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EFFECT OF BIOREMEDIATION OF OIL DERIVATIVES IN SOIL ON *Pterostichus* sp. (COLEOPTERA, CARABIDAE) OCCURRENCE

WPŁYW BIOREMEDIACJI ROPOPOCHODNYCH W GLEBIE NA WYSTĘPOWANIE *Pterostichus* sp. (COLEOPTERA, CARABIDAE)

Abstract: The research aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of *Pterostichus* sp. (Coleoptera, Carabidae) occurrence. The following objects were established in two series (natural and supported bioremediation): control – unpolluted soil; soil polluted with petrol; soil polluted with diesel fuel and soil polluted with used engine oil (dose: 6000 mg of fuel · kg^{-1}d.m. of soil). Epigeal fauna was trapped using Barber's traps. During the periods from June to October 2010, from May to October 2011 and in May and June 2012 the traps were emptied once a week.

Soil pollution with petrol inhibits *Pterostichus* sp. beetles activity for about 3 months, whereas diesel oil may reveal a negative effect even after 14 months, and engine oil after 13 months from the moment of pollution. Bioremediation process of soil contaminated with oil derivatives generally does not affect *Pterostichus* beetles during the first 5 months after its initiation, but after a year it may contribute to increased activity under conditions of soil polluted with diesel oil. The COD data indicate that the process of bioremediation occurs in all of the tested soil samples. It was the most intensively in the case of soil polluted with used engine oil.

Keywords: oil derivatives, soil, bioremediation, Carabidae, *Pterostichus* sp.

Oil derivatives belong to widespread soil pollutants. Their source may be leaks from storage containers, pipeline damage, road accidents, mechanization of agriculture,

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industries, etc. [1]. Moreover, for many years petroleum sprays were used as insecticides [2].

The effect of oil derivatives on invertebrates depends on pollutant concentration, their kind, the time during which animals are exposed to them and on animal species [3, 4]. Investigations conducted so far revealed that oil derivatives in soil negatively affect the representatives of epigeal fauna, including carabid beetles. Their considerably limited presence was observed in polluted areas [5–8] whereas the time of negative influence depended on the pollutant.

Carabid beetles of *Pterostichus* sp. genus are counted among the most common species, both on arable fields and in forests. They are typical predators, although their occasional uptake of plant feed was also reported [9]. Research on pollutant influence of these invertebrates focuses mainly on heavy metal effect. They reveal that detoxification methods used by *Pterostichus* sp. beetles allow to limit heavy metal accumulation [10, 11], however some metals, *eg* copper depressed locomotor activity, which may reduce the fitness of the animal under field conditions. Investigations on the effect of petrol, diesel fuel and engine oil on life and biochemical parameters of *Pterostichus cupreus* L. species demonstrated declined survivability at the exposure to diesel fuel and changes in activity of some detoxifying enzymes under the influence of diesel and engine oil [12]. Microbial biopreparations used to support natural process of oil derivative pollutants degrading may affect the subsequent links of trophic chain through changes of bacterial flora and formation of short chained intermediates in result of their activity. Changes in number of some arachnid groups were registered after their application [13, 14].

The work aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of *Pterostichus* sp. (Coleoptera, Carabidae) occurrence.

Material and methods

The investigations were conducted in 2009–2012 at the Experimental Station of the University of Agriculture in Mydlniki near Krakow. In autumn 2009 indigenous soil was placed in 1 m^3 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. The soil in containers was left untouched for 8 months to allow it to restore its natural biological efficiency. Subsequently, in June 2010 the soil in containers was polluted with the following oil derivatives: petrol, diesel oil and used engine oil in the amounts equal to 6000 mg of fuel $\cdot$ kg^{-1} d.m. of soil. A week later half of the containers was subjected to bioremediation with the use of ZB-01 biopreparation, specially prepared for this purpose. Four objects were identified in each of the two series (natural and supported bioremediation): 1. Control – unpolluted soil, 2. Soil with simulated petrol leak, 3. Soil with simulated diesel oil leak and 4. Soil with simulated used engine oil leak. The whole experiment was conducted in 4 replications according to randomized block design. Epigeal fauna was trapped using Barber's traps (0.9 dm^3 jars dug even with the soil level and protected against atmospheric

precipitation with a plastic roof) placed in the central point of each container. During the periods from June to October 2010, from May to October 2011 and in May and June 2012 the traps were emptied once a week. Trapped carabid beetles were classified using appropriate keys [15].

