates even 5 years after the moment of contamination [50]. In the case of soil contamination with diesel fuel, the remediation process was not completed according to the P_{co} values. The process occurred the most slowly in the section with the engine oil. The application of biopreparation ZB-01 caused a marked acceleration of the bioremediation process, which was apparent in both the oil derivatives content in the soil [38] and the nematodes ability to kill and penetrate test insects. Ropek and Gospodarek [51] found that the use of a microbial preparation had a beneficial effect on the natural occurrence of entomopathogenic nematodes in contaminated soils, as they were isolated earlier than in the sections undergoing the process of natural remediation.

Considering the potential application of *S. feltiae* nematodes as indicators of soil pollution with oil derivatives, it should be noted that a good indicator could be the penetration intensity of the test insects by *S. feltiae* nematodes IJs. Pco also seems to be a good indicator of *S. feltiae* virulence as it combines both the mortality of insects and penetration rate of IJs into insect bodies. However, an observation of the mortality of test insects caused by nematodes was also an effective indicator. In the case of test insect mortality, it should be emphasized that high mortality was observed in conditions of soil severely polluted with oil derivatives, but this was caused by the toxic effect of the pollutants and not by the penetration of nematodes. For this reason, it is necessary to confirm the cause of insect death through dissection to isolate penetrating IFs.

5. Conclusions

Soil pollution with oil derivatives caused a significant reduction in the ability of *S. feltiae* nematodes to kill test insects.

The negative effects of soil pollution with oil derivatives on *S. feltiae* virulence persisted longest when engine oil and diesel fuel were used as pollutants.

Application of the biopreparation reduced a negative effect of soil contamination with engine oil on the enthomopathogenic abilities of *S. feltiae* nematodes.

The mortality of test insects caused by *S. feltiae* nematodes is a sufficient indicator of the progress of degradation of petroleum pollutants, such as diesel oil and engine oil. However, it is necessary to confirm the cause of death of the test insects.

