Int. J. Environ. Res., 10(3):449-458, Summer 2016
ISSN: 1735-6865

The Occurrence of Springtails (Collembola) Andspiders (Araneae) as an Effectiveness Indicator of Bioremediation of Soil Contaminated by Petroleum-Derived Substances

Rusin, M.* and Gospodarek, J.

Department of Agricultural Environment Protection, University of Agriculture in Kraków, Al. Mickiewicza 21, 31-120 Krakow, Poland

Received 20 April 2016; Revised 2 June 2016; Accepted 9 June 2016

ABSTRACT: The objective of the study was to determine the effect of the process of natural bioremediation, and bioremediation supported by ZB-01 microbiological preparation on the occurrence of Collembola andAraneaein conditions of soil contaminated by petroleum-derived substances. The studies were conducted in 2010-2012, in the area of the Experiment Station of the University of Agriculture, near Kraków. In June 2010, the soil surface was contaminated with petrol, diesel fuel and engine oil with amount of 6 000 mg per kg of dry mass of soil. The studies were conducted in two series. The first employed microbiological bioremediation using ZB-01 biopreparation, and the second – without applying this process. The catching of invertebrates was done by usingpitfall traps. The results of the experiment show thatthe representatives of the EntomobryidaeandIsotomidae families are suitable for assessing the rate of bioremediation. Epigeic spiders do not react negatively to soil contamination.Biopreparationcaused the acceleration of the recolonisation of the soil by representatives of the Isotomidae and Hypogastruidae families. With regard to the spiders, the application of biopreparation eliminated the aforementioned effect of increasing the numbers of the Lycosidae family in the object contaminated with engine oil.

Key words: Bioremediation process, Fuels, Petrol, Collembola, Araneae

INTRODUCTION

Soil contamination with petroleum and petroleum-derived substances can adversely affect living organisms inhabiting the soil (Couceiroet al., 2007).The compounds present in the contaminants can enter body cavities and ducts, and – additionally – they can coat their bodies, preventing gas exchange, energy exchange, uptake of nutrients, as well as receiving stimuli from the surroundings. The adverse effects of petroleum-derived substances on soil and terrestrial invertebrates have been confirmed by many authors(Blakely et al., 2002;Gospodarek et al., 2016).

Springtails, being commonly distributed, and – at the same time – ecologically adapted organisms, can be good indicators of the quality of the environment (Fiera, 2009).Moreover, this group is relatively well studied both in terms of their taxonomy and ecology, showing little dispersion potential, and sedentary way of life. Springtails react relatively quickly to any changes in soil structure and to any alterations in its physical and chemical composition resulting from the presence of xenobiotics in the soil environment (Gillet and Ponge, 2003).

For many years, spiders have been model organisms in a great number of studies in the field of ecology. They also play an important role in bioindication. They are mostly well known in terms of their systematics, which are very numerous, easily collected, monitored and identified (Hore andUniyal, 2008), and very sensitive to even minor changes in the natural environment, including those changes caused by human activities (Aspetti et al., 2010). Furthermore, spiders are important indicators of heavy metal pollution and – as consumers – they participate in the biomagnification of many pollutants (Jung et al., 2008).

Soil organisms (especially springtails and spiders) may be useful in the environmental change monitoring because they provide objective data that integrate physical, chemical, and biological properties of the soil. Thus, they provide a more accurate and feasible

*Corresponding author E-mail: milena_rusin@wp.pl

measure of chemical contaminants (such as petroleum-derived substances) biotoxicity, thanchemical methods.Despite the great importance of Collembola andAraneaein the natural environment, and principally their major role as bioindicators, the number of studies dealing with the effects of petroleum and petroleum-derived substances on the occurrence of these invertebrates is very low. Moreover, in published references, little information is available on the effect on contaminated soil of biopreparations containing live microorganisms used in order to accelerate the decomposition of petroleum-derived substances upon the occurrence of soil and epigeic invertebrates. Our studies to date devoted to this topic have demonstrated that the application of bioremediation in soil contaminated with petrol, diesel fuel, and engine oil accelerated the process of soil recolonisation by soil invertebrates but also depended on the group of fauna studied, as well as on the type of contaminating substance. It should also be stressed that the analysis of TPH (total petroleum hydrocarbons) content in soils contaminated with petrol and diesel fuel did not show differences in uncontaminated soil at the end of the study period while the differences in the density of soil fauna were still recorded. It confirms its higher 'sensitivity' as an indicator of the state of environment than that offered by chemical analyses (Gospodarek et al., 2016).In the mentioned experiment Collembola were particularly sensitive and showed a reaction to both pollution and bioremediation process. In the case of petrol both with and without using biopreparation delayednegative effect on the total number of springtail was observed (persisting for more than 28 months after contamination). After 25 months from the soil contamination with diesel fuel and after 14 months from the contamination with engine oil the number of trappedspringtailswasequated with the control. However, in the first case, the use of biopreparation accelerated the process of recolonization, and the second – extended this period for over 28 months.This suggests significant differences in this group of invertebrates in response to the process of hydrocarbons biodegradation. In the case of spidersour earlier studies showed that engine oil and petrol even stimulated their occurrence on the soil surface(Gospodarek et al. 2012).It has become a premisefor a detailed analysis of Collembola and Araneae families.

