

*Original Research*

# Effect of Soil Polluted with Petroleum-Derived Substances during Bioremediation on the Occurrence of Collembola and Acarina

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## Abstract

The research aimed at investigating the effect of soil contamination with various petroleum-derived substances (PDSs, e.g., spent engine oil, diesel fuel, and petrol) on springtail (Collembola) and mite (Acarina) occurrence over 28 months from the moment of pollution. We also assessed the effect of the bioremediation process supported by ZB-01 preparation on the process of soil settlement by the above-mentioned invertebrates. We tried to determine whether the occurrence and activity of these organisms may be useful as a bioindicator or monitoring tool for assessing PDS presence in the soil and speed of remediation process (both natural and supported). A combined method, i.e., pitfall trapping and analysis of soil samples, was used for assessing the presence of Collembola and Acarina. Considering the investigated PDSs, petrol revealed the shortest (about a year) negative effect on Collembola presence on the soil surface (measured by pitfall trapping), whereas it was visible for the longest time (2 years) for diesel fuel. Although initiating a bioremediation process using ZB-01 preparation on soil polluted with petrol (in the first year) and diesel fuel (in the second) resulted in a significant increase in Collembola activity on the soil surface, it limited this parameter in soil contaminated with engine oil and in the control soil (in the second and third years of the experiment). Both diesel fuel and engine oil revealed a strong negative effect on the occurrence of springtails and mites in soil (soil sample analysis) – perceptible even after 28 months from the moment of soil pollution. At the same time, a visible positive response to applied bioremediation supported by biopreparation ZB-01 was registered. Analysis of soil samples polluted with PDSs for springtail and mite presence may be a potentially useful indicator for assessing the degree of soil self-cleaning, since a perceptible response was noticed, dependent both on the PDS and the course of the supported bioremediation process.

**Keywords:** oil derivatives, soil, bioremediation, Collembola, Acarina

## Introduction

Soil pollution with petroleum-derived substances (PDSs) entails a strong negative effect on both its physicochemical properties [1, 2] and biological activity [3-7], as well as causing far-reaching changes in organisms indirectly exposed to these pollutants (e.g., herbivores) [8-10]. The components that are especially dangerous to the environment are polycyclic aromatic hydrocarbons (PAHs) [11]. Efficient methods for their safe neutralization are still being sought through bioaugmentation or adding surfactants [12, 13], optimization of fertilization [14], rhizoremediation [15] or electrobioremediation [16]. Among the methods mentioned above, the use of specially selected microorganisms breaking down organic compounds gives beneficial results in cleaning soils contaminated mainly by medium and low PAH concentrations [17].

Among microinvertebrates, Collembola and Acarina are mentioned as potential bioindicators of changes in the soil environment, mainly due to their common occurrence and the role they play in the processes of matter cycling in nature [18-20]. Collembola are characterized by high sensitivity to soil pollution with heavy metals showing lower densities in the soils with higher metal concentrations [21, 22]. On the other hand, oribatid mite communities (which dominate among invertebrates inhabiting the topsoil) display a great variety of responses to heavy metals [23]. Features such as fertility and survival rate of Collembola are mentioned as greatly sensitive indicators of soil contamination with PAHs [24, 25]. We also investigated Collembola and Acarina occurrence in soils polluted to various degrees with PAHs [26, 27]. The mortality of springtails were also used as one of the tests for assessing the ecotoxicological usability of various bioremediation techniques of soils contaminated with oil derivatives, indicating a rapid reduction of toxicity [28]. Among ecological indicators, abundance and density of invertebrates are regarded as features that reflected the trend of soil chemical and physical characteristics to a greater extent than taxa richness [22]. Some researchers have also suggested that the ratio between the number of mites and collembolans is a useful indicator for the assessment of human activity effect upon the soil environment. The high value of this indicator suggests high soil quality [29].

So far little attention has been paid to a potential application of the analysis of the above-mentioned invertebrate groups occurrence for the assessment of the rate of natural bioremediation and microbial biopreparation-supported process on soils polluted with PDSs [30]. Scientific literature provides the results of research into the effects of PDSs on different elements of the environment; however, they usually pertain to soil environmental changes occurring soon after contamination. There is a lack of data on the more long-term consequences of these pollutants, especially in field conditions. On the other hand, in

the available literature we can find data on the impact of distant in time petroleum soil contamination on soil fauna [27]. However, these in turn do not take into account the effect immediately after pollution. It would be interesting to study how over a longer period of time, starting from the moment of pollution, the possibilities of soil settlement by mentioned soil fauna in field-realistic conditions changed. It is also worth emphasizing that the analysis of the occurrence of springtails and mites has not yet been considered as a simple way to assess the speed of self-cleaning soil polluted with PDSs and subjected to bioremediation. The latest research conducted in sub-Antarctic areas indicates the usefulness of assessing the occurrence of native springtails and mites in the environmental risk assessment of soils contaminated with petroleum hydrocarbons [31, 32].

The aim of our paper was to determine: a) the effect of soil contamination with various PDSs (petrol, diesel fuel and used engine oil) on springtail (Collembola) and mite (Acarina) occurrence over 28 months from the moment of pollution and b) the effect of bioremediation process supported by ZB-01 microbial preparation on the process of soil settlement by the above-mentioned invertebrates. We sought to determine whether the occurrence and activity of these organisms may be useful as a bioindicator or monitoring tool for assessing PDS presence in the soil and speed of remediation process (both natural and supported). We also tried to compare two methods of analyzing microfauna occurrence, i.e., pitfall trapping and soil sample analysis.

