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## BIOREMEDIATION OF SOIL POLLUTED WITH OIL DERIVATIVES AND ITS EFFECT ON COLEOPTERA, CARABIDAE OCCURRENCE

### ODDZIAŁYWANIE BIOREMEDIACJI GLEBY ZANIECZYSZCZONEJ ROPOPOCHODNYMI NA WYSTĘPOWANIE BIEGACZOWATYCH (COLEOPTERA, CARABIDAE)

**Abstract:** The investigations were conducted to determine the effect of oil derivatives (petrol, diesel fuel and used engine oil) during the process of their bioremediation on dynamics of Carabidae occurrence. Carabidae activity is to various extent limited in conditions of soil pollution with oil derivatives depending on the kind of applied substance. Petrol reveals negative effect for the shortest period of time (*ca* 3 months), whereas both diesel oil and engine oil act for a much longer period: diesel fuel may have a negative effect for 12 months, whereas engine oil for 15 months from the time of soil pollution. The process of bioremediation of soil polluted with oil derivatives does not influence Carabiade activity during the first five months after its initiation, whereas in the later period (after a year) it may contribute to increased activity of Carabidae in conditions of soil polluted with diesel fuel, at the same time neutralizing the negative effect of the above-mentioned substance.

**Keywords:** oil derivatives, soil, bioremediation, Carabidae

#### Introduction

Carabidae are often mentioned among invertebrates as indicators of soil pollution. Their occurrence was analyzed among others with reference to the efficiency of remediation of soils for many years subjected to anthropogenization [1], as well as on soils polluted with heavy metals [2-4]. The available literature contains little information about the influence of oil derivatives on these organisms. Carabidae were one of invertebrate groups studied from the perspective of domestic sewage effect and oil spills on edaphic invertebrates occurrence on periodically flooded stream banks [5]. Their considerably limited of their number was found in samples from the most polluted area. Carabidae activity in conditions of soils polluted with petrol, diesel fuel or engine oil also became considerably limited [6]. A diversification of response was noted depending on the kind of pollution but also on the beetle species.

The efficiency of microbiological biopreparations application in order to accelerate the process of natural recultivation of the ground polluted with oil derivatives was proved [7]. However, there is still no information how the bioremediation process affects the organisms living on the soil surface.

The work aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of Carabidae occurrence.

#### Materials and methods

Experiment was conducted at the Experimental Station of the University of Agriculture in Krakow situated in Mydlniki near Krakow, Poland. It was conducted in four replications

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and comprised the following objects in two series (with bioremediation and without bioremediation): 1. Control - unpolluted soil (C); 2. Soil artificially polluted with petrol (P); 3. Soil artificially polluted with diesel fuel (DF) and 4. Soil artificially polluted with used engine oil (EO). In autumn 2009, the indigenous soil was placed in cubic containers (1 m<sup>3</sup>) specially adapted for this purpose. The containers were placed into the ground. Doses of 6 000 mg of oil derivative per 1 kg soil d.m. were applied in June 2010 by pouring onto soil in containers. After one week a half of the containers with experimental soil were subjected to bioremediation using a ZB-01 preparation designed for bioremediation of soils polluted with oil derivatives. Detailed description of experiment was presented in other paper [8]. Coleoptera Carabidae were trapped using Barber's traps placed in the central part of each container (a 0.9 dm<sup>3</sup> jar dug in level with the ground surface and covered against rainfall water by a plastic roof). The traps were emptied once a week in growing seasons during two years from the moment of soil contamination (until end of June 2012). Statistical computations were conducted using Statistica 9.0 PL programme. Means were differentiated using LSD Fisher test on significance level  $\alpha = 0.05$ .