In order to monitor total pollutant load, measurements of *chemical oxygen demand* (COD) were made in samples according to Polish Standard [Polska Norma PN-74 C-04578/03]. For this reason the soil was dried until constant weight at room temperature. Air-dried soil was averaged and rubbed through a sieve with 0.1 mm mesh. Subsequently an appropriately selected soil weighed portion was suspended in distilled water. The assessments were made using cuvette tests LCK-ChZT (with measuring range 1000–60000 mg/dm^3) made by Hach-Lange. The required sample amount (from 0.2–0.5 cm^{3}of sample depending on measuring range of applied cuvette tests) with suspended soil was removed to measuring vial, which was then placed in Hach-Lange mineralizer for mineralization (at 148 ^{o}C for 2 hours). In order to inhibit oxidation reaction, the cuvettes were cooled under cold running water. Subsequently, COD measurement was conducted by means of DR 5000 Spectrophotometer (Hach-Lange). The final results were given in mg of oxygen per 1 kg of air-dried soil.

Statistical computations concerning *Pterostichus* sp. beetles occurrence in the subsequent months from the moment of soil pollution were made using Statistica 9.0 PL computer programme. Means were diversified using LSD Fisher test at significance level $\alpha = 0.05$.

Results and discussion

Pterostichus sp. beetles revealed the highest activity in August (Fig. 1 and 2). At that time, on average even 6 specimens per week were trapped into one trap.

In the 2010 season, *ie* after 0 to 5 months from the moment of soil pollution, beetles were more numerously trapped under conditions of control soil (Fig. 1). At the same time a negative effect of applied supported bioremediation on their activity under conditions of unpolluted soil was visible. The applied ZB-01 biopreparation includes the nutrients and substrate for microorganisms. The relatively low concentration of petroleum as a substrate can influence the activities of *Pterostichus* sp. in terms of their settle in unpolluted control soil. As can be seen in Table 1, the above-mentioned effect is noticeable up to four months of the experiment's duration. After that time the influence of the substrate disappears and the soil regains the properties as a control one (0R). Soil contamination with petrol and diesel fuel contributed to limiting *Pterostichus* sp. beetles activity for 3 months, whereas in case of engine oil a marked decline in the number of trapped beetles was noted in the second and third month. In the last above-mentioned object applied ZB preparation reduced the activity of investigated animals in the second month after its application, whereas in the other objects with contaminated soil no significant effect of supported bioremediation was observed. Beetles' activity in the 4th and 5th month (*ie* September and October) was slight and therefore no marked influence of the applied oil derivatives or applied bioremediation

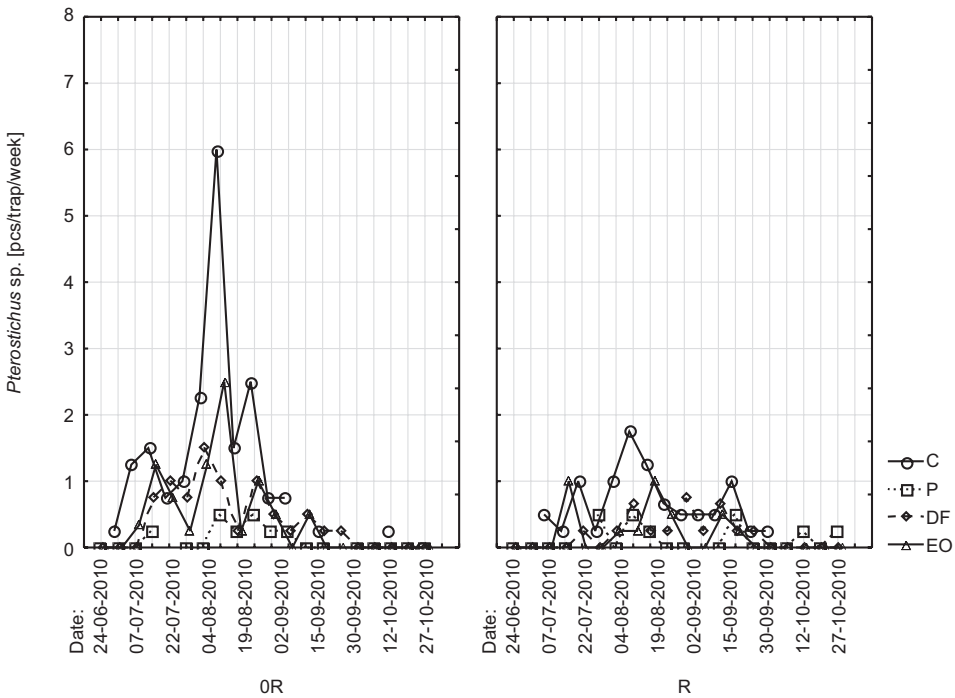

Fig. 1. Course of dynamics of *Pterostichus* sp. occurrence trapped using Barber's traps in 2010. EO – soil contaminated with used engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – unpolluted soil, 0R – series with natural bioremediation, R – series with supported bioremediation