## Material and Methods

The investigations were conducted at the Experimental Station of the University of Agriculture in Mydlniki near Krakow (50°5'5,04"N 19°51'13,47"E). In autumn 2009 indigenous soil (detailed description in the earlier paper [33]) was placed in 1m<sup>3</sup> containers with maintained natural layers arrangement. The containers were then dug into the soil so that their upper area was on the same level with the surrounding soil. Additionally, the upper section of the container sides was perforated to enable the invertebrates free penetration of the polluted soil. Perforated plastic tubes were also placed in all containers (every 30 cm, 4 pieces per container) for proper soil aeration in the gravitation system, which is a prerequisite for proper course of the bioremediation process. The soil in containers was left untouched for 8 months in order to allow it to restore its natural biological efficiency. Subsequently, in June 2010 the soil in containers was polluted (by pouring) with the following oil derivatives: petrol, diesel oil and used engine oil in the amounts equal to 6 000 mg of fuel · kg<sup>-1</sup> d.m. of soil (i.e., a typical oil derivative concentration in medium-polluted soils). Four objects were identified: 1. Control – unpolluted soil,

2. Soil with a simulated petrol leak (P), 3. Soil with a simulated diesel fuel (DF) leak and 4. Soil with a simulated used engine oil leak (EO). A week later half of the containers were subjected to bioremediation. The process of oil derivatives bioremediation was initiated and continued by adding ZB-01 preparation to the soil, which was especially prepared for this purpose and contained selected procaryotic microorganisms, mainly bacteria: *Stenotrophomonas*, *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, *Ochrobactrum*, *Comamonas*, *Burkholderia*, *Corynebacterium*, and *Oligella*. The treatment was applied by sprinkling, maintaining 60% of soil sorption moisture. Prior to the biopreparation application, a multicomponent fertilizer (Azofoska; 13.6% N, 2.8% P and 15.8% K) dosed 100 g per container was applied to the soil surface in the containers that were intended to undergo this treatment. The activity of breakdown of organic and aromatic compounds contained in oil derivative pollutants by the suggested biopreparation was verified and proved in the environmental practice, as well as published [6]. After a year (in spring 2011), bioremediation treatment was repeated following the same procedure as in 2010.

The whole experiment was conducted in 4 replications according to randomized block design.

A combined method, i.e., pitfall trapping and analysis of soil samples, was used to assess the presence of Collembola. The application of this method using Collembola is recommended in large-scale biodiversity assessments since it allows us to obtain a more complete picture of the collembolan community on site than either method alone [34]. One pitfall trap (0.9 dm<sup>3</sup> jar dug even with the soil level and protected against atmospheric precipitation with a plastic roof) was placed in the central point of each container. The traps were emptied once a week during the periods from June to October 2010, and from May to October 2011 and 2012. Moreover, soil samples were collected once a month during growing season, thoroughly viewed under a binocular and identified fauna (Acarina and Collembola) was counted and classified. A split corer was used for sample extraction (according to ISO standard 23611-2-2006 [35]). The diameter and depth of the sample were 5 cm and 15 cm, respectively. The volume of a sample was 294.52 cm<sup>3</sup>. Soil was sampled separately from three evenly distributed places in