## Results and discussion

Dynamics of the number of insect capturings conducted during the period of investigations points to a higher activity of Carabidae during the late summer (August, beginning of September). The number of specimens trapped at that time reached even 20 per trap during a week, whereas in the other period of time it was only several pieces (Figs. 1-3). During capturing conducted in the 2010 season, *ie* during the period from 0 to 5 months from the moment of pollution, a higher number of captured insects might be observed in the unpolluted soil (Fig. 1) almost during the whole time. Dynamics of insect capturing in conditions of polluted soil pointed to particularly negative effect of applied engine and diesel oils. Activity of Carabidae in conditions of soil subjected to bioremediation and in non-remediated soil was similar. Statistical analysis conducted in subsequent months after soil pollution in 2010 revealed significantly less Carabidae trapped in conditions of soil polluted with petrol for the first three months and in diesel oil contaminated soil for 4 months (Table 1) in comparison with unpolluted soil. In soil polluted with engine oil, significant differences were registered in the months from the second to the fourth. In the fifth month from the moment of pollution, *ie* in October, very low activity of Carabidae was observed and therefore no significant differences were noted between studied objects. In none of the above-mentioned months was any marked effect of applied bioremediation noted on the number of trapped Carabidae. The dependencies described above were also reflected in the average per season number of captured Carabidae (Fig. 4).

In the 2011 season, *ie* between 11 and 16 months from the moment of soil pollution, the dynamics of Carabidae activity in the individual objects was different in comparison with the previous year (Fig. 2). The main difference concerned the object where the soil was polluted with petrol. In this case for the whole season the number of captured insects was similar to unpolluted soil. Also an increase in the number of trapped insects was observed in conditions of soil polluted with diesel oil and subjected to remediation. Statistical analysis of the number of trapped insects in the individual months of 2011 revealed a lack of significant effect of soil pollution with petrol (Table 1). A negative effect

of diesel fuel was statistically proved still in the 11<sup>th</sup> and 12<sup>th</sup> month after the soil contamination. In case of engine oil a significant, in comparison with the control, decline in the number of captured insects was noted in the 11<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> month from the moment of soil contamination, however much fewer of these invertebrates were trapped also in the other months of 2011. Applied bioremediation of the petrol soil polluted contributed to a decrease in the number of Carabidae trapped in some months (11<sup>th</sup> and 15<sup>th</sup>), but only to the level similar as in conditions of unpolluted soil. During whole period of the insect capturing conducted in the 2011 season, more numerous Carabidae were noted in the traps placed in the soil contaminated with diesel fuel and subjected to bioremediation than in the soil where this measure was not applied, therefore the analysis of the data for the whole 2011 season revealed a marked positive effect of this measure (Fig. 4). On the other hand no significant effect of bioremediation on Carabidae activity was noticed in conditions of soil polluted with engine oil.