was observed. In previous research using oil derivatives dose of 2 dm^3/m^2 *Pt. vulgaris* species reduced its activity in the area polluted with petrol and diesel fuel for one month, whereas in the area polluted with engine oil in one of two years of investigations, the first beetles were trapped only after 3 months [8]. Laboratory analyses of the oil derivative effect on life and biochemical parameters of *Pt. cupreus* beetles using the soil from the presented experiment, collected in August 2010 (3^{rd} month from the moment of soil pollution) revealed that diesel oil contributed to lowering their survivability by 31 % in comparison with the control, soil contamination with engine oil proved less toxic (6 % of the insects died), whereas petrol had no effect on this beetle survival. The investigated substances did not have any influence on beetle body weight, but they caused a decline in the activity of microsomal enzyme ECOD (*Ethoxycoumarin-O-deethylase*). Moreover, in comparison with control data, a decrease in superoxide dismutase and HSP70 proteins level in beetles exposed to used engine oil and increase in glutathione transferase activity in beetles exposed to diesel oil were noticed [12].

In the 2011 season, *ie* after 11–16 months from the moment of soil pollution, the dynamics of *Pterostichus* sp. beetles occurrence pointed to consistent negative influence of soil pollution with diesel and engine oil, whereas the number of beetles trapped under

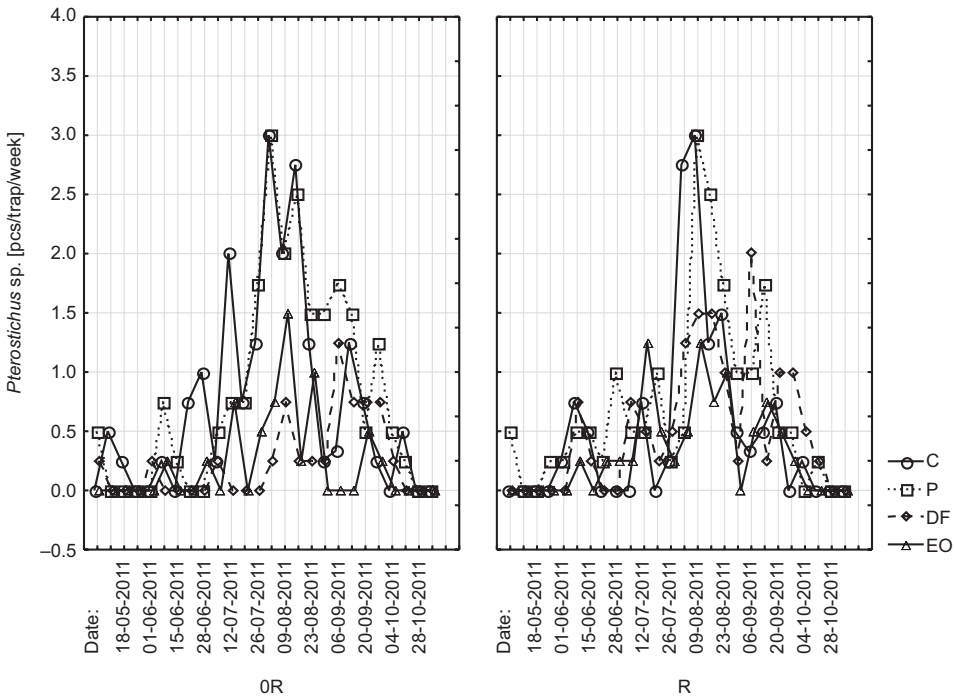

Fig. 2. Course of dynamics of *Pterostichus* sp. occurrence trapped using Barber's traps in 2011. The symbols as in Fig. 1

conditions of petrol polluted soil was similar as in the control (Fig. 2). A significant decrease in the number of trapped beetles was registered under conditions of soil contaminated with diesel oil in the months from 12 to 14 and in engine oil polluted soil in the 13th month (*ie* during the period of their highest activity) (Table 1). Starting from the 12th month a beneficial effect of supported bioremediation applied on the soil polluted with diesel oil towards the number of trapped beetles was visible. At the same time slightly more of the investigated animals were trapped also under conditions of soil contaminated with engine oil and inoculated with ZB-01 preparation as compared with the same object where the treatment was not applied.