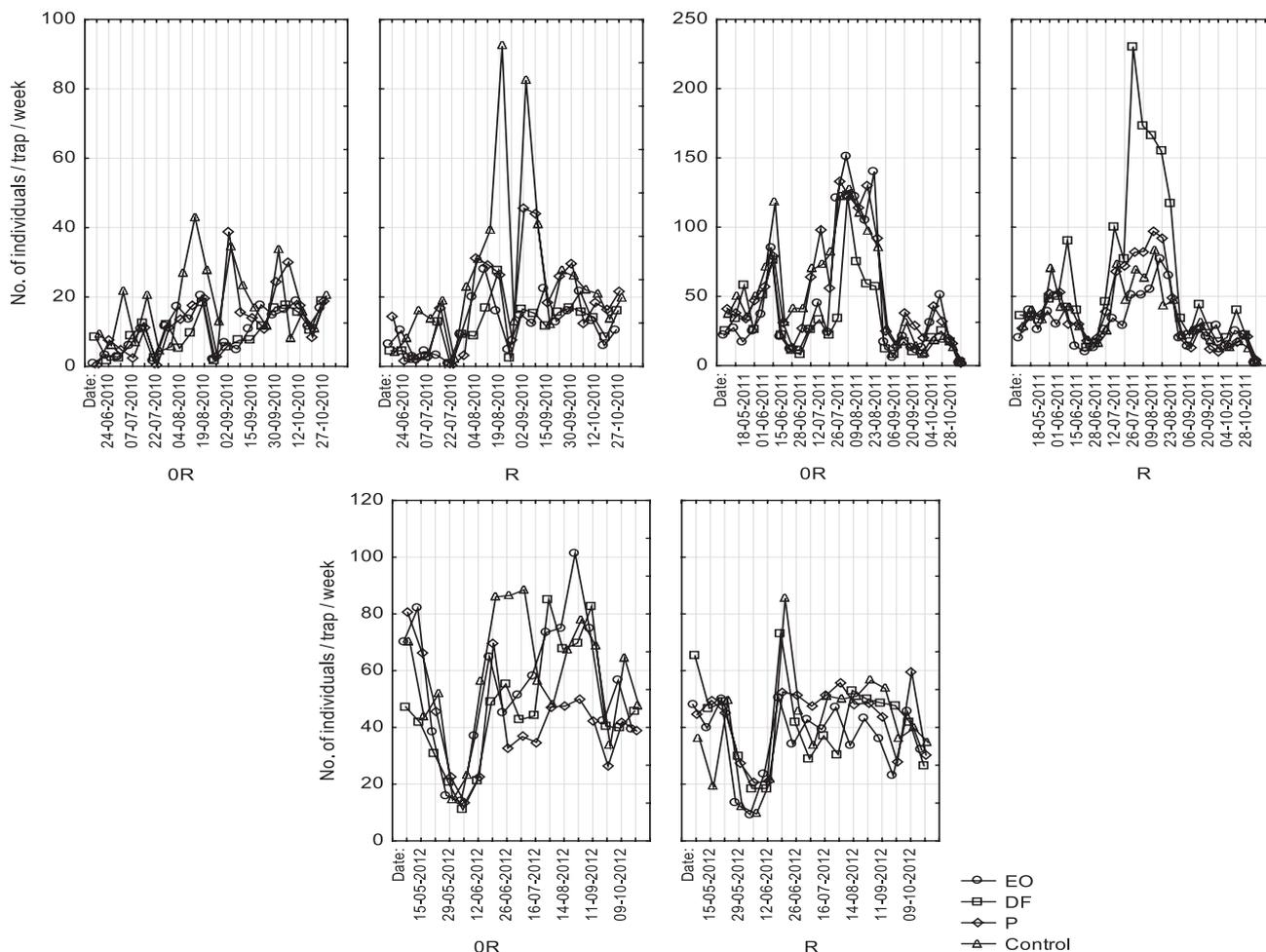


Fig. 1. Occurrence dynamics of Collembola trapped using pitfall traps in the years 2010, 2011 and 2012. EO - soil contaminated with used engine oil. DF - soil contaminated with diesel fuel. P - soil contaminated with petrol. Control - unpolluted soil. OR – series without ZB-01, R – series with ZB-01.

each container. The total sample from each container was  $\approx 300$  g of soil. In addition, the ratio between the total numbers of Acarina and Collembola (A/C) was calculated due to the usefulness of the above-mentioned bioindicator for soil quality assessment as confirmed by the literature of the subject [22].

Statistical computations were made using Statistica 13.1 PL software. ANOVA two-way analysis were carried out. Means were diversified using Fisher's LSD test at significance level  $\alpha = 0.05$ .

## Results and Discussion

The dynamics of springtails occurrence on the soil surface (measured by pitfall trapping) in individual years of investigations indicates their peak activity in summer months (July-August) (Fig. 1). A similarly dynamic occurrence of these invertebrates was registered also in previous investigations [36, 37]. Pouring the PDSs to the soil caused a considerable decline in the number of springtails caught using this method (Table 1). Differences in comparison with the control were particularly visible under conditions of soil polluted with engine oil. A decrease in the number

of Collembola caught in the soils polluted with petrol and engine oil was apparent even 12 months after soil contamination. On the other hand, a negative effect of diesel fuel remained even for 2 years after soil pollution. Bioremediation of petrol-polluted soil in the initial period (2010 season) contributed to an increase in springtail activity, and a similar effect was registered also in 2011 for the soil polluted with diesel fuel (Table 1, Fig. 2). On the other hand, regarding the soil polluted with engine oil and control soil, bioremediation limited Collembola activity in 2011 and 2012 seasons. This negative effect was also observed in the second season of investigations (2011) in soil polluted with petrol.

The analysis of the soil samples for Collembola presence (second method) revealed that they were almost absent in the soil polluted with PDSs during the first season of research (2010) (Table 2). Single specimens were registered only 4 months after contamination in the soil polluted with engine oil, but later, until the end of research (i.e., 28 months from the time of pollution) their presence was not spotted even once in the analyzed soil samples. Similarly, in the object where the soil was contaminated with diesel fuel, springtail presence was registered only

Table1. Occurrence of Collembola trapped using pitfall traps in individual months after soil contamination.