The objective of the study was to determine the effect of the process of natural bioremediation, and bioremediation supported by ZB-01 microbiological preparation upon the dynamics of occurrence ofthe representatives of particular families of springtails (Collembola) and spiders (Araneae) in conditions of soil contaminated by petroleum-derived substances, such as: petrol, diesel fuel, as well as by used engine oil. We also wanted to find out which representatives of the above listed groups of invertebrates would be most useful as potential bioindicators for the assessment of the rate of bioremediation as well as the ecological risk of supported bioremediation.

MATERIAL & METHODS

The studies were carried out between the beginning of July 2010 and the end of September 2012, in the area of the Experiment Station of the University of Agriculture, situated in Mydlniki near Kraków(Poland; 50.0815°N, 19.84730°E). It was an area left out of agricultural use, overgrown by grass which was cut twice a year during the vegetation season. The experiment was set in a randomized block system with four repetitions. The studies were conducted in two series. The first employed microbiological bioremediation using ZB-01 biopreparation, and the second – without applying the process of supported bioremediation. Four objects were set up in each series: the control, i.e. uncontaminated soil, soil artificially contaminated with petrol, diesel fuel, and used engine oil.

In November 2009, native soil was placed, with the natural system of layers retained, in purpose-built $1m^3$ containers. The characteristics of the native soil as well as detailed description of containers and their adaptation to the objectives of the experiments, as well as their method of distribution on the study area were given in an earlier paper(Gospodarek et al., 2016).The containers were dug into the ground in such a way that their upper edge was even with the surface of the soils, and thence it was not an obstacle for the movement of studied organisms. Additionally, the upper sections of the container sides were perforated in order to enable the independent penetration of contaminated soil by invertebrates.

The contamination procedure was conducted on 10 June 2010, i.e. after the period allowing conditions in containers to approximate natural conditions. The soil surface was artificially contaminated with petrol, diesel fuel, and used engine oil with amount of 6 000mg per kg of dry mass of soil (by pouring), i.e. in concentration of petroleum-derived substances occurring in moderately contaminated soils.Then, after one week, half of the containers were subjected to bioremediation by adding ZB-01 biopreparation to the soil. The ZB-01 biopreparation, especially produced for the purpose of the experiments, with its composition including selected prokaryotic microorganisms, principally bacteria of *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Oligella*, *Ochrobactrum*, *Comamonas*, *Burkholderia*, *Stenotrophomonas*, and

*Corynebacterium*genera. After one year, in the spring of 2011, the bioremediation exercise was repeated.

The catching of springtails andspiders was done by usingpitfall traps.It is a commonly used method for measuring the activity of these terrestrial fauna (Jung et al., 2008; Quernerand Bruckner, 2010).They were single glass jars of 0.9 litre capacity dug in the central part of each container, with their upper edge even with the soil surface, with plastic shields protecting them from atmospheric precipitation. The trapping was conducted during the vegetation season that is from May to October in 2010 – 2012 (except 2010, when the first trapping session was conducted at the end of June). Traps were emptied once a week. The detailed analysis were performed for the material obtained twice in a given vegetation season, i.e. in spring (in May, except for 2010, when the material for analysis was collected within one month after the contamination exercise – at the beginning of July) and in autumn (in September). These dates were after 1, 3, 11, 15, 23, and 27 months, respectively, from the time of contamination and the first application of the ZB-01 biopreparation. The collected samples of fauna were identified to the level of families by using the appropriate keys (Fjellberg, 1998;Fjellberg, 2012;Nentwig et al., 2015;Platnick, 2015).

The obtained results were then subjected to analysis by STATISTICA 10.0 software. The significance of differences between the means were tested by two-factor (contamination x remediation)variance analysis, and the means were differentiated by Fisher's LSD test at α = 0.05.

RESULTS& DISCUSSION

Throughout the duration of the experiments, a total of 7,886 individuals of springtails were caught in the study area (Table 1). The highest number was trapped in the control object, whereas almost half twice less – in the objects contaminated with diesel fuel and engine oil. Applying the ZB-01 biopreparation contributed to a minor decrease in the numbers of individuals trapped in the control, whereas in other objects it resulted in an increase of numbers of Collembola. In all objects analysed, Entomobryidae, Isotomidae, and Hypogastruidae families were represented by the highest numbers of individuals. Apart from these, also individuals of Onychiuridae, Katianidae, Bourletiellidae, and Dicyrtomidae families were recorded, although only sporadically.

Table 2. Occurrence of Collembola from Hypogastruidae family in individual months after soil contamination [pcs./trap]In one month after the contamination, only engine oil caused asignificant decrease in the number of trapped springtails of the Hypogastruidae family, but after three months all the applied pollutants significantly inhibited the occurrence of the studied soil invertebrates (Table 2). After 11 months, such an effect was recorded only with respect of diesel fuel and engine oil, while after the passage of 15 months no adverse effects on the occurrence of these springtails were recorded for any of the petroleum-derived substances used. The application of the ZB-01 biopreparation in the object contaminated with diesel fuel caused a significant increase in the numbers of trapped springtails after 11 and 15 months from the time of contamination. Similar regularity was noted also in the object contaminated with petrol after the passage of 11 months. In the case of the control, after one month the biopreparation caused a significant decrease in the number of trapped individuals of the Hypogastruidae family.

