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EFFECT OF SOIL POLLUTION WITH OIL DERIVATIVES ON THE OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES

WPŁYW ZANIECZYSZCZENIE GLEBY SUBSTANCJAMI ROPOPOCHODNYMI NA WYSTĘPOWANIE NICIENI OWADOBÓJCZYCH

Abstract: The aim of the research was to evaluate the effect of oil derivatives on the occurrence of entomopatogenic nematodes during the bioremediation process. The experiment was carried out in field conditions in two series (with bioremediation and without bioremediation) at the Experimental Station in Mydlniki near Krakow. Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil (dose: 6 000 mg of fuel · kg^{-1} d.m. of soil). Unpolluted soil was used as control. The extraction of nematodes naturally occurring in the soil was done with trap method. Larvae of *Tenebrio molitor* were used to isolate nematodes. Soil samples were taken once in each season during two year experiment. Contamination of soil with oil derivatives caused significant reduction of entomopathogenic nematodes occurrence in soil environment. The most long-lasting effect on entomopathogenic nematodes population naturally occurring in soil had used engine oil and diesel oil. Application of the biopreparation into contaminated soil had a favorable effect on enthomopathogenic nematodes occurrence. On objects where biopreparation was applied enthomopathogenic nematodes were isolated significantly faster after initial contamination than on objects where only natural remediation process were carried out. Occurrence of entomopathogenic nematodes seems to be suitable indicator of bioremediation process effectiveness in soil environment contaminated with oil derivatives.

Keywords: entomopathogenic nematodes, petrol, engine oil, diesel fuel, bioremediation

Introduction

Oil derivatives as petrol or diesel oil are widely used and are often unintentionally spilled into the environment thus being an important threat to the environment [1]. Oil pollution may affect almost all living organisms present in soil [2, 3]. There are evidence that oil derivatives have an adverse effect on soil invertebrates community [4].

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In contaminated soil diesel oil and petrol has among others a negative impact on the nitrification process [5].

It is difficult to predict the impact of oil derivatives pollutions on soil environment. Oil derivatives may evaporate, become diluted or transformed in physical, chemical or biological processes. Durability of soil contamination with oil derivatives depends on these processes and type of these products. Contaminated soil often requires special treatment as bioremediation techniques to fasten degradation of non-volatile fractions of oil derivatives [6]. There are not many researches concerning the effect of oil derivatives contamination on soil mesofauna and especially on nematodes [2, 7–9]. However soil mesofauna is often used as an indicator of environment pollution with heavy metals [10]. Entomopathogenic nematodes commonly occurs in soil environment [11, 12] as a free-living, third stage *infective juveniles* (IJs). Nematodes are also regarded as possible indicators of the environment contamination *eg* heavy metals [13, 14]. It is important to determine the efficiency of soil restoration not only with chemical parameters but also with biological criteria [7].

The aim of the research was to evaluate the effect of oil derivatives during the process of their bioremediation on natural occurrence of entomopatogenic nematodes. It was assumed that nematodes may be sensible indicators of soil contamination with oil derivatives and effectiveness of bioremediation process.

Material and methods

The research was conducted at the Experimental Station in Mydlniki near Krakow. The experiment was set up in autumn 2009 on an agricultural land used as a hay meadow. The containers (*ca* 1 m^3) were dug into the soil at the ground level and filled with indigenous soil, and the natural soil layer arrangement was preserved. For six months the soil was left in the containers to return to its natural density and biological stability. After that period in June 2010 the soil was artificially polluted with petrol, diesel fuel and used engine oil. Oil derivatives were poured onto the surface of soil in the amount equal to 6 000 mg of fuel per 1 kg of soil dry mass. In the control unpolluted soil was placed in containers.