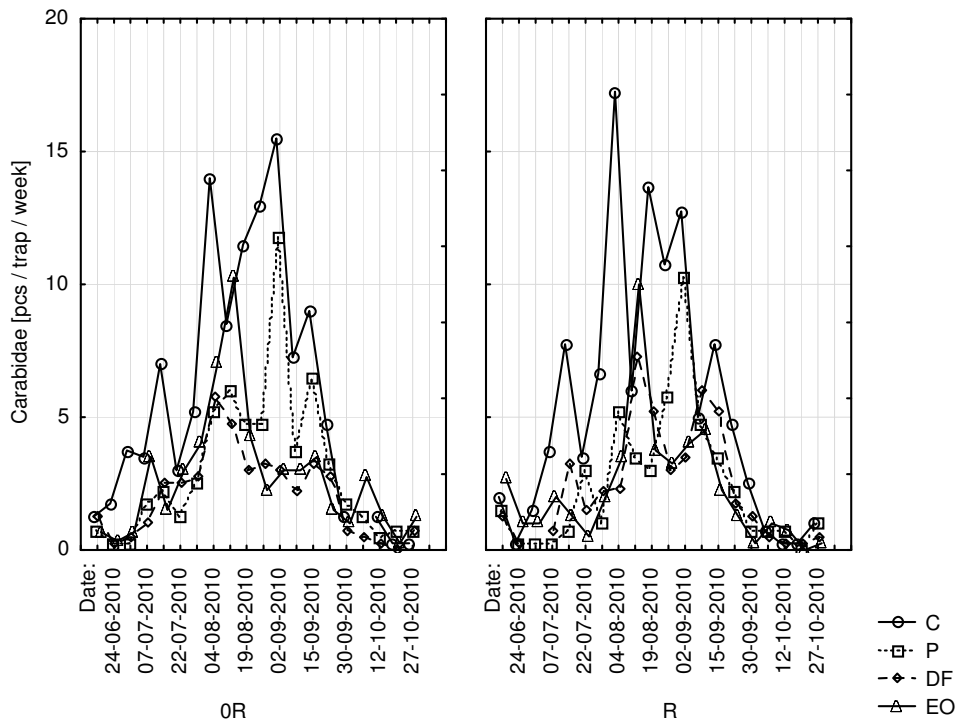


Fig. 1. Course of dynamics of Carabidae occurrence trapped using Barber's traps in 2010. EO - soil contaminated with used engine oil, DF - soil contaminated with diesel fuel, P - soil contaminated with petrol, C - unpolluted soil, OR - series without bioremediation, R - series with bioremediation

In the 2012 season the insects were captured in May and June (*ie* in the 23<sup>rd</sup> and 24<sup>th</sup> month after the moment of soil contamination). Activity of Carabidae in this period was

low (Fig. 3). No marked differences were registered among the studied objects dependent on the kind of pollution or applied bioremediation.

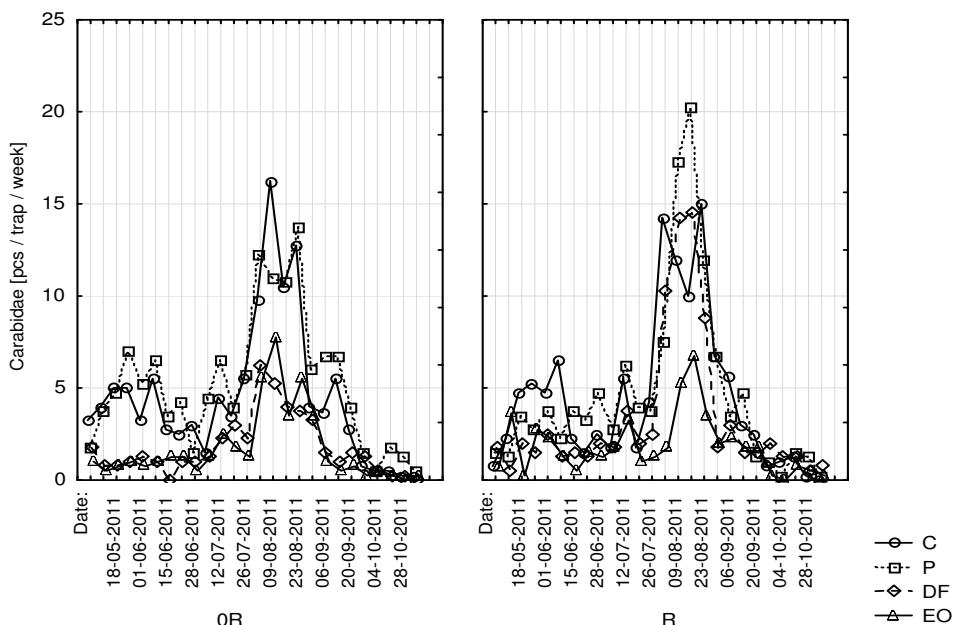


Fig. 2. Course of dynamics of Carabidae occurrence trapped using Barber's traps in 2011. The symbols as in Figure 1