After one month from the soil contamination, both petrol as well as engine oil caused significant limitations

Table 1. Occurrence of representatives from Collembola families under the control soil and soil contaminated with petroleum substances subjected and not subjected to bioremediation process [pcs.]

Family	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
Hypogastruidae	166	155	154	311	10	57	31	171
Onychiuridae	0	1	0	4	1	0	1	0
Isotomidae	326	275	259	232	158	184	176	197
Entomobryidae	760	713	605	609	550	526	507	687
Katianidae	3	4	11	4	1	1	0	2
Bourletiellidae	4	1	12	12	0	2	2	0
Dicyrtomidae	0	0	0	1	0	0	0	0
Sum	1259	1149	1041	1173	720	770	717	1057

C – control soil, P – soil contaminated with petrol, EO - soil contaminated with engine oil, DF - soil contaminated with diesel fuel, 0R - series without ZB-01, R - series with ZB-01.

Table 2. Occurrence of Collembola from Hypogastruidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	0.8^{b}*	0.0^{a}	0.3^{ab}	0.3^{ab}	0.0^{a}	0.0^{a}	0.3^{ab}	0.0^{a}
3	5.8^{c}	6.3^{c}	2.3^{ab}	2.8^{b}	0.0^{a}	1.0^{ab}	0.3^{ab}	0.8^{ab}
11	31.8^{bc}	27.0^{bc}	33.8^{c}	73.5^{d}	2.0^{a}	10.3^{ab}	2.3^{a}	34.0^{c}
15	0.8^{a}	2.0^{ab}	1.5^{a}	1.3^{a}	0.0^{a}	0.3^{a}	1.5^{a}	7.5^{b}
23	1.3^{a}	1.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.3^{a}	0.0^{a}	0.0^{a}
27	1.3^{ab}	2.5^{ab}	0.8^{ab}	0.0^{a}	0.3^{a}	2.5^{ab}	3.5^{b}	0.5^{ab}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at $\alpha=0.05$; factors contamination x remediation

Table 3. Occurrence of Collembola from Isotomidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	13.8^{b}	13.8^{b}	1.5^{a}	2.3^{a}	3.5^{ab}	1.5^{a}	2.0^{a}	2.3^{a}
3	3.0^{a}	6.3^{ab}	7.8^{ab}	11.3^{b}	2.0^{a}	4.8^{a}	5.0^{a}	3.3^{a}
11	47.8^{a}	32.0^{a}	43.5^{a}	32.3^{a}	26.3^{a}	27.0^{a}	33.0^{a}	32.8^{a}
15	3.6^{ab}	2.5^{ab}	6.0^{b}	1.8^{a}	2.3^{ab}	4.0^{ab}	0.5^{a}	2.3^{ab}
23	5.3^{d}	3.8^{bcd}	2.3^{abc}	4.5^{cd}	1.3^{ab}	2.8^{abcd}	0.8^{a}	4.5^{cd}
27	9.0^{bc}	10.5^{c}	3.8^{a}	6.0^{abc}	4.3^{ab}	6.0^{abc}	2.8^{a}	4.3^{ab}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at $\alpha=0.05$; factors contamination x remediation.

of the numbers of trapped springtails of the Isotomidae family (Table 3). However, the analyses performed 3, 11, and 15 months after the time of the contamination exercise found no significant effect of any of the applied petroleum-derived substances. After the passage of 23 months, significantly fewer springtails were trapped in all objects contaminated with petroleum-derived substances than in the control object. In the cases of objects contaminated with petrol and diesel fuel, this relationship continued also after 27 months. Most often, the application of the ZB-01 biopreparation did not significantly affect the studied feature and only in the object contaminated with petrol did it cause an decrease in the number of trapped individuals after 15 months, whereas in the object contaminated with diesel fuel there was an almost fivefold increase after 23 months.

A significant decrease in the number of trapped springtails of the Entomobryidae family was noted one month after the contamination of soil with engine oil and diesel fuel(Table 4). A similar relationship was again noted in this object after 3 and 11 months. After 11 months in the object contaminated with engine oil, more than three times fewer springtails were trapped than in the control, whereas in the object contaminated with diesel fuel - more than half. The negative effect of petrol on the analysed invertebrates was noted only after one month. In the cases of control and petrol-contaminated objects, applying ZB-01 biopreparation led to a significant decrease in the numbers of trapped springtails. In the remaining cases, it did not show any effect on the analysed feature.