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References

1. Lv, Y.; Bao, J.; Zhu, L. A comprehensive review of recent and perspective technologies and challenges for the remediation of oil-contaminated sites. *Energy Rep.* **2022**, *8*, 7976–7988. [CrossRef]
2. Meuser, H. Contaminated urban soil. *Environ. Pollut.* **2010**, *19*, 320. [CrossRef]
3. Najar-Rodriguez, A.J.; Lavidis, N.A.; Mensah, R.K.; Choy, P.T.; Walter, G.H. The toxicological effects of petroleum spray oils oninsects–Evidence for an alternative mode of action and possible new control options. *Food Chem. Toxicol.* **2008**, *46*, 3003–3014. [CrossRef]
4. Zhang, J.M.; Liu, F.; Huang, H.; Wang, R.J.; Xu, B.L. Occurrence, risk and influencing factors of polycyclic aromatic hydrocarbonsin surface soils from a large-scale coal mine, Huainan, China. *Ecotoxicol. Environ. Saf.* **2020**, *192*, 110269. [CrossRef] [PubMed]
5. Gonzáles-Doncel, M.; Gonzáles, L.; Fernández-Torija, C.; Navas, J.M.; Tarazona, J.V. Toxic effects of an oil spill of fish early life stages may not be exclusively associated to PAHs: Studies with *Prestige* oil and medaka (*Oryzias latipes*). *Aquat. Toxicol.* **2008**, *87*, 280–288. [CrossRef] [PubMed]
6. Stroomberg, G.J.; Zappey, H.; Steen, R.J.; van Gestel, C.A.; Ariese, F.; Velthorst, N.H.; van Straalen, N.M. PAH biotransformation in terrestrial invertebrates–a new phase II metabolite in isopods and springtails. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2004**, *138*, 129–137. [CrossRef] [PubMed]
7. Dominguez-Rosado, E.; Pichtel, J. Phytoremediation of Soil Contaminated with Used Motor Oil: II. Greenhouse Studies. *Environ. Eng. Sci.* **2004**, *21*, 169–180. [CrossRef]
8. Khan, S.; Tripathi, A.K.; Srivastava, R.; Saleem, M.S.; Yeremenko, S.; Sydorenko, V. Bioremediation of Petroleum Contamination: A Short Review. *Ecol. Quest.* **2022**, *33*, 43–52. [CrossRef]
9. Stepanova, A.Y.; Gladkov, E.A.; Osipova, E.S.; Gladkova, O.V.; Tereshonok, D.V. Bioremediation of Soil from Petroleum Contamination. *Processes* **2022**, *10*, 1224. [CrossRef]
10. Sui, X.; Wang, X.; Li, Y.; Ji, H. Remediation of Petroleum-Contaminated Soils with Microbial and Microbial Combined Methods: Advances, Mechanisms, and Challenges. *Sustainability* **2021**, *13*, 9267. [CrossRef]
11. Kalia, A.; Sharma, S.; Semor, N.; Babele, P.K.; Sagar, S.; Bhatia, R.K.; Walia, A. Recent advancements in hydrocarbon bioremediation and future challenges: A review. *3 Biotech* **2022**, *12*, 135. [CrossRef] [PubMed]
12. Kour, D.; Kaur, T.; Devi, R.; Yadav, A.; Singh, M.; Joshi, D.; Suyal, D.C.; Kumar, A.; Rajput, V.D.; Yadav, A.N.; et al. Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: Present status and future challenges. *Environ. Sci. Pollut. Res.* **2021**, *28*, 24917–24939. [CrossRef] [PubMed]