The experiment was set up in two variants: in the first ZB-01 preparation designed for bioremediation of soils polluted with oil derivatives was added, whereas in the second variant bioremediation process was running naturally with no preparation supplied. The biopreparation was applied several days after the soil contamination with oil derivatives. The soil bioremediation process was chemically controlled [15]. The experiment was set up in four replications. Four times a year (in each season) soil samples were collected from each container form the depth of 0–20 cm by means of Ebner's stick. The soil was placed in plastic sterile containers (120 cm^3) and than 10 larvae of trap insect *Tenebrio molitor* were put into them. Death rate of trap insects was checked every 24 hours. Dead *T. molitor* larvae were removed into sterile Petri dishes and stored at 25 ^oC for 48 hours. After two days since the test insect death they were dissected under a binocular magnifying glass to count the number of nematodes able to actively penetrate into their bodies. Rate of male and female of nematodes entering insect body was also calculated.

The results were analysed statistically using the Statistica 10,0 PL programme. ANOVA analysis was conducted and the Newman-Keuls critical intervals were computed. The value of the final step was used for differentiating means at the significance level $p < 0.05$.

Results and discussion

The occurrence of entomopathogenic nematodes in soil was performed with the use of trap insects [16]. The mortality of trap insects was observed and the cause of death was determined. High mortality of test insects on contaminated soil was observed (Fig. 1). However, no infective juveniles were found inside the insects. It suggest that the test insect death was caused by oil derivatives applied into the soil or other microorganism *eg* entomopathogenic fungi.

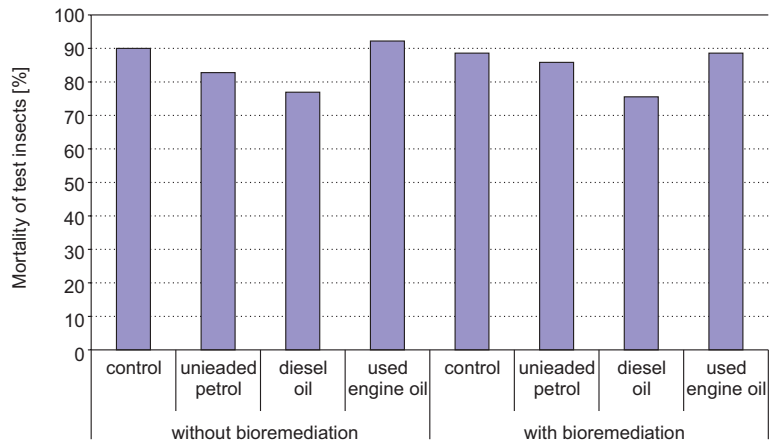

Fig. 1. Mortality of test insects – average for the investigated period

Soil samples were taken before the experiment set up to evaluate natural occurrence of entomopathogenic nematodes. It was confirmed that entomopathogenic nematodes were present in the soil where the experiment was conducted. Only one species *Steinernema feltiae* was isolated from the soil, which is one of the most often occurring entomopathogenic nematode in agricultural and urban ecosystems in Poland [11, 17]. In uncontaminated soil (control) entomopathogenic nematode was isolated in all seasons during the two year experiment (Table 1, Fig. 2). Contamination of the soil with oil derivatives significantly affected nematode occurrence. In contaminated soil nematode was not isolated for at least several months from the moment of soil pollution. The adverse effect of used engine oil application were apparent during the whole period of the experiment – no entomopathogenic nematodes were isolated from the moment of soil pollution to the end of the experiment. Soil contamination with unleaded petrol and diesel oil did not influenced entomopathogenic nematodes occurrence to the same

Table 1

The effect of soil pollution with oil derivatives on the occurrence of entomopathogenic nematodes