Throughout the duration of the experiments, a total of 1,074 individuals of spiders were caught in the study area (Table 5).The highest number was noted in the object contaminated with engine oil, whereas the lowest - in the object contaminated with petrol. Most often, the application of biopreparation contributed to the increase in numbers of Araneae trapped, and only in the object contaminated with engine oil did it cause a more than twofold decrease in the number of spiders compared with the object where biopreparation was not used. In all objects analysed, spiders of the Linyphiidae, Lycosidae, and Theridiidae families

Table 4. Occurrence of Collembola from Entomobryidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	18.8^{b}	19.3^{b}	1.8^{a}	3.3^{a}	2.8^{a}	2.0^{a}	1.5^{a}	2.5^{a}
3	30.0^{d}	23.3^{cd}	20.5^{bcd}	26.3^{d}	5.3^{a}	13.0^{ab}	8.3^{a}	13.5^{abc}
11	34.5^{c}	21.0^{ab}	29.0^{bc}	14.8^{a}	11.0^{a}	17.3^{a}	15.8^{a}	20.0^{ab}
15	18.8^{ab}	25.0^{ab}	41.5^{b}	17.8^{ab}	18.8^{ab}	30.5^{ab}	12.3^{a}	27.5^{ab}
23	19.5^{ab}	18.0^{ab}	23.0^{ab}	31.5^{b}	16.0^{a}	15.3^{a}	25.5^{ab}	27.3^{ab}
27	68.5^{ab}	77.0^{ab}	35.5^{a}	58.8^{ab}	83.8^{b}	53.5^{ab}	63.5^{ab}	81.0^{b}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at α=0.05; factors contamination x remediation.

Table. 5. Occurrence of Araneae families under the control soil and soil contaminated with petroleum substances subjected to bioremediation process

Family	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
Linyphiidae	71	56	40	55	82	41	69	91
Lycosidae	42	54	55	42	81	42	55	58
Araneidae	0	2	4	0	2	0	0	1
Tetragnathidae	0	0	3	0	2	0	0	0
Thomisidae	0	0	4	0	5	0	0	2
Theridiidae	5	13	5	17	20	15	9	27
Pholicidae	2	1	0	0	0	0	0	1
Sum	120	126	111	114	192	98	133	180

Symbols as in Table 1.

predominated. Apart from these, the presence of individuals of the Araneidae, Tetragnathidae, Thomisidae, and Pholicidae families were recorded.

Throughout the whole period of the experiments, none of the applied petroleum-derived substances had a significant impact on the numbers of spiders of the Linyphiidae family(Table 6). Most often, the applied biopreparation also did not affect the analysed feature and only in the object contaminated with diesel fuel did it cause a significant increase in the number of trapped individuals after 15 months from the time of contamination.

Among all the applied petroleum-derived substances, it was only engine oil which caused a significant increase in the number of the Lycosidae family after 11 months from the time of contamination (Table 7). After the passage of three months from the contamination exercise, ZB-01 biopreparation led to a significant decrease in the numbers of individuals trapped in the experimental object involving contamination with diesel fuel, and, in turn, to its increase in the case of the object contaminated with petrol. Additionally, over the period of eight subsequent months it led to a more than tenfold decrease in the number of spiders trapped in the object contaminated with engine oil. After 27 months, the biopreparation also contributed to a significant limitation of the occurrence of analysed invertebrates in the control object.

No significant effects were noted for any of the applied contaminants upon the occurrence of spiders of the Theridiidae family at all dates when the studies were conducted(Table 8). Similarly, in no case, did the biopreparation used have any significant effect on the analysed feature.

Springtails andspiders live in various soil and terrestrial habitats but the changes in their numbers and biological diversities under the impact of the petroleum-derived substances present, are still poorly documented. The experiments performed in the presented study proved that these compounds greatly affect the occurrence of Collembola, and the strength

Table. 6. Occurrence of Araneae from Linyphiidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	0.3^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.5^{a}
3	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.5^{a}	0.0^{a}	0.5^{a}	0.0^{a}
11	9.0^{a}	7.3^{a}	3.0^{a}	5.8^{a}	11.3^{a}	4.0^{a}	5.5^{a}	7.8^{a}
15	0.3^{ab}	0.0^{a}	0.3^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.8^{b}
23	7.0^{ab}	5.5^{a}	6.5^{ab}	6.5^{ab}	5.8^{a}	4.7^{a}	8.3^{ab}	11.5^{b}
27	1.3^{ab}	1.3^{ab}	0.0^{a}	1.5^{ab}	3.0^{b}	0.8^{ab}	3.0^{b}	2.3^{ab}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at α=0.05; factors contamination x remediation.

Table 7. Occurrence of Araneae from Lycosidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	2.0^{a}	1.5^{a}	5.0^{a}	2.5^{a}	4.3^{a}	1.0^{a}	5.0^{a}	2.3^{a}
3	0.3^{ab}	0.3^{ab}	0.0^{a}	0.8^{b}	0.3^{ab}	0.0^{a}	0.8^{b}	0.0^{a}
11	2.3^{a}	7.0^{ab}	3.8^{ab}	4.0^{ab}	10.5^{b}	1.0^{a}	5.0^{ab}	6.5^{ab}
15	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
23	4.8^{a}	4.8^{a}	4.3^{a}	2.5^{a}	4.5^{a}	3.8^{a}	2.5^{a}	5.0^{a}
27	1.3^{b}	0.0^{a}	0.8^{ab}	0.5^{ab}	0.8^{ab}	0.8^{ab}	0.5^{ab}	0.8^{ab}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at α=0.05; factors contamination x remediation