In an extensive literature on the effect of oil spills on aquatic invertebrates diversified responses of the animals are emphasized, which depend on their taxonomic position, way of feeding, age, *etc.* [9, 10]. Despite the fact that terrestrial arthropods are regarded as efficiently metabolizing harmful components of oil derivatives, among others PAHs [11], still it does not denote that this type of pollution has no effect on their occurrence. While analyzing occurrence of invertebrates in soil samples from the banks of seasonally flooding streams and to various extent exposed to pollution, Couceiro et al [5] found even 7 times fewer representatives of Carabidae in the samples from the banks of a stream exposed to pollution due to oil spills in comparison with the stream not prone to pollution, whereas in the samples from the area close to a stream exposed to pollution with domestic sewage they were about twice less numerous. Using of pitfall traps is regarded and commonly reported as a good indicator of terrestrial invertebrate activity [1, 12]. In previous investigations using them, at about thrice lower dose of applied oil derivatives, a decrease in the number of captured Carabidae was observed for a period of 4 months from the moment of soil contamination. Like in the presented research, Carabidae avoided contact with petrol polluted soil for the shortest period of time [6]. On the other hand, 2 and 3 years after the moment of soil pollution with oil derivatives in result of a road accident, no negative effect of these substances on general activity of Carabidae was registered [13].

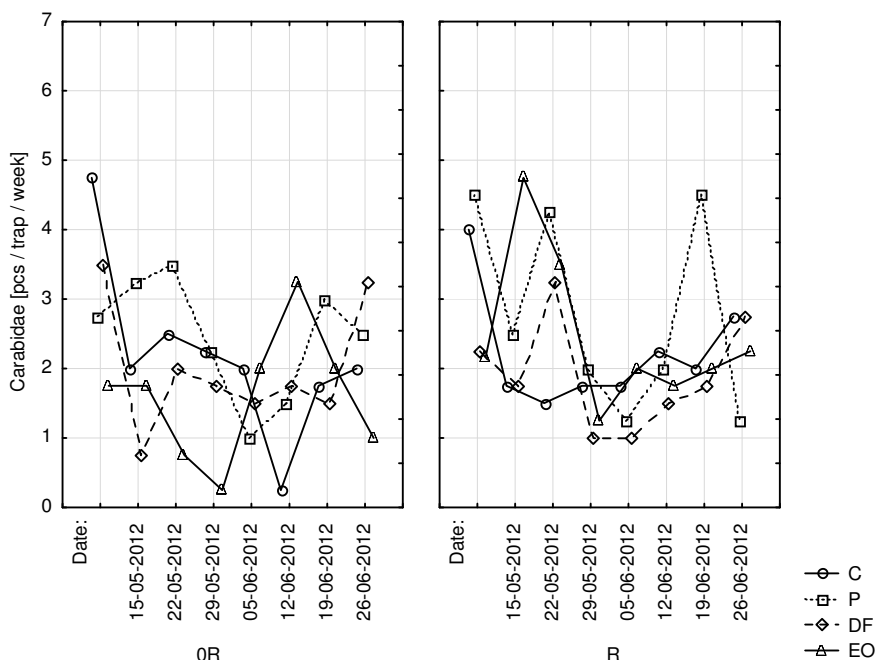


Fig. 3. Course of dynamics of Carabidae occurrence trapped using Barber's traps in 2011. The symbols as in Figure 1

Table 1  
Occurrence of Carabidae trapped using Barber's traps in individual months after soil contamination.  
The symbols as in Figure 1