Date	Mortality of test insects caused by entomopathogenic nematodes [%]							
	Without bioremediation				With bioremediation			
	Control	Unleaded petrol	Diesel oil	Used engine oil	Control	Unleaded petrol	Diesel oil	Used engine oil
Spring 2010	72.5	0	0	0	70	0	0	0
Summer 2010	75	0	0	0	80	0	0	0
Autumn 2010	65	0	0	0	70	0	0	0
Winter 2011	65	0	0	0	65	0	0	0
Spring 2011	75	0	0	0	72.5	0	17.5	0
Summer 2011	80	0	0	0	77.5	0	15	0
Autumn 2011	72.5	0	15	0	67.5	0	17.5	0
Winter 2012	65	25	12.5	0	65	22.5	20	0
Spring 2012	77.5	12.5	15	0	72.5	17.5	12.5	7.5
LSD ($\alpha = 0.05$) factor date × contamination x remediation – 15.45								

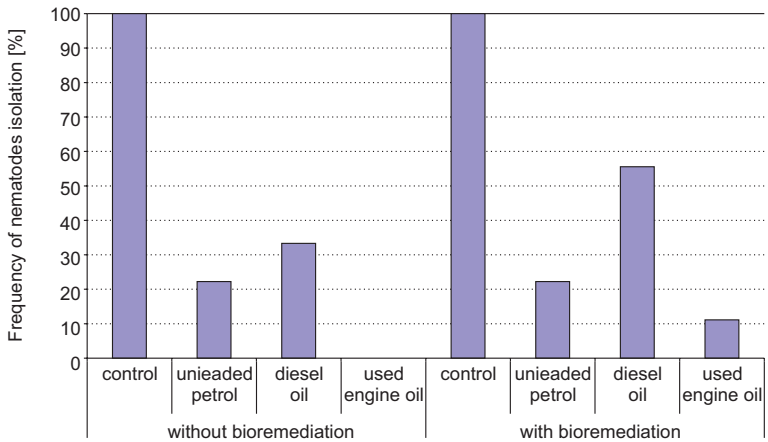

Fig. 2. Frequency of nematodes isolation from the contaminated soil – average for the investigated period

extent. In the objects contaminated with diesel oil *S. feltiae* was isolated in Autumn 2011 (15 months after contamination) and in objects with unleaded petrol in Winter 2012 (18 months after contamination). Application of the biopreparation markedly limited the adverse effect oil derivatives on entomopathogenic nematodes occurrence. This phenomena was particularly evident in objects contaminated with diesel oil.

In unpolluted objects death rate of trap insects caused by entomopathogenic nematodes was about 65–80 % (Table 1). It indicates that the population of entomopathogenic nematodes was quite numerous in the soil. The activity of nematode changed with the season and was higher in summer and lower in winter. In the objects polluted with oil derivatives mortality of trap insects caused by *S. feltiae* was significantly lower than in control and ranged from 7.7 to 25 %. Application of the biopreparation in the polluted objects caused that the trap insect were earlier infected with *S. feltiae* than in objects were bioremediation process ran naturally. However, mortality rate of trap insects in the objects with or without biopreparation supplement was very similar.

Aside from test insects mortality, also the intensity of test insects infestation by *S. feltiae* was investigated (Table 2, Fig. 3). On the uncontaminated objects without biopreparation mean intensity of trap insect infestation was 6.3–19.8 IJs and on objects with biopreparation 6.8–20.8 IJs, respectively. Application of oil derivatives caused that trap insects were not infested with entomopathogenic nematodes for at least several months after contamination. In months in which nematodes killed trap insects infestation intensity ranged 2.8–4.8 IJs (unleaded petrol) and 2.5–3.0 IJs (diesel oil). On the objects contaminated with used engine oil no entomopathogenic nematodes infested trap insects. Application of biopreparation did not influenced intensity of test insects infestation by *S. feltiae* but significantly affected time when first infective juveniles were isolated from contaminated soil.