Table 8. Occurrence of Araneae from Theridiidae family in individual months after soil contamination [pcs./trap]

Number of months from soil contamination	C		P		EO		DF	
	0R	R	0R	R	0R	R	0R	R
1	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}
3	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
11	1.0^{ab}	1.5^{ab}	0.5^{a}	2.5^{ab}	2.0^{ab}	3.3^{ab}	1.8^{ab}	4.5^{b}
15	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}
23	0.3^{a}	1.0^{a}	0.8^{a}	1.0^{a}	2.0^{a}	0.0^{a}	0.5^{a}	2.0^{a}
27	0.0^{a}	0.8^{a}	0.0^{a}	0.8^{a}	0.5^{a}	0.0^{a}	0.0^{a}	0.3^{a}

Symbols as in Table 1. *Means in lines marked with the same letters do not differ significantly according to LSD test at α=0.05; factors contamination x remediation.

of this impact depends on the length of time that passed from the contamination event, and on the family of the particular individuals involved.

Jaworska and Gospodarek (2006) proved that the negative effect of petroleum-derived substances upon the occurrence of springtails can continue from five weeks to even as long as five months after the introduction of contamination. In our experiment it was noted that engine oil and diesel fuel limited the occurrence of springtails of the Entomobryidae and Hypogastruidaefamilies even after the 11 months from the time of contamination.Kireeva et al. (2005) proved that the contamination of soil with Tyumen oil at a dose of 1% of soil weight resulted in an increased mortality and shortened length of life among Collembola, whereas doses of 5 and 10% contribute to the complete mortality of springtails irrespective of the time of soil incubation. Additionally, these authors noted that with the increased time of incubation, the negative effect of Tyumen oil on the studied invertebrates lessened. It stems from the fact that the most toxic, light fraction of petroleum are able to evaporate from soil during incubation. It was confirmed by the results obtained in the presented study for petrol - here the effect on the occurrence of springtails of the Entomobryidae and Hypogastruidae families was maintained for the shortest period (1- 3 months). Among the studied petroleum-derived substances, petrol contains the highest amounts of substances undergoing fast vaporisation. Moreover, Szarlip et al. (2014) concluded, on the basis of their studies, that the rate of biodegradation of petrol was higher than diesel fuel.

Blakely et al.(2002) studied the effects of polycyclic aromatic hydrocarbons (whose major quantities are also present in petroleum-derived substances) on the occurrence of soil invertebrates. These authors found that these compounds negatively affect the springtails of the Isotomidaefamily, but did not note this kind of relationship in the case of other families of Collembola. In the experiment presented in our study, the engine oil and diesel fuel also contributed to the decrease in numbers of trapped individuals of Isotomidae family, and this effect was still discernible after such a long time as 27 months. PAHs contribute to an increase of bulk density of the soil which, in turn, causes the shrinking of the habitats available to springtails, and additionally involves reduction in the quantity of dead organic matter in soil (Blakely et al., 2002). Other authors demonstrated however, that minor quantities of PAHs in soil (5.28 - 80.46 mg/kg) could stimulate the occurrence of Collembola(Erstfeld and Snow-Ashbrook, 1999). These compounds can contribute to increases in the activity of soil microorganisms utilising the petroleum-derived substances as a source of carbon and energy.

Petroleum-derived substances contribute to the increased contents of heavy metals such as zinc (Benka-Coker andEkundayo, 1995),lead and copper (Ujowundu et al., 2011) in the soil and plants (Rusin et al., 2015) and also result in far-reaching physicochemical changes (Adekunle, 2011).Syrek et al.(2006) determinde the effect of soil contamination with heavy metals upon the occurrence and species diversity of springtails living in forest soil. The authors found that high concentrations of zinc and lead could contribute to the complete elimination of many species of the Entomobryidaeandthe Isotomidae family. In our experiment, contamination with engine oil and diesel fuel were also the most often cause of the limited occurrence of springtails of these families. In turn, Gillet and Ponge (2003)proved that soil contamination with zinc, on the one hand the number of springtails increased, but - on the other hand - their species richness decreased. Heavy metals can affect springtails directly, or indirectly - by contaminating their sources of food. Some Collembola species are relatively resistant to metals as they are capable of eliminating them from their bodies during the moulding process. Additionally, heavy metals can also show toxic effects towards predators or competitors which may ultimately result in an increase in the number of springtails in the contaminated area (Fiera, 2009). It should, however, be emphasised that epigeic springtails are less vulnerable to contaminants than springtails living in the soil. The difference stems chiefly from their higher mobility and less direct contact with contaminants (Fountain andHopkin, 2004 a). Additionally, the effect of xenobiotics on epigeic organisms depends on a number of factors such as the type of soil,coverage of ground (Fountain andHopkin, 2004 b),anthropogenic activities (Chernova andKuznetsowa, 2000).