Number of months from the moment of soil contamination	Collembola [No. of individuals / trap / month]							
	Control		Petrol		Diesel fuel		Engine oil	
	0R	R	0R	R	0R	R	0R	R
1	43.50 <sup>c*</sup>	41.75 <sup>bc</sup>	15.25 <sup>abc</sup>	21.00 <sup>abc</sup>	21.25 <sup>abc</sup>	13.75 <sup>ab</sup>	13.00 <sup>a</sup>	23.83 <sup>abc</sup>
2	51.58 <sup>ab</sup>	74.42 <sup>b</sup>	36.50 <sup>a</sup>	51.83 <sup>ab</sup>	32.00 <sup>a</sup>	31.42 <sup>a</sup>	41.25 <sup>a</sup>	33.50 <sup>a</sup>
3	117.50 <sup>c</sup>	227.08 <sup>d</sup>	79.00 <sup>abc</sup>	109.00 <sup>bc</sup>	35.75 <sup>a</sup>	63.25 <sup>abc</sup>	42.50 <sup>ab</sup>	64.00 <sup>abc</sup>
4	85.00 <sup>bc</sup>	106.25 <sup>c</sup>	64.75 <sup>ab</sup>	118.25 <sup>c</sup>	43.75 <sup>a</sup>	59.33 <sup>ab</sup>	48.25 <sup>a</sup>	63.75 <sup>ab</sup>
5	54.50 <sup>a</sup>	76.50 <sup>a</sup>	75.25 <sup>a</sup>	68.75 <sup>a</sup>	62.75 <sup>a</sup>	54.00 <sup>a</sup>	63.75 <sup>a</sup>	51.25 <sup>a</sup>
11	243.00 <sup>c</sup>	213.25 <sup>bc</sup>	217.25 <sup>bc</sup>	206.50 <sup>bc</sup>	193.50 <sup>abc</sup>	203.75 <sup>abc</sup>	128.25 <sup>a</sup>	156.00 <sup>ab</sup>
12	231.50 <sup>c</sup>	105.00 <sup>a</sup>	149.75 <sup>b</sup>	99.00 <sup>ab</sup>	117.25 <sup>ab</sup>	159.50 <sup>b</sup>	129.25 <sup>ab</sup>	79.00 <sup>a</sup>
13	347.00 <sup>bc</sup>	215.25 <sup>ab</sup>	352.00 <sup>bc</sup>	261.75 <sup>ab</sup>	115.00 <sup>a</sup>	453.25 <sup>c</sup>	216.25 <sup>ab</sup>	140.50 <sup>a</sup>
14	445.50 <sup>ab</sup>	258.25 <sup>a</sup>	483.00 <sup>ab</sup>	343.00 <sup>a</sup>	327.00 <sup>a</sup>	645.00 <sup>b</sup>	535.74 <sup>ab</sup>	269.00 <sup>a</sup>
15	68.50 <sup>a</sup>	85.67 <sup>a</sup>	102.00 <sup>a</sup>	62.00 <sup>a</sup>	43.75 <sup>a</sup>	111.00 <sup>a</sup>	54.75 <sup>a</sup>	90.50 <sup>a</sup>
16	53.25 <sup>a</sup>	46.50 <sup>a</sup>	94.00 <sup>a</sup>	56.50 <sup>a</sup>	64.25 <sup>a</sup>	84.50 <sup>a</sup>	102.75 <sup>a</sup>	64.00 <sup>a</sup>
23	179.75 <sup>ab</sup>	116.50 <sup>a</sup>	214.75 <sup>b</sup>	166.50 <sup>ab</sup>	140.17 <sup>ab</sup>	191.00 <sup>ab</sup>	206.50 <sup>b</sup>	144.00 <sup>ab</sup>
24	251.50 <sup>b</sup>	162.25 <sup>ab</sup>	138.58 <sup>a</sup>	145.25 <sup>a</sup>	136.00 <sup>a</sup>	151.75 <sup>a</sup>	160.75 <sup>ab</sup>	117.50 <sup>a</sup>
25	192.50 <sup>c</sup>	134.50 <sup>abc</sup>	118.75 <sup>ab</sup>	154.50 <sup>abc</sup>	171.75 <sup>bc</sup>	96.50 <sup>a</sup>	183.33 <sup>bc</sup>	128.92 <sup>abc</sup>
26	145.25 <sup>cd</sup>	107.50 <sup>abc</sup>	97.50 <sup>ab</sup>	96.75 <sup>ab</sup>	137.17 <sup>bcd</sup>	103.00 <sup>ab</sup>	176.25 <sup>d</sup>	76.67 <sup>a</sup>
27	102.00 <sup>cde</sup>	89.92 <sup>a-d</sup>	68.58 <sup>ab</sup>	71.50 <sup>abc</sup>	123.17 <sup>c</sup>	96.33 <sup>b-e</sup>	117.00 <sup>de</sup>	59.25 <sup>a</sup>
28	112.25 <sup>b</sup>	74.42 <sup>a</sup>	80.75 <sup>ab</sup>	89.75 <sup>ab</sup>	85.50 <sup>ab</sup>	68.50 <sup>a</sup>	95.75 <sup>ab</sup>	77.50 <sup>a</sup>

\*Means in lines marked with the same letters do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation. 0R – series without ZB-01, R - series with ZB-01.