In the 2012 season insects were trapped in May and June (23rd and 24th month since the moment of the soil contamination). Carabid activity at that time was slight (Fig. 3). In most cases no significant differences were observed between studied objects depending on the kind of pollution, or applied supported bioremediation. Only in the object where the soil was polluted with diesel fuel, more trapped beetles were noted than under conditions of unpolluted soil.

Previous investigations revealed that in the area contaminated with crude oil in result of a road accident and subjected to bioremediation, even after 3 years from the moment of pollution occasionally lower number of trapped *Pterostichus* sp. beetles was noted than on the control [16].

Table 1

Occurrence of *Pterostichus* sp. trapped using Barber's traps in individual months after soil contamination. The symbols as in Fig. 1

Number of months from the moment of soil contamination	*Pterostichus* sp. [pcs/trap/month]							
	Control		Petrol		Diesel fuel		Engine Oil	
	0R	R	0R	R	0R	R	0R	R
1	3.00 c*	0.75 ab	0.25 ab	0.00 a	0.75 ab	0.00 a	1.58 bc	1.00 ab
2	9.08 d	4.00 bc	0.50 a	1.00 ab	4.25 bc	1.08 ab	4.75 c	0.50 a
3	5.50 c	2.92 b	1.25 ab	0.25 a	2.00 ab	1.50 ab	1.75 ab	1.50 ab
4	0.25 a	2.00 b	0.00 a	0.50 ab	1.00 ab	1.17 ab	0.50 ab	0.75 ab
5	0.25 a	0.00 a	0.00 a	0.50 a	0.00 a	0.00 a	0.00 a	0.00 a
11	0.75 a	0.25 a	0.50 a	1.00 a	0.50 a	0.00 a	0.00 a	0.00 a
12	2.00 b	1.25 ab	1.00 ab	2.25 b	0.00 a	1.00 ab	0.50 ab	0.75 ab
13	4.25 c	1.00 a	3.75 bc	2.25 abc	0.25 a	2.00 ab	1.25 a	2.25 abc
14	9.25 bc	9.00 bc	10.50 c	8.75 bc	1.75 a	5.50 abc	3.50 ab	3.50 ab
15	2.58 abc	1.58 ab	5.00 c	3.75 abc	3.50 abc	4.25 bc	0.75 a	2.00 abc
16	0.50 a	0.25 a	0.75 a	0.25 a	0.25 a	0.75 a	0.00 a	0.00 a
23	0.50 a	0.50 a	1.25 ab	1.00 ab	1.92 b	1.50 ab	0.25 a	0.75 ab
24	0.75 a	3.25 a	2.50 a	2.25 a	2.25 a	1.50 a	1.50 a	2.75 a