Soil contamination can involve serious negative effects on spiders as well. The majority of publications pertains to the effects of heavy metals which are also one of the components of petroleum-derived substances. Chen et al.(2011) proved that the contamination of soil with lead and zinc resulted in a decrease of body mass in Pardosaastigera(Lycosidaefamily) as well as in lengthening the duration of the reproductive cycle, and - in the case of females - also in the lower number of eggs laid. Similar regularities, but with regards to cadmium, were also noted by other authors (Jung et al., 2005). Spiders very often uptake heavy metals with ingested food (with their prey). They are, however, capable of detoxification, sequestering them in the intercellular pellet of midget diverticulae. Owing to that capability, the chances of survival of these invertebrates in contaminated soil increase, but - all the same - the protective mechanism of this type can result in slowing down their growth and development and can disturb the reproductive process (Chen et al., 2011). The contamination of soil with petroleum-derived substances often has a nature of point event(road accidents, leakages from pipelines,), thus it can be expected that the effects on life parameters of these animals will not be so acute as the aforementioned ones. The protective mechanism for spiders, being very mobile animals, can be, in this case, the strategy of avoiding contact with contaminated soil. However, as shown in earlier studies, these animals do not react to the presence of such substances with decreases in their number (Jaworska andGospodarek, 2006;Gospodarek et al., 2012),and some of the contaminants can even stimulate the occurrence of arachnids on the soil surface. In our study, petroleum-derived substances most often had no adverse effect on the numbers of Araneae. This phenomenon can be explained by the fact that the changes in the environment resulting from the presence of xenobiotics can result in creating new ecological niches suitable for new species of spiders(Brändle et al., 2001),or - as it has been suggested in earlier studies - that petroleum-derived substances can contain attractants for these animals (Gospodarek et al., 2012).

A number of authors emphasise that bioremediation supported by adding selected microorganisms to the soil results in the degradation of hydrocarbons contained in the soil contaminated with petroleum-derived substances (Wang et al., 2009),not that many studies have been conducted to date on the topic of the effect of supported bioremediation process on the occurrence of Collembola andAraneae. Kireeva et al. (2005) proved that the application of Basispecin (a preparation containing strains of Bacillus sp. 739) on the soil contaminated by minor doses of Tyumen oil positively affects the survival rate of springtails. Basispecin accelerates the decomposition of hydrocarbons in soil and - as a consequence - reduces the concentration of the xenobiotic by 25-40%, depending on the dose.In our experiment, ZB-01 biopreparation caused periodical increases in the numbers of springtails of the Hypogastruidae and Isotomidae families in the object contaminated with petrol and diesel fuel. However, on the other side it slightly limited the occurrence of springtails of the Entomobryidae family in the control object and in the object contaminated with petrol.

Gospodarek et al. (2012) proved that the bioremediation of soil contaminated with petroleum-derived substances, when it is supported by the use of selected prokaryotic organisms over a period of five months after the contamination event, does not significantly affect the total number of arachnids. In our experiment, the application of ZB-01 biopreparation most often failed to affect the number of trapped spiders. The only significant effects were sporadically noted in the case of the Linyphidae family and in the case of the Lycosidae family (a periodical increase in the object contaminated with petrol but the limitation of numbers in the case of soil contaminated with engine oil, diesel fuel and in the control object).

The different effects of the studied biopreparation ZB-01 on Collembola andAranaein various objects could be explained in part by the fact that the effectiveness of the bioremediation process in the conditions of contamination by petroleum-derivatives depends on a number of factors, including the type of contaminating substance. The substances with complex structures are most often less vulnerable to the effects of this process (Van Hamme et al., 2003). The effectiveness of the bioremediation process also depends on moisture content in the soil, temperature, pH, and the bioavailability of substrates (Dindar et al., 2013).Various authors also show different stands on the possibility of using supported bioremediation to clean soils of xenobiotics. Some regard it as the fastest and most effective method to decontaminate soils (Bento et al., 2005),while others maintain that the method only works in laboratory conditions, and that biostimulation involving environmental modifications (supplying nutrients, aeration of soil) aimed at increasing the effectiveness of decomposition of harmful substances by native microflora is much more effective (Kogbara, 2008).

Springtails and arachnids are often listed as potential bioindicators of contamination of the natural environment with heavy metals (Fiera, 2009)as these animals react with a rapid decrease in their numbers when xenobiotics are present. Furthermore, Collembola are regarded as good indicators of the intensification of ground use(Sousa et al., 2004),types of forest management (Cassagne et al., 2006)and pesticide use (Frampton, 1997). In our experiments, it was proved that some families of the studied invertebrates can also be used for assessing the rate of bioremediation, both natural and supported. Springtails seem to be the most sensitive group, particularly those of the Entomobryidae and Isotomidaefamilies. Entomobryidae were trapped in great numbers and significant effects in the cases of engine oil and diesel fuel were discernible from the contamination event itself to as long as 11 months after it. Isotomidae were trapped less frequently and the effect was not that unambiguous in the initial period after the contamination exercise, but it continued to be noted even 23 - 27 months after the contamination exercise, whereas the results of chemical analysis for TPH content in the case of diesel fuel did not show significant difference compared with the control soil as early as 12 months after the contamination event (Gospodarek et al., 2016).