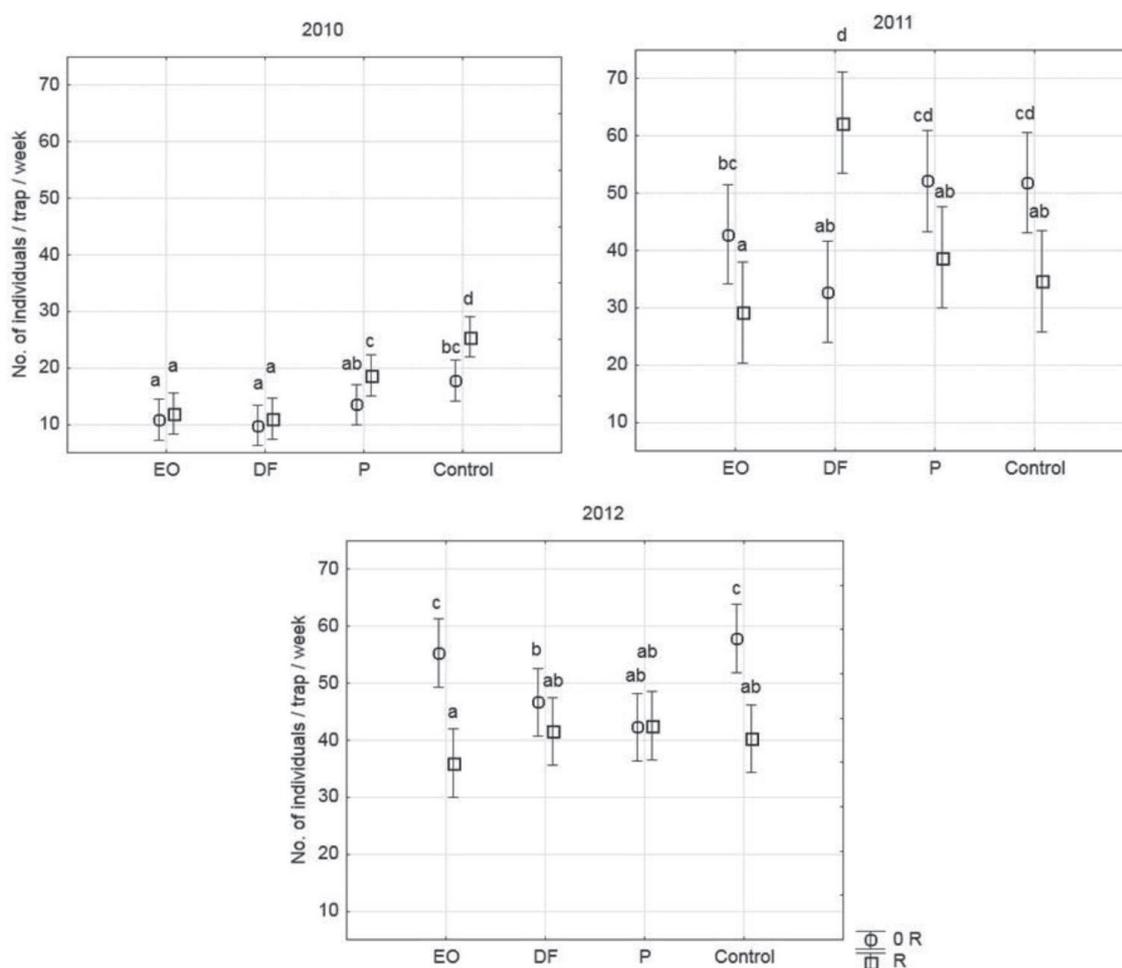


Fig. 2. Occurrence of Collembola trapped using pitfall traps in the years 2010, 2011, and 2012. Explanation as in Fig. 1. Means marked with the same letters do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation.  $\perp$  Mean $\pm$ 0.95 confidence interval.

once – in the 25<sup>th</sup> month after contamination. The earliest appearances of these invertebrates, in similar number as in the control, were spotted in the object with soil contaminated with petrol (11<sup>th</sup> months after contamination). It may be explained by a great number of volatile components in petrol, which quickly evaporated, as demonstrated by Kireeva et al. [24], considerably diminishing the oil derivative negative effect on survival rate and life span of springtails. A positive effect of artificially initiated bioremediation on the soil polluted with petrol was registered already in the second month after contamination – the first live specimens were noticed in the object. Regarding the soil polluted with diesel fuel, the application of ZB-01 preparation became visible about the 12<sup>th</sup> month after contamination, leading to further constant registration of Collembola presence until the end of the experiment, wherein in the 2012 season the number of Collembola registered in this object was similar to their number in the unpolluted soil (Fig. 3a). In the third season of research, also a beneficial effect of bioremediation was noticed in soil contaminated with engine oil, although the differences in the number of registered springtails

in comparison with the object without bioremediation were not statistically proven.

Springtails occurrence was investigated depending on the degree of soil contamination with polycyclic aromatic hydrocarbons (PAHs), whose source was pollution with creosote for 50 years [27] or industry [26], however obtained results point to diversified responses. In soils containing low amounts of PAHs (5.28-80.46 mg kg<sup>-1</sup>) positive associations of Collembola presence with PAH concentrations were registered [26], but no direct association with PAHs originating from creosote (where they occurred in a wide range from 5 to 37 500 ppm) was noted [27]. In the latter case the authors as a more important indicate indirect influence of altering habitat of microinvertebrates and their prey, fungi and bacteria, than direct toxicity. Although these studies (similar to ours) took into account the long-term effects of soil contamination with PAHs, they were only carried out during a small period of time, i.e., within one season of 50 years after contamination. Also, research conducted by Santarufu et al. [22] points to a considerable Collembola sensitivity to changes of soil properties. While researching the effect of heavy metals,

Table 2. Occurrence of Collembola in soil samples in individual months after soil contamination.