Table 2

The effect of soil pollution with oil derivatives on the intensity of trap insect infestation with entomopathogenic nematodes

Date	Number of IJs entering test insect body							
	Without bioremediation				With bioremediation			
	Control	Unleaded petrol	Diesel oil	Used engine oil	Control	Unleaded petrol	Diesel oil	Used engine oil
Spring 2010	6.3	0.0	0.0	0.0	6.8	0.0	0.0	0.0
Summer 2010	8.8	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Autumn 2010	13.8	0.0	0.0	0.0	12.0	0.0	0.0	0.0
Winter 2011	15.5	0.0	0.0	0.0	12.8	0.0	0.0	0,0
Spring 2011	19.8	0.0	0.0	0.0	16.8	0.0	2.5	0.0
Summer 2011	18.8	0.0	0.0	0.0	20.8	0.0	4.0	0.0
Autumn 2011	13.3	0.0	2.5	0.0	12.0	0.0	2.5	0.0
Winter 2012	13.3	2.8	3.0	0.0	12.0	2.8	2.3	0.0
Spring 2012	17.3	4.8	2.8	0.0	18.3	3.5	2.8	0.3
LSD ($\alpha = 0.05$) factor date × contamination × remediation – 5.65								

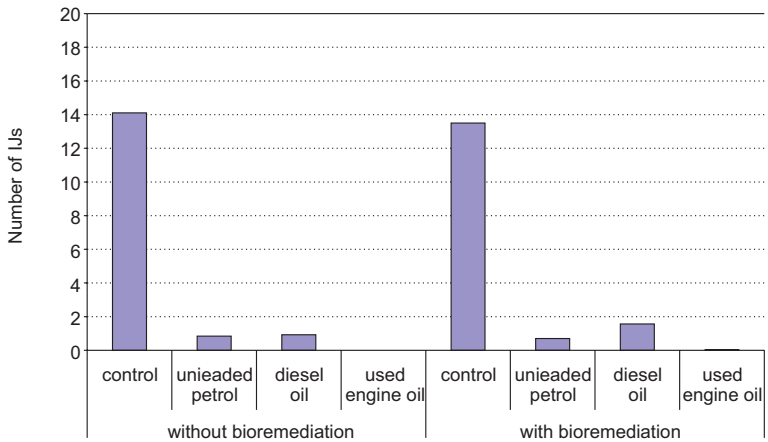

Fig. 3. Intensity of test insects infestation by entomopatogenic nematodes – average for the investigated period

Not only the intensity of test insects infestation by entomopathogenic nematodes was affected by oil derivatives but also the ratio female/male of IJs entering test insect body (Fig. 4). In contaminated soil number of male nematodes isolated from insects was rather low. In soil contaminated with oil derivatives female nematodes were more abundant and were able to infest test insects. It suggests that IJs which develop inside insects into females are less sensitive to oil derivatives than the larvae developing into males.

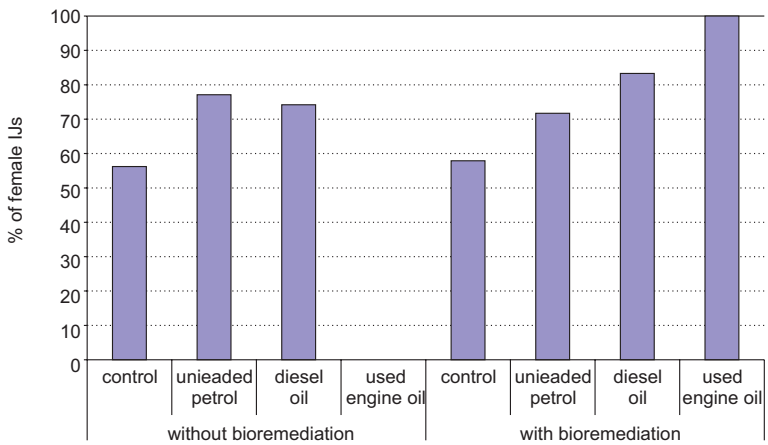

Fig. 4. Female/male ratio of *S. feltiae* isolated from dead insects in soil contaminated with oil derivatives

Entomopathogenic nematodes were not isolated from soil contamination with oil derivatives which indicates that these contaminates significantly affected soil environment. Trap insect method allows isolation of living and active IJs. The adverse effect of

oil derivatives on nematodes could be associated both with death of IJs and with inability of nematodes to locate a potential host.