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

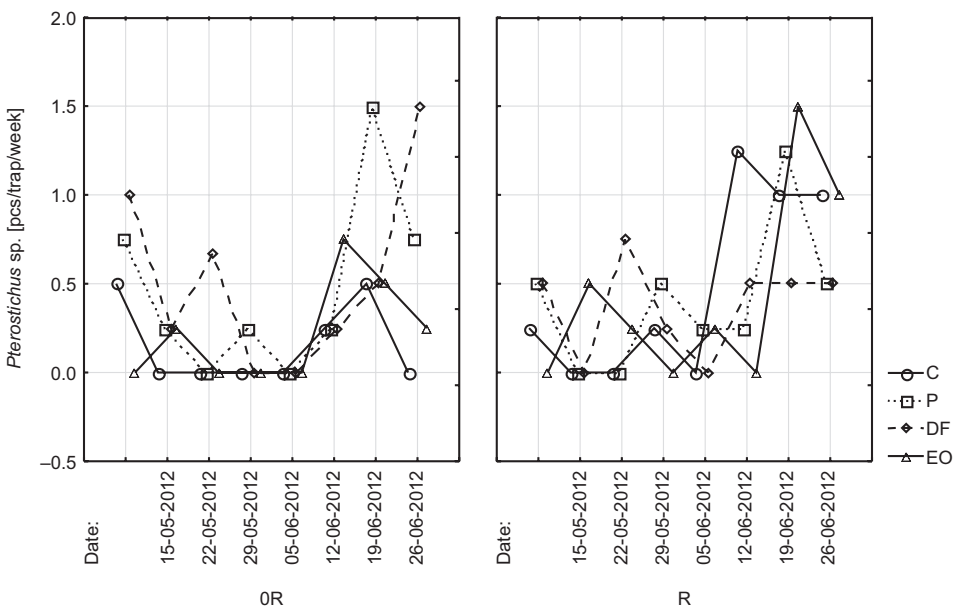

Fig. 3. Course of dynamics of *Pterostichus* sp. occurrence trapped using Barber's traps in 2011. The symbols as in Fig. 1

Figure 4 shows changes of COD parameter in the soil contaminated with used engine oil, diesel oil and petrol in comparison with the control sample (unpolluted soil). In case

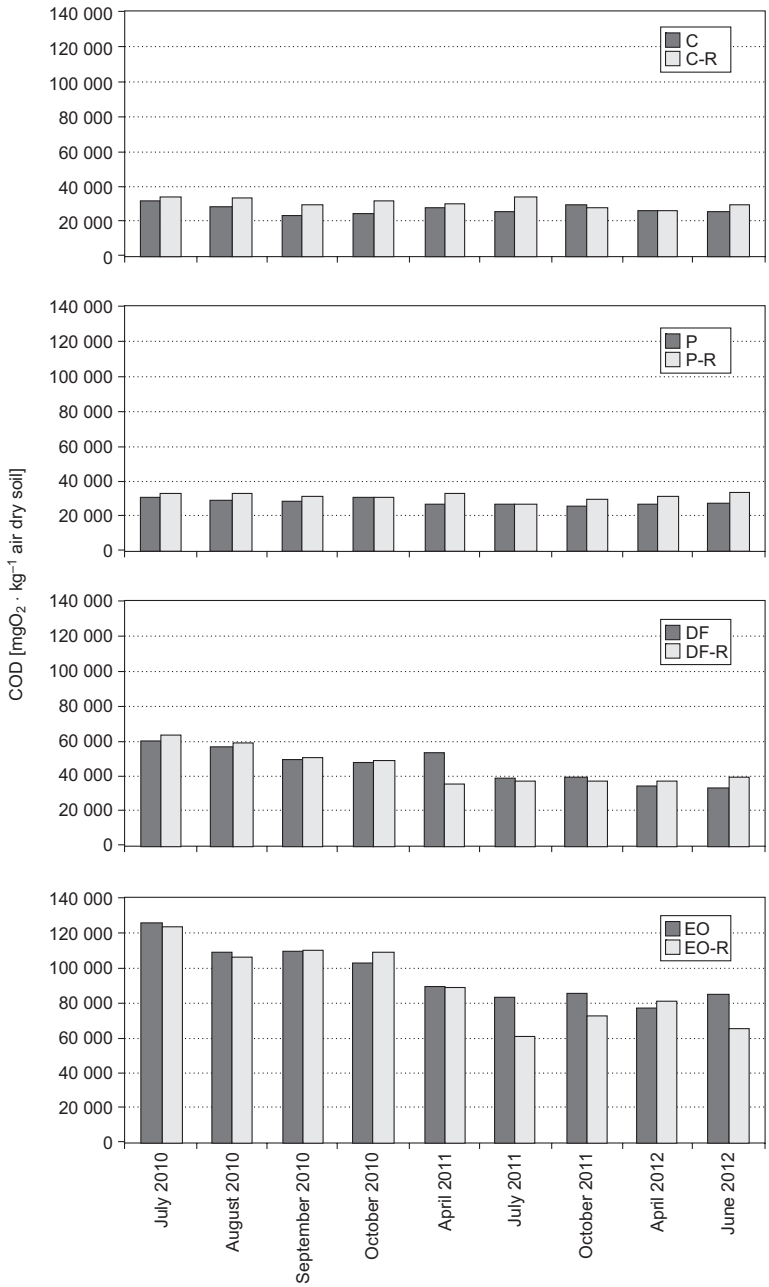

Fig. 4. Dynamics of COD changes during biodegradation process. The symbols as in Fig. 1