Number of months from the moment of soil contamination	Carabidae [pcs/trap/month]							
	Control		Petrol		Diesel fuel		Engine oil	
	0R	R	0R	R	0R	R	0R	R
1	10.25 b*	7.50 ab	3.00 a	2.35 a	3.00 a	2.25 a	5.17 ab	6.67 ab
2	27.00 b	35.17 b	11.25 a	10.00 a	13.50 a	9.25 a	15.50 a	7.25 a
3	48.50 c	43.17 bc	27.25 ab	22.50 a	14.00 a	19.00 a	19.75 a	21.00 a
4	22.25 c	20.00 bc	15.25 abc	11.25 a	9.00 a	14.25 ab	9.00 a	8.25 a
5	1.75 a	2.25 a	3.25 a	2.50 a	1.50 a	1.50 a	5.50 a	2.00 a
11	20.50 cd	17.75 cd	22.50 d	12.75 bc	5.50 ab	8.25 ab	4.00 a	9.54 ab
12	13.75 cd	12.75 bcd	15.75 d	14.00 cd	3.00 a	6.00 abc	4.00 ab	4.50 ab
13	15.00 ab	13.25 ab	20.75 b	16.75 ab	8.75 a	10.00 ab	6.75 a	7.25 a
14	53.25 a	58.00 a	53.75 a	63.75 a	22.50 a	49.50 a	25.74 a	19.25 a
15	12.67 bc	11.92 bc	19.00 c	10.25 b	5.25 ab	7.75 ab	2.50 a	6.00 ab
16	1.50 ab	2.50 ab	4.00 b	3.25 ab	1.00 a	3.50 ab	1.00 a	1.50 ab
23	11.50 ab	9.00 ab	11.75 b	13.25 b	8.00 ab	8.25 ab	4.50 a	11.58 ab
24	6.00 a	8.75 a	8.00 a	9.00 a	8.00 a	7.00 a	8.25 a	8.00 a