Number of months from the moment of soil contamination	Collembola [No. of individuals / sample]							
	Control		Petrol		Diesel fuel		Engine oil	
	0R	R	0R	R	0R	R	0R	R
1	2.00 <sup>b*</sup>	1.25 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
2	0.50 <sup>a</sup>	1.75 <sup>b</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
3	2.75 <sup>b</sup>	3.00 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
4	0.25 <sup>ab</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>a</sup>
11	1.25 <sup>bc</sup>	1.50 <sup>c</sup>	1.50 <sup>c</sup>	0.50 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
12	4.00 <sup>bc</sup>	3.50 <sup>abc</sup>	2.25 <sup>ab</sup>	6.00 <sup>c</sup>	0.00 <sup>a</sup>	1.50 <sup>ab</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>
13	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.75 <sup>ab</sup>	1.50 <sup>b</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
14	0.25 <sup>ab</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.25 <sup>ab</sup>
15	0.25 <sup>ab</sup>	0.50 <sup>ab</sup>	1.25 <sup>b</sup>	0.25 <sup>ab</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>a</sup>	0.25 <sup>ab</sup>
16	0.25 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
23	0.50 <sup>ab</sup>	1.25 <sup>b</sup>	0.75 <sup>ab</sup>	0.25 <sup>ab</sup>	0.00 <sup>a</sup>	1.25 <sup>b</sup>	0.00 <sup>a</sup>	0.25 <sup>ab</sup>
24	0.50 <sup>ab</sup>	1.75 <sup>c</sup>	0.00 <sup>a</sup>	1.50 <sup>bc</sup>	0.00 <sup>a</sup>	1.00 <sup>abc</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>
25	0.75 <sup>ab</sup>	0.25 <sup>ab</sup>	1.00 <sup>b</sup>	0.50 <sup>ab</sup>	0.25 <sup>ab</sup>	0.25 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
26	0.00 <sup>a</sup>	0.25 <sup>ab</sup>	0.25 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.75 <sup>b</sup>	0.00 <sup>a</sup>	0.25 <sup>ab</sup>
27	0.25 <sup>a</sup>	0.25 <sup>a</sup>	0.25 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>
28	0.00 <sup>a</sup>	1.00 <sup>a</sup>	0.50 <sup>a</sup>	1.00 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>

\*Means in lines marked with the same letters do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation. 0R – series without ZB-01, R - series with ZB-01.

pesticides and fuels on Collembola populations in soil, Antonioli et al. [38] found in laboratory conditions that in the presence of fuels (burned lubricating oil – 5, 50 and 100 mL kg<sup>-1</sup> soil and diesel oil – 5, 50 and 100 mL kg<sup>-1</sup> soil) these organisms presented an incapacity to procreate regardless of the dose applied to the soil. The presence of burned oil and diesel oil in the soil inhibited Collembolan development in the soil. During our research, starting with the moment of soil pollution and continuing for 28 months in field conditions, considerable differences in the Collembola number trapped using pitfall traps and spotted in the soil samples were registered. A clearer response to applied toxic substances was obtained through an analysis of springtails occurrence in the soil samples, which indicates the usefulness of this method in particular to assess the quality of soil contaminated with PDSs. The effect measured using pitfall traps was apparent mainly at the initial period after soil pollution, which might have resulted from a toxic effect of applied PDSs on vegetal cover. Springtails are particularly sensitive to soil shadowing, showing strong dependence on the presence and density of vegetation [39, 40].

A positive effect of applied ZB-01 preparation on accelerating bioremediation process in the polluted soil was registered, visible as a considerable fastening

of recolonization by springtails of the soil polluted by petrol or diesel fuel. Similarly, in previous investigations the same preparation exhibited a favorable effect on entomopathogenic nematodes occurrence [41]. On objects where biopreparation was applied, entomopathogenic nematodes were isolated significantly faster after initial contamination than on objects where only a natural remediation process was carried out. Only few researchers focused on the influence of supported bioremediation on soil fauna. Investigations carried out so far have covered the effect of these measurements on life parameters of springtails, such as survival rate or life span [24, 28] and did not include the occurrence of these insects in bioremediated soil in field conditions. For instance, the application of Basispecin preparation containing *Bacillus* sp. 739 strain to soil to various degrees (0, 1, 2, 5 and 10% of soil weight) polluted with Tyumen oil visibly affected springtail survival rate [24]. Already after 3 days of incubation at low oil concentrations, the survival rate of springtails in the object where Basispecin was added was higher than in the untreated soil. At higher oil concentrations in soil, the Basispecin effect was visible only after 180-day incubation, causing increased survival rate and life span of springtails. At the same time, this preparation effect