of samples of soil contaminated with used engine oil, both subjected to bioremediation supported with ZB-01 preparation and natural bioremediation, the highest initial value of COD parameter was registered on the level of *ca* 126 000 $mgO_2 \cdot kg^{-1}$ of air-dried soil. In course of microbial bio-cleaning process, a dynamic decline of this parameter value to the level of *ca* 85 000 and 65 000 $mgO_2 \cdot kg^{-1}$ of air –dried soil was registered respectively for the soil subjected to natural bioremediation and supported bio-remediation. In the variant with diesel oil, like in the soil contaminated with used engine oil, a dynamic decrease in COD parameter value was noticed (from *ca* 60 000 to 35 000 $mgO_2 \cdot kg^{-1}$ of air-dried soil).

Kinetics of COD value changes deserves attention, since it shows that in case of soil subjected to supported bioremediation process (introducing ZB-10 preparation inoculums), already in the 10^{th} month COD value was significantly lower than COD value for soil sample from natural bioremediation process (without inoculation). It may indirectly point to more efficient biodegradation of oil derivatives in case of the sample treated with ZB-01 biopreparation.

Observation of changes of COD parameter for the soil contaminated with petrol points to its fluctuations on the level of *ca* 30 000 (for not inoculated soil) and 32 000 (for inoculated soil). These changes seem to be random and are not connected with process of oil derivatives degradation.

Conclusions

1. Soil pollution with petrol inhibits *Pterostichus* sp. beetles activity for about 3 months, whereas diesel oil may reveal a negative effect even after 14 months, and engine oil after 13 months from the moment of pollution.

2. Bioremediation process of soil contaminated with oil derivatives generally does not affect *Pterostichus* beetles during the first 5 months after its initiation, but after a year it may contribute to increased activity under conditions of soil polluted with diesel oil.

3. The COD data indicate that the process of bioremediation occurs in all of the tested soil samples. However, in the case of soil contaminated with petrolum and diesel oil the COD measurments provide the information about the orginal content of the organic matter and the one being artificially introduced into soil. The above conclusion has been drawn out on the basis of weight method (data not shown). Only when the soil is contaminated with engine oil, do COD data illustrate the bioremediation process with more oxygen being required for the oxidation rection of organic matter.

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WPŁYW BIOREMEDIACJI ROPOPOCHODNYCH W GLEBIE NA WYSTĘPOWANIE *Pterostichus* sp. (COLEOPTERA, CARABIDAE)

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Abstrakt: Celem pracy było zbadanie oddziaływania substancji ropopochodnych w trakcie procesu bioremediacji gleby na dynamikę występowania chrząszczy z rodzaju *Pterostichus* sp. (Coleoptera, Carabidae).

W ramach doświadczenia utworzono następujące obiekty w dwóch seriach (z naturalną bioremediacją i stymulowaną bioremediacją): 1. Kontrola – gleba niezanieczyszczona. 2. Gleba z symulowanym wyciekiem benzyny, 3. Gleba z symulowanym wyciekiem oleju napędowego, 4. Gleba z symulowanym wyciekiem zużytego oleju silnikowego w dawce 6000 mg substancji ropopochodnej · kg^{-1} s.m. gleby. Odłowy fauny naziemnej prowadzono z wykorzystaniem pułapek Barbera. W okresach od czerwca do października 2010, od maja do października 2011 oraz w maju i czerwcu 2012 r. pułapki opróżniano raz w tygodniu.

Zanieczyszczenie gleby benzyną ogranicza aktywność chrząszczy z rodzaju *Pterostichus* przez okres ok. 3 miesięcy, natomiast olej napędowy może wykazywać negatywny wpływ jeszcze po upływie 14 miesięcy, a silnikowy po upływie 13 miesięcy od momentu zanieczyszczenia. Proces bioremediacji gleby zanieczyszczonej ropopochodnymi na ogół nie wpływa na aktywność biegaczowatych z rodzaju *Pterostichus* w ciągu pierwszych 5 miesięcy po jego zainicjowaniu, natomiast po upływie roku może przyczyniać się do wzrostu aktywności w warunkach gleby zanieczyszczonej olejem napędowym. Analiza parametru ChZT wskazuje, że proces bioremediacji przebiegał we wszystkich próbkach gleby, najintensywniej w przypadku gleby zanieczyszczonej olejem silnikowym.

Słowa kluczowe: ropopochodne, gleba, bioremediacja, Carabidae, *Pterostichus* sp.