In soils contaminated with used engine oil entomopathogenic nematodes were isolated only on objects treated with biopreperation. However nematodes were isolated not until two years after initial contamination. On objects contaminated with unleaded petrol and diesel oil the toxic effect was less sustained. First nematodes were isolated 18 months after contamination. It indicates that among investigated oil derivatives used engine oil has most toxic effect on entomopatogenic nematodes community in the soil. Reisolation of entomopathogenic nematodes from the contaminated soil may be related with reactivation of nematode's IJs, which survived in soil or migration of new population from neighboring areas. Entomopathogenic nematodes can actively migrate in soil in search for a new host [18] or be passively carried by infested insects. As it was shown in other paper some representatives of invertebrates may penetrate soil surface shortly after initial pollution [19, 20], thus contributing to nematodes recolonization of contaminated area.

It is important to include biological indicators of soil pollution with oil derivatives. Entomopathogenic nematodes seems to be suitable indicators of bioremediation process in soil environment.

Conclusions

1. Entomopathogenic nematodes were not isolated from soil contaminated with oil derivatives for several months after application of contaminates.
2. The most negative effect on entomopathogenic nematodes had used engine oil.
3. Application of the biopreparation accelerated bioremediation process and reduced a negative effect of soil contamination with oil derivatives on the occurrence of enthomopathogenic nematodes.
4. Occurrence of entomopathogenic nematodes seems to be suitable indicator of effectiveness of bioremediation process in soil environment contaminated with oil derivatives.

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WPŁYW ZANIECZYSZCZENIA GLEBY SUBSTANCJAMI ROPOPOCHODNYMI NA WYSTĘPOWANIE NICIENI OWADOBÓJCZYCH

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Abstrakt: Celem badań było poznanie wpływu wybranych substancji ropopochodnych na występowanie nicieni owadobójczych w środowisku glebowym w trakcie procesu bioremediacji. Doświadczenie przeprowadzono w warunkach polowych w dwóch seriach (z bioremediacją i bez bioremediacji) w Stacji Doświadczalnej w Mydlnikach koło Krakowa. Gleba w każdej serii została sztucznie zanieczyszczona następującymi substancjami ropopochodnymi: benzyną, olejem napędowym i zużytym olejem silnikowym (dawka 6 000 mg paliwa · kg^{-1} s.m. gleby). Kontrolę stanowiła gleba niezanieczyszczona. Nicienie owadobójcze naturalnie występujące w glebie izolowano metodą owadów pułapkowych, przy wykorzystaniu larw mącznika młynarka. Analizę prowadzono przez okres dwóch lat, pobierając glebę do analizy w każdej porze roku. Zanieczyszczenie gleby ropopochodnymi wpłynęło negatywnie na występowanie nicieni owadobójczych w środowisku glebowym. Z gleby zanieczyszczonej substancjami ropopochodnymi nicienie

owadobójcze nie były izolowane. Najbardziej długotrwały, niekorzystny wpływ na nicienie owadobójcze naturalnie występujące w glebie miało zastosowanie oleju silnikowego i oleju napędowego. Zastosowanie biopreparatu miało korzystny wpływ na występowanie nicieni owadobójczych. W obiektach, w których zastosowano biopreparat mikrobiologiczny, nicienie owadobójcze izolowano istotnie wcześniej po ich skażeniu niż w obiektach, w których proces remediacji zachodził w sposób naturalny. Analiza występowania naturalnej populacji nicieni owadobójczych w glebie może być wykorzystana do monitorowania przebiegu procesu bioremediacji gruntu zanieczyszczonego substancjami ropopochodnymi.

Słowa kluczowe: nicienie owadobójcze, benzyna, olej napędowy, olej silnikowy, bioremediacja