CONCLUSIONS

The analysis of occurrence of Collembola as indicators of the rate of natural bioremediation of soils contaminated with petroleum-derived substances points to the possibility of the existence of their negative impact as late as 27 months after the contamination event in the case of oils (engine oil and diesel fuel) and 3 months in the case of petrol. The representatives of various families of Collembola display different reactions to various types of petroleum-derived substances. Among the studied families, the representatives of the Entomobryidae as well as the Isotomidae families are characterised by their greatest sensitivity to the presence of contamination which can suggest that they are suitable for assessing the rate of bioremediation, both natural and supported by the use of biopreparations.

Epigeic spiders do not react negatively to soil contamination with petroleum-derived substances, and these compounds can even stimulate the occurrence of some of their representatives - stimulated the periodical occurrence of spiders of the Lycosidae family. The analysis of occurrence of Collembola in the objects subjected to bioremediation supported by the use of ZB-01 biopreparation indicated the acceleration of the recolonisation of the soil by representatives of the Isotomidae and Hypogastruidae families, particularly in the object contaminated with diesel fuel.With regard to the representatives of spiders, the application of biopreparation somewhat eliminated the aforementioned effect of increasing the numbers of the Lycosidae family in the object contaminated with engine oil.

ACKNOWLEDGEMENTS

Scientific work financed from the funds for science in 2009-2013 as a research project (N N305 151537). Preparation of the article financed from the funds for designated subsidy to conduct research, development work and related tasks, contributing to the development of young scientists and University of Agriculture phD students financed under competition procedure in 2015. No topic 4172.

REFERENCES

Adekunle, I. M. (2011). Bioremediation of soils contaminated with Nigerian petroleum products using composted municipal wastes. Bioremediation Journal, **15(4),** 230-241.

Aspetti, G. P.,Boccelli,R.,Ampollini, D., Del Re Attilio, A. M. and Capri, E.(2010). Assessment of soil-quality index based on microarthropods in corn cultivation in Northern Italy. Ecological Indicators,**10,** 129-135.

Benka-Coker, M. O. and Ekundayo, J. A.(1995). Effects of an oil spill on soil physico-chemical properties of a spill site in the Niger Delta Area of Nigeria. Environmental Monititoringand Assessment, **36(2),** 93-104.

Bento, F. M., Camargo, F. A. O.,Okeke, B. C. and Frankenberger, W. T.(2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation,biostimulation and bioaugmentation. Bioresource Technology, **96(9),** 1049-1055.

Blakely, J. K.,Neher, D. A. and Spongberg, A. L.(2002). Soil invertebrate and microbial communities and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. Applied Soil Ecology, **21,** 71-88.

Brändle, M.,Amarell, U.,Auge, H., Klotz, S. and Brand, R.(2001). Plant and insect diversity along a pollution gradient: understanding species richness across trophic levels. Biodiversity and Conservation, **10,** 1497-1511.

Cassagne, N.,Gauquelin, T., Bal-Serin, M. C. and Gers, C.(2006). Endemic Collembola, privileged bioindicators of forest management. Pedobiologia, **50(2),** 127-134.

Chen, X. Q., Zhang, Z. T., Liu, R., Zhang, X. L., Chen, J. and Peng, Y.(2011). Effects of the metals lead and zinc on the growth, development and reproduction of Pardosaastrigera (Araneae: Lycosidae). Bulletin of Environmental Contamination and Toxicology, **86(2),** 203-207.

Chernova, N. M. and Kuznetsova, N. A.(2000). Collembolan community organisation and its temporal predictability. Pedobiologia, **44(3-4),** 451-66.

Couceiro, S. R. M., Hamada, N., Ferreira, R. L. M., Forsberg, B. R. and da Silva, J. O. (2007). Domestic sewage and oil spills in streams: effects on edaphic invertebrates in flooded forest. Manaus. Amazonas. Brazil. Water Air and Soil Pollution,**180(1-4),** 249-259.

Dindar, E.,Sagban, F. O. T. and Baskaya, H. S.(2013). Bioremediation of petroleum-contaminated soil. Journal of Biodiversity and Environmental Sciences, **7(19),** 39-47.

Erstfeld, K. M. and Snow-Ashbrook, J.(1999). Effects of chronic low-level PAH contamination on soil invertebrate communities. Chemosphere, **39(12),** 2117-2139.

Fiera, C. (2009). Biodiversity of Collembola in urban soils and their use as bioindicators for pollution. PesquisaAgropecuáriaBrasileira, **44(8),** 868-873.

Fjellberg, A. (1998). The Collembola of Fennoscandia and Denmark. Part I: Poduromorpha. Fauna EntomologicaScandinavica,**35,** 1-184.

Fjellberg, A. (2012). The Collembola of Fennoscandia and Denmark. Part II: Entomobryomorpha and Symphypleona. Fauna EntomologicaScandinavica,**42,** 1-252.

Fountain, M. T. and Hopkin, S. P.(2004 a). A comparative study of the effects of metal contamination on Collembola in the field and in the laboratory. Ecotoxicology, **13,** 573-587.