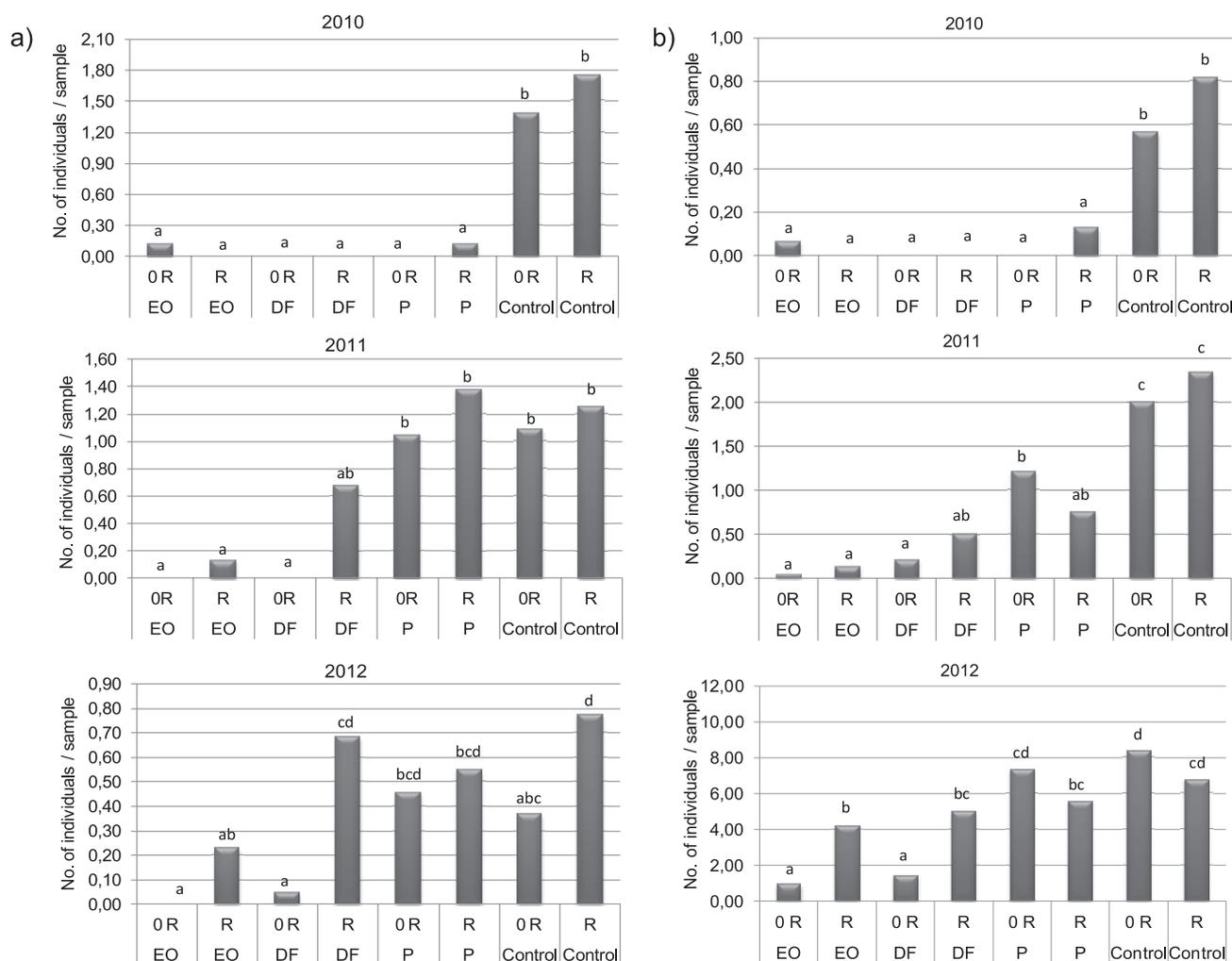


Fig. 3. Occurrence of Collembola a) and Acarina b) in soil samples mean in the years 2010, 2011, and 2012. Explanations as in Fig. 1. Means marked with the same letters do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation.

of oil content being reduced in oil-polluted soils was demonstrated through normalization of enzymatic and microbiological parameters of the soil. In this case the biopreparation supplement caused a decrease in residual oil component by 25-40%. In our investigation inoculation with ZB-01 biocenosis allowed for the degradation of PDSs present in the soil contaminated with diesel fuel and engine oil with 82.3% and 75.4% efficiency, respectively, (after 24 months) [6]. Covino et al. [28] also found that after a 60 d mycoaugmentation with *Pseudoallescheria* sp. of the clay soil historically contaminated by mid- and long-chain aliphatic hydrocarbons (AH), a 79.7% AH reduction and a significant detoxification, inferred by a drop in mortality of *Folsomia candida* springtail from 90 to 24%, were observed. Although the measurement of the survival and fertility of springtails is a commonly used ecotoxicological test, an analysis of the occurrence of these invertebrates in soil under natural conditions can reveal the impact of pollution more comprehensively, i.e., on the animal's habitat and their behavior strategy.

Soil pollution with oil derivatives led to a total destruction of mites in the soil samples (Table 3). Their presence was not observed for the first three months following soil contamination in any of the polluted objects. Live specimens were noticed for the first time in soil polluted with engine oil in the 4<sup>th</sup> month after pollution. However, for most of the second growing season their presence was not found in polluted soil. In the soil contaminated with diesel fuel and petrol their presence was spotted only in the subsequent vegetation season, i.e., in the 11<sup>th</sup> month after pollution. The population of Acarina soil started to reconstruct visibly in the soil polluted with petrol, although they reached a number similar to the control soil only in 2012 (Fig. 3b). The bioremediation process started on petrol-polluted soil caused live Acarina specimens to be spotted there already in the 3<sup>rd</sup> month after pollution. On the other hand, in the soil polluted with diesel fuel, the process caused the number of Acarina observed in the soil samples in the 2012 season to be on a similar level as in the control soil. In the same season the number of the above-mentioned invertebrates increased considerably

Table 3. Occurrence of Acarina in soil samples in individual months after soil contamination.