13. Ghosh, S.; Sharma, I.; Nath, S.; Webster, T.J. Chapter 2—Bioremediation—The natural solution. In *Microbial Ecology of Wastewater Treatment Plants*; Shah, M., Rodriguez-Couto, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 11–40, ISBN 9780128225035. [CrossRef]
14. Bala, S.; Garg, D.; Thirumalesh, B.V.; Sharma, M.; Sridhar, K.; Inbaraj, B.S.; Tripathi, M. Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics* **2022**, *10*, 484. [CrossRef]
15. Dzionek, A.; Wojcieszyńska, D.; Guzik, U. Natural carriers in bioremediation: A review. *Electron. J. Biotechnol.* **2016**, *23*, 28–36. [CrossRef]
16. Orellana, R.; Cumsille, A.; Piña-Gangas, P.; Rojas, C.; Arancibia, A.; Donghi, S.; Stuardo, C.; Cabrera, P.; Arancibia, G.; Cárdenas, F.; et al. Economic Evaluation of Bioremediation of HydrocarbonContaminated Urban Soils in Chile. *Sustainability* **2022**, *14*, 11854. [CrossRef]
17. Kołoczek, H.; Kaszycki, P. Bioremediacja zanieczyszczeń rafineryjnych w środowisku gruntowo-wodnym. In *Metody Usuwania Zanieczyszczeń Węglowodorowych ze Srodowiska Gruntowo-Wodnego*; Rychlickiego, S., Ed.; Uczelniane Wyd. Nauk.-Dydakt., AGH: Kraków, Poland, 2006.
18. Chan, H. Biodegradation of petroleum oil achieved by bacteria and nematodes in contaminated water. *Sep. Purif. Technol.* **2011**, *80*, 459–466. [CrossRef]
19. Hentati, O.; Lachhab, R.; Ayadi, M.; Ksibi, M. Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates Environ. *Monit. Assess.* **2013**, *185*, 2989–2998. [CrossRef] [PubMed]
20. Al-Mutairi, N.; Bufarsan, A.; Al-Rukaibi, F. Ecorisk evaluation and treatability potential of soils contaminated with petroleum and plant bioassays. hydrocarbon-based fuels. *Chemosphere* **2008**, *74*, 142–148. [CrossRef]
21. Trishala, K.; Rawtani, P.D.; Agrawal, Y.K. Bioindicators: The natural indicator of environmental pollution. *Front. Life Sci.* **2016**, *9*, 110–118. [CrossRef]
22. Parikh, G.; Rawtani, D.; Khatri, N. Insects as an Indicator for Environmental Pollution. *Environ. Claims J.* **2021**, *33*, 161–181. [CrossRef]
23. García-Segura, D.; Castillo-Murrieta, I.M.; Martínez-Rabelo, F.; Gomez-Anaya, A.; Jacobo Rodríguez-Campos Hernández-Castellanos, B.; Contreras-Ramos, S.M.; Barois, I. Macrofauna and mesofauna from soil contaminated by oil extraction. *Geoderma* **2018**, *332*, 180–189. [CrossRef]
24. Blakely, J.K.; Neher, D.A.; Spongberg, A.L. Soil invertebrate and microbial communities and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. *Appl. Soil Ecol.* **2002**, *21*, 71–88. [CrossRef]
25. Raymond, R.L.; Hudson, J.O.; Jamison, V.W. Oil degradation in soil. *Appl. Environ. Microbiol.* **1976**, *31*, 522–535. [CrossRef] [PubMed]