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

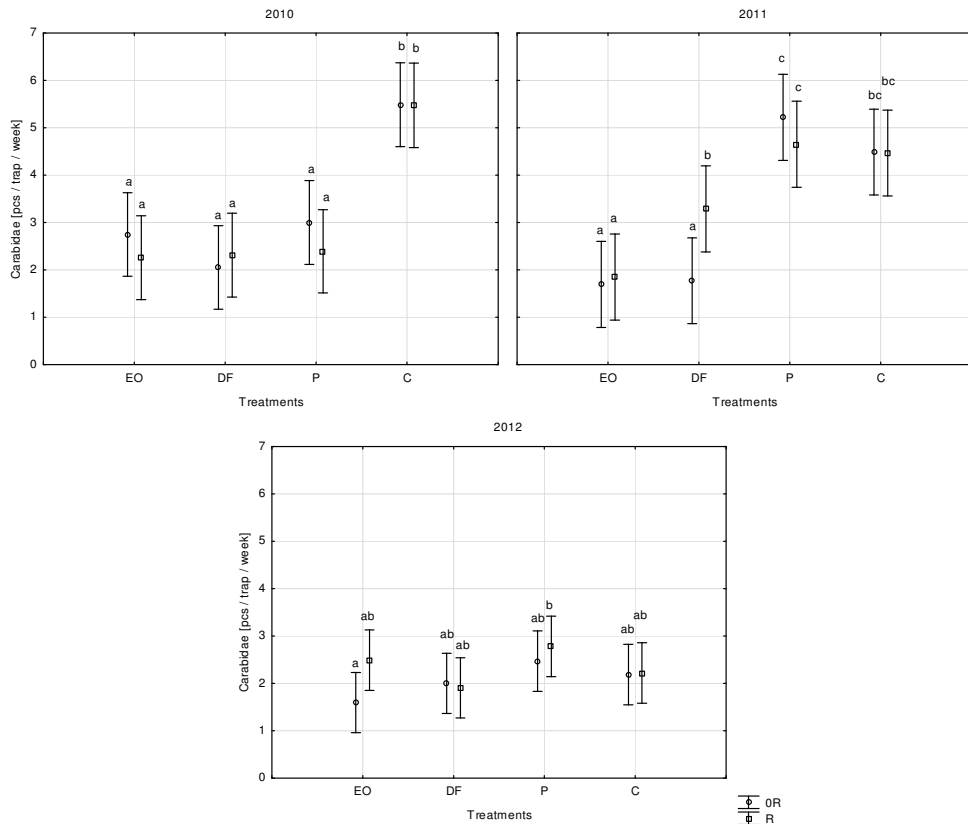


Fig. 4. Occurrence of Carabidae trapped using Barber's traps mean in the years 2010, 2011, 2012. The symbols as in Figure 1. Means marked with the same letters in a given year do not differ significantly according to LSD test at  $\alpha = 0.05$ ; factors contamination x remediation.  $\pm$  Mean  $\pm$  0.95 confidence interval

No influence of bioremediation process on Carabidae presence was observed for a most part of the period of investigations. Similarly, bioremediation did not affect the occurrence of a total of arachnid representatives trapped using Barber's traps, although a diversification within Arachnida orders was registered [8]. Bioremediation of soil polluted with petrol contributed to limited occurrence of Opiliones, especially during the first month after its completion, whereas a beneficial effect of this measure on the presence of terrestrial mites was noted in the third month after the soil contamination. Advantageous effect of bioremediation of soil polluted with petrol visible as increase in the number of captured insects already immediately after its beginning (0-5 months) was registered for Gastropoda representatives [14]. Like in case of Carabidae, during the period from 11 to 16 months after contamination, more snails were found in conditions of soil polluted with diesel fuel and subjected to remediation, than when this measure was not applied. In both cases it may result from a beneficial effect of bioremediation on overgrowing polluted soil with vegetation. Carabidae, like snails prefer shadowed sites, covered with vegetation. This

factor was mentioned as among others often conditioning greater activity of Carabidae when chemical plant protection is applied [15].

## Conclusions

1. Carabidae activity is to various extent limited in conditions of soil pollution with oil derivatives depending on the kind of applied substance. Petrol reveals negative effect for the shortest period of time (*ca* 3 months), whereas both diesel oil and engine oil act for a much longer period: diesel fuel may have a negative effect for 12 months, whereas engine oil for 15 months from the time of soil pollution.
2. The process of bioremediation of soil polluted with oil derivatives does not influence Carabidae activity during the first five months after its initiation, whereas in the later period (after a year) it may contribute to increased activity of Carabidae in conditions of soil polluted with diesel fuel, at the same time neutralizing the negative effect of the above-mentioned substance.

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## **ODDZIAŁYWANIE BIOREMEDIACJI GLEBY ZANIECZYSZCZONEJ ROPOPOCHODNYMI NA WYSTĘPOWANIE BIEGACZOWATYCH (COLEOPTERA, CARABIDAE)**

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**Abstrakt:** Celem pracy było zbadanie oddziaływania substancji ropopochodnych (benzyny, oleju napędowego, zużytego oleju silnikowego) w trakcie procesu bioremediacji gleby na przebieg dynamiki występowania biegaczowatych. Aktywność biegaczowatych w warunkach zanieczyszczenia gleby ropopochodnymi jest ograniczana w różnym stopniu zależnie od rodzaju użytej substancji. Benzyna wykazuje negatywny wpływ najkrócej (ok. 3 miesiące), znacznie dłużej oddziałują oleje - napędowy może wykazywać negatywny wpływ jeszcze po upływie 12 miesięcy, natomiast silnikowy po upływie 15 miesięcy od momentu zanieczyszczenia. Proces bioremediacji gleby zanieczyszczonej ropopochodnymi nie wpływa na aktywność biegaczowatych w ciągu pierwszych 5 miesięcy po jego zainicjowaniu, natomiast w późniejszym okresie (po upływie roku) może przyczyniać się do wzrostu aktywności Carabidae w warunkach gleby zanieczyszczonej olejem napędowym, niwelując tym samym negatywny wpływ wymienionej substancji.

**Słowa kluczowe:** ropopochodne, gleba, bioremediacja, Carabidae