Number of months from the moment of soil contamination	Acarina [No. of individuals / sample]							
	Control		Petrol		Diesel fuel		Engine oil	
	0R	R	0R	R	0R	R	0R	R
1	0.75 <sup>b*</sup>	0.75 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
2	0.50 <sup>a</sup>	1.50 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
3	1.00 <sup>b</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
4	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>
11	1.25 <sup>bc</sup>	1.75 <sup>c</sup>	1.00 <sup>abc</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
12	5.00 <sup>d</sup>	4.00 <sup>cd</sup>	2.75 <sup>bcd</sup>	2.25 <sup>abc</sup>	0.00 <sup>a</sup>	1.00 <sup>ab</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>
13	2.50 <sup>b</sup>	3.25 <sup>b</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.25 <sup>a</sup>	1.00 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>
14	1.75 <sup>c</sup>	1.25 <sup>bc</sup>	0.25 <sup>ab</sup>	0.75 <sup>abc</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
15	1.00 <sup>a</sup>	3.50 <sup>b</sup>	2.75 <sup>b</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>
16	0.50 <sup>a</sup>	0.25 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
23	3.50 <sup>a</sup>	1.75 <sup>a</sup>	2.00 <sup>a</sup>	3.00 <sup>a</sup>	0.50 <sup>a</sup>	3.75 <sup>a</sup>	1.75 <sup>a</sup>	1.25 <sup>a</sup>
24	2.00 <sup>abc</sup>	4.25 <sup>d</sup>	2.50 <sup>bcd</sup>	3.50 <sup>cd</sup>	0.50 <sup>ab</sup>	2.75 <sup>cd</sup>	0.00 <sup>a</sup>	2.25 <sup>bcd</sup>
25	2.75 <sup>b</sup>	2.00 <sup>ab</sup>	1.75 <sup>ab</sup>	0.25 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>
26	2.25 <sup>c</sup>	0.50 <sup>ab</sup>	0.50 <sup>ab</sup>	1.25 <sup>bc</sup>	0.25 <sup>ab</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.75 <sup>ab</sup>
27	0.25 <sup>a</sup>	0.50 <sup>a</sup>	1.50 <sup>b</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.75 <sup>ab</sup>
28	0.50 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>

\*Means in lines marked with the same letters do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation. 0R – series without ZB-01, R - series with ZB-01.

also in the object contaminated with engine oil after bioremediation process application (although the differences were not statistically proven).

Research of Blakely et al. [27] on the use of soil invertebrates, including Acarina as indicators of soil pollution with PAHs, revealed a reduction in the abundance of total Acarina with PAH contamination and confirmed the previous results obtained by Erstfeld and Snow-Ashbrook [26]. The authors gave two possible explanations of the observed phenomenon, i.e., pointing to a fungicidal role of PAHs which may lead to elimination of microarthropod's fungal or detrital food, or to increase in bulk density due to PAH contamination,

which in turn leads to a reduction of habitat space for these animals. Also, research by Garcia-Segura et al. [42] showed that Acari were the groups that were most negatively correlated to the different hydrocarbons in soil contaminated by oil spills in Mexico.

In summary, our long-term research indicates that both Collembola and Acarina are good indicators of the speed of PDS-contaminated soil self-cleaning. However, taking soil samples and their analysis in the laboratory gives a more reliable answer than the use of pitfall traps. It is worth noting that the analysis of the occurrence of soil fauna in the soil samples is a very cheap and easy to carry out method, which does not require specialized

Table 4. Mean of the Acarina and Collembola ratios (A/C) in soil samples in individual years after soil contamination.

Year	A/C							
	Control		Petrol		Diesel fuel		Engine oil	
	0R*	R	0R	R	0R	R	0R	R
2010	0.41	0.46	-	1.00	-	-	0.5	-
2011	1.85	1.87	1.16	0.55	-	0.75	-	1
2012	5.50	2.12	3.33	2.83	5.00	1.87	-	4.2

\*0R – series without ZB-01, R – series with ZB-01.

equipment or labor-intensive rearing. It may be a useful alternative for other ecotoxicological tests in assessing PDS presence in the soil and speed of remediation process.

The A/C ratio differed between the investigated seasons, also on the control soil. However, in the final 2012 season it revealed higher values in the objects without supported bioremediation than in the objects where the treatment was applied (Table 4). The exception was the object with the soil polluted with engine oil and subjected to bioremediation process, where the indicator was similar to control without bioremediation. The high value of the Acarina/Collembola ratio suggests high soil quality, which is associated with a decrease in Acarina species number in degraded soils. However, research conducted by Santarufo et al. [22] on soil fauna occurrence in five urban soil in Naples, Italy revealed the highest A/C ratio value in the soil characterized by the highest content of heavy metals, such as Pb, Cu and Zn, which confirms previous findings of Jacomini et al. [29] showing that the indicator cannot be used in all ecosystems. The results of our research also indicate that the use of the A/C indicator is not very suitable for assessing the condition of soil contaminated with PDSs and the speed of the self-cleaning process.

### Conclusions

1. Considering the investigated PDSs, petrol revealed the shortest (about a year) negative effect on Collembola presence on the soil surface (measured by pitfall trapping), whereas it was visible for the longest time (2 years) for diesel fuel. Initiating a bioremediation process using ZB-01 preparation on the soil polluted with petrol (in the first year) and diesel fuel (in the second), resulting in a significant increase in Collembola activity on the soil surface; however, it limited this parameter in soil contaminated with engine oil and in the control soil (in the second and third years of the experiment).
2. Both diesel fuel and engine oil revealed a strong negative effect on the occurrence of springtails and mites in soil (measured by soil sample analysis), perceptible even after 28 months from the moment of soil pollution. At the same time, a visible positive response to applied bioremediation supported by biopreparation was registered.
3. Analysis of soil samples polluted with oil derivatives for springtail and mite presence may be a potentially useful indicator for assessing the degree of soil self-cleaning, since a perceptible response was noticed – dependent both on the oil derivative and the course on the supported bioremediation process.

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### Conflict of Interest

The authors declare no conflict of interest.

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