26. Remelli, S.; Rizzo, P.; Celico, F.; Menta, C. Natural Surface Hydrocarbons and Soil Faunal Biodiversity: A Bioremediation Perspective. *Water* **2020**, *12*, 2358. [CrossRef]
27. Brázová, T.; Kováčik, P.; Matoušková, M.; Oros, M. Nematodes as soil stress indicators for polycyclic aromatic hydrocarbons: A review. *Helminthologia* **2022**, *59*, 117–126. [CrossRef] [PubMed]
28. Tomalak, M. Potencjał nicieni owadobójczych w biologicznym zwalczaniu szkodliwych błonkówek atakujących drzewa owocowe i parkowe. *Prog. Plant Prot.* **2006**, *46*, 249–255.
29. Patil, J.; Linga, V.; Vijayakumar, R.; Subaharan, K.; Navik, O.; Bakthavatsalam, N.; Mhatre, P.H.; Sekhar, J. Biocontrol potential of entomopathogenic nematodes for the sustainable management of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *Pest Manag. Sci.* **2022**, *78*, 2883–2895. [CrossRef]
30. Nouh, G.M.; Adly, D. Evaluation of the virulence of entomopathogenic nematodes as a biological control agents against *Gryllotalpa gryllotalpa* (Gryllotalpidae). *J. Appl. Entomol.* **2021**, *145*, 1050–1056. [CrossRef]
31. Manochaya, S.; Udikeri, S.; Srinath, B.S.; Sairam, M.; Srinivas, V.; Bandlamori, S.V.; Ramakrishna, K. In vivo culturing of entomopathogenic nematodes for biological control of insect pests: A review. *J. Nat. Pestic. Res.* **2022**, *1*, 100005. [CrossRef]
32. Jayashree Ramakrishnan, E.J.; Salame, L.; Ahmed Nasser, A.; Glazer, I.; Ment, D. Survival and efcacy of entomopathogenic nematodes on exposed surfaces. *Sci. Rep.* **2022**, *12*, 4629. [CrossRef]
33. Grewal, P.; Lewis, E.; Gaugle, R.; Campbell, J. Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* **1994**, *108*, 207–215. [CrossRef]
34. Hao, W.; Li, Q.; Zhang, J.; Jiang, Y.; Liang, W. Utility of nematode *Acrobeloides nanus* for assessing subacute toxicity of heavy metals. *Environ. Monit. Assess.* **2010**, *164*, 273–278. [CrossRef] [PubMed]
35. Ropek, D.; Gondek, K. Occurrence and virulence of entomopathogenic nematodes and fungi in soil contaminated with heavy metals near petroleum refinery and thermal power plant in Trzebinia. *Ecol. Chem. Eng.* **2002**, *9*, 447–454.
36. Ropek, D.; Gospodarek, J. The effect of oil derivatives on the ability of entomopathogenic nematode Steinernema feltiae to find host. *Ecol. Chem. Eng. A* **2013**, *20*, 857–865.
37. Maurya, S.; Abraham, J.S.; Somasundaram, S.; Toteja, R.; Gupta, R.; Makhija, S. Indicators for assessment of soil quality: A mini-review. *Environ. Monit. Assess.* **2020**, *192*, 604. [CrossRef] [PubMed]
38. Gospodarek, J.; Petryszak, P.; Kołoczek, H. The effect of the bioremediation of soil contaminated with petroleum derivatives on the occurrence of epigeic and edaphic fauna. *Bioremediation J.* **2016**, *20*, 38–53. [CrossRef]
39. Kaszycki, P.; Petryszak, P.; Pawlik, M.; Koloczek, H. Ex situ bioremediation of soil polluted with oily waste: The use of specialized microbial consortia for process bioaugmentation. *Ecol. Chem. Eng. S* **2011**, *18*, 83–92.
40. Kaszycki, P.; Supel, P.; Petryszak, P. Bacterial population dynamics of biostimulated auto- and allochthonous microflora in waste oily emulsions from the metal-processing industry. *J. Ecol. Eng.* **2014**, *15*, 14–22.
41. Kaszycki, P.; Petryszak, P.; Supel, P. Bioremediation of a spent metalworking fluid with auto- and allochthonous bacterial consortia. *Ecol. Chem. Eng. S* **2015**, *22*, 285–299. [CrossRef]
42. Kaszycki, P.; Szumilas, P.; Kołoczek, H. Biopreparat przeznaczony do likwidacji środowiskowych skażeń węglowodorami i ich pochodnym. *Inżynieria Ekol.* **2001**, *4*, 15–22.
43. Gospodarek, J.; Kołoczek, H.; Petryszak, P. Effect of bioremediation process of soil contaminated with oil derivatives on *Coleoptera, Carabidae* representatives. *Ecol. Chem. Eng. A* **2013**, *20*, 545–554.
44. Kaya, H.K.; Stock, S.P. Techniques in insect nematology. In *Manual of Techniques in Insect Pathology*; Lacey, L.A., Ed.; Academic Press: Cambridge, MA, USA, 1997; pp. 281–324, ISBN 9780124325555. [CrossRef]
45. Chachina, S.B.; Voronkova, N.A.; Shadrin, M.A.; Evdokimov, N.S. The recultivation of the soils, contaminated with petrol and diesel fuel, with the help of earthworms Dendrobena veneta and the complex of microorganisms. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *315*, 052065. [CrossRef]
46. Hawrot-Paw, M.; Koniuszy, A.; Zając, G.; Szyszlak-Bargłowicz, J. Ecotoxicity of soil contaminated with diesel fuel and biodiesel. *Sci. Rep.* **2020**, *10*, 16436. [CrossRef]
47. Li, Y.-Y.; Zheng, X.-L.; Li, B.; Ma, Y.-X.; Cao, J.-H. Volatilization behaviors of diesel oil from the soils. *J. Environ. Sci.* **2004**, *16*, 1033–1036.
48. Wołejko, E.; Wydro, U.; Odziejewicz, J.I.; Koronkiewicz, A.; Jabłonska-Trypuc, A. Biomonitoring of Soil Contaminated with Herbicides. *Water* **2022**, *14*, 1534. [CrossRef]
49. Dahiya, U.R.; Das, J.; Bano, S. Biological Indicators of Soil Health and Biomonitoring. In *Advances in Bioremediation and Phytoremediation for Sustainable Soil Management*; Malik, J.A., Ed.; Springer: Cham, Switzerland, 2022. [CrossRef]
50. Gospodarek, J.; Rusin, M.; Barczyk, G.; Nadgórska-Socha, A. The Effect of Petroleum-Derived Substances and Their Bioremediation on Soil Enzymatic Activity and Soil Invertebrates. *Agronomy* **2021**, *11*, 80. [CrossRef]
51. Ropek, D.; Gospodarek, J. Effect of soil pollution with oil derivatives on the occurrence of entomopathogenic nematodes. *Ecol. Chem. Eng. A* **2013**, *20*, 157–166.