Fountain, M. T. and Hopkin, S. P.(2004 b). Biodiversity of Collembola in urban soils and the use of Folsomia candida to assess soil "quality". Ecotoxicology, **13,** 555-572.

Frampton, G. K. (1997). The potential of Collembola as indicators of pesticide usage: evidence and methods from the UK arable ecosystem. Pedobiologia, **41,** 179-184.

Gillet, S. and Ponge, J. F.(2003). Changes in species assemblages and diets of Collembola along a gradient of metal pollution. Applied Soil Ecology, **22,** 127-138.

Gospodarek, J., Ko?oczek, H. and Petryszak P. (2012). Dynamics of arachnid occurrence in soil contaminated with petrol, diesel fuel and engine oil during bioremediation process. Ecological Chemistry and Engineering. A, **19(9),** 1099-1106.

Gospodarek, J.,Petryszak, P. andKo?oczek, H. (2016). The effect of the bioremediation of soil contaminated with petroleum derivatives on the occurrence of epigeic and edaphic fauna. Bioremediation Journal, **20(1),** 38-53.

Hore,U. and Uniyal, V. P.(2008).Use of spiders (Araneae) as indicator for monitoring of habitat conditions in Terai Conservation Area. India. Indian Forester,**134(10),**1371-1380.

Jaworska, M. and Gospodarek, J. (2006). Occurrence of selected groups of soil invertebrates in conditions of soil polluted with oil derivatives. Ecological Chemistry and Engineering, **13(6),** 521-528.

Jung, C. S., Lee, S. B., Jung, M. P., Lee. J. H., Lee, S. and Lee, S. H.(2005). Accumulated Heavy Metal Content in Wolf Spider.Pardosaastrigera (Araneae: Lycosidae), as a Bioindicator of Exposure. Journal of Asia-Pacific Entomology,**8(2),** 185-192.

Jung, M. P., Kim, S. T., Kim, H. and Lee, J. H.(2008). Species diversity and community structure of ground-dwelling spiders in unpolluted and moderately heavy metal-polluted habitats. Water Air and Soil Pollution,**195(1),** 15-22.

Kireeva, N. A.,Khanislamova, G. M. and Tarasenko, E. M. (2005). Biological testing of oil-polluted and reclaimed soils based on the survival of springtails (Collembola). Russian Journal of Ecology,**36,** 361-364.

Kogbara, R. B. (2008). Ranking agro-technical methods and environmental parameters in the biodegradation of petroleum-contaminated soils in Nigeria. Electron. Journal of Biotechnology,**11(1),** 113-125.

Nentwig, W.,Blick, T.,Gloor, D.,Hänggi, A. and Kropf, C.(2015). Spiders of Europe. www.araneae.unibe.ch Version 09.2015.

Platnick, N.I. (2015). World Spider Catalog. Natural History Museum Bern. www.wsc.nmbe.ch version 16.5.

Querner, P. and Bruckner,A. (2010). Combining pitfall traps and soil samples to collect Collembola for site scale biodiversity assessments. AppliedSoil Ecology, **45,** 293-297.

Rusin, M., Gospodarek, J. and Nadgórska-Socha, A.(2015). The effect of petroleum-derived substances on the growth and chemical composition of Viciafaba L. Polish Journal of Environmental Studies,**24(5),** 2157-2166.

Sousa, J. P., de Gama, M. M., Pinto, C., Keating. A.,Calhôa, F.,Lemos, M., Castro, C., Luz, T.,Leitão, P. and Dias, S. (2004). Effects of land-use on Collembola diversity patterns in a Mediterranean landscape. Pedobiologia, **48(5-6),** 609-622.

Syrek, D., Weiner. W. M., Wojtylak, M., Olszowska, G and Kwapis, Z.(2006). Species abundance distribution of collembolan communities in forest soils polluted with heavy metals. Applied Soil Ecology, **31,** 239-50.

Szarlip, P., Stelmach, W., Jaromin-Glen, K., Bieganowski, A., Brzezi?ska, M., Trembaczowski, A., Ha?as S. and ?agód, G. (2014). Comparison of the dynamics of natural biodegradation of petrol and diesel oil in soil. Desalin. Water Treatment,**52(19-21),** 3690-3697.

Ujowundu, C. O.,Kalu, F. N.,Nwaoguikpe, R. N.,Kalu, O. I.,Ihejirika, C. E.,Nwosunjoku, E. C. and Okechukwu, R. I.(2011). Biochemical and physical characterization of diesel petroleum contaminated soil in southeastern Nigeria. Research Journal of Chemistry Sciences, **1(8),** 57-62.

Van Hamme, J. D., Singh, A. and Ward, O. P.(2003). Recent advances in petroleum microbiology. Microbiology and Molecular Biology Reviews,**67(4),** 503-549.

Wang, F., Su, Z. C., Yang,H., Li,X. J., Yang, G. P. and Dong, D. B.(2009). Microbial degradation of soil polycyclic aromatic hydrocarbons (PAHs) and its relations to soil bacterial population diversity. Ying Yong Sheng Tai XueBao,**20(12),** 3020-3026.