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## EFFECT OF SOIL POLLUTION WITH OIL DERIVATIVES ON THE OCCURRENCE OF ISOPODA

### ODDZIAŁYWANIE ZANIECZYSZCZENIA GLEBY ROPOPOCHODNYMI NA WYSTĘPOWANIE ISOPODA

**Abstract:** The research aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of Isopoda 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<sup>-1</sup> 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 2012 the traps were emptied once a week. Activity of Isopoda order representatives was reducing under the influence of soil pollution with engine oil - the result was discernible even after 14 months from the moment of contamination. Pollution with petrol generally did not affect the occurrence of Isopoda, whereas contamination with diesel oil even favoured their presence after two years from the contamination moment. Supported bioremediation applied to the soil contaminated with diesel and engine oils was regulating, *ie* contributed to increase in the number of trapped specimens in places where the oil derivatives limited their occurrence (the object polluted with engine oil) or to diminish their activity where the soil pollution favoured the occurrence of crustaceans (the object where the soil was contaminated with diesel oil in the 2012 season). In result, the number of trapped Isopoda was similar as in the unpolluted object.

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

Representatives of Isopoda order inhabiting the soil environment are counted among decomposing soil macrofauna. They feed mainly on dead plant material. They contribute to accelerating the decomposition processes by mechanical and chemical crushing of plant tissues which they enrich in microorganisms during transport through their bowels [1, 2]. Due to their key role in the matter cycling, common occurrence and ease with which they are collected, they are frequently used in research on the environment risk assessment [3-5]. The investigations on the effect of water polluting oil derivatives point to their strong toxic effect on macroinvertebrate fauna, including crustaceans [6]. Considerably less information is available about this type of pollution effect on terrestrial crustaceans. As reported by Van Brummelen et al [7] mainly due to the food they consume, Isopods are exposed to toxic effect of PAHs, regarded as the most dangerous oil derivative substances. Strategies of avoiding the polluted soil by Isopoda are also well known, which argues for their possible use as bioindicators of pollution and assessment of bioremediation progress [4].

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

### Materials 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

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placed in 1 m<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. The soil in containers was left untouched for 8 months to allow it to restore its natural biological efficiency. After this period (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 · kg<sup>-1</sup> 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. Isopods were trapped using Barber's traps (0.9 dm<sup>3</sup> 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 2012 the traps were emptied once a week. Statistical computations concerning Isopoda occurrence in the subsequent months and years from the moment of soil pollution were made using Statistica 10.0 PL computer programme. Means were diversified using NIR Fisher test at significance level  $\alpha = 0.05$ .

## Results and discussion

The dynamics of Isopoda trapping during the conducted research did not reveal the maximum of their occurrence during the vegetation season (Figs. 1-3). In 2010 the most numerous were trapped at the beginning of September, in 2011 by the end of May, at the turn of July and August and by the end of October, whereas in 2012 the course of the trappings fluctuated greatly. Isopoda activity is often connected with the substratum moisture. During the first three months following the soil contamination with oil derivatives, no significant effect on the number of trapped Isopoda representatives was registered (Table 1). In the fourth month significantly lower number of these crustaceans were noted in conditions of soil polluted with engine oil in comparison with the control, however next month more were caught in the polluted soil. Supported bioremediation applied on the soil polluted with diesel oil, as soon as in the fourth month after pollution revealed a marked effect contributing to increased number of trapped Isopoda.

In the second research period, *ie* 11-16 months after the contamination moment, a distinctive reduction of these invertebrate activity was observed under the influence of engine oil during their intensified occurrence (14<sup>th</sup> month following the contamination moment). Analysis of the oil derivative compounds content at that time pointed to their still about 5-fold higher content than the content of compounds extracted by light petroleum from the unpolluted soil (unpublished data). Supported bioremediation did not affect significantly Isopoda occurrence during this period. Analysis of results obtained for the entire vegetation period revealed a significant negative effect of engine oil, slightly reduced by the conducted supporting remediation. On the other hand, in case of soil polluted with petrol and subjected to supporting bioremediation, markedly smaller number of Isopoda were trapped than in the unpolluted soil (Fig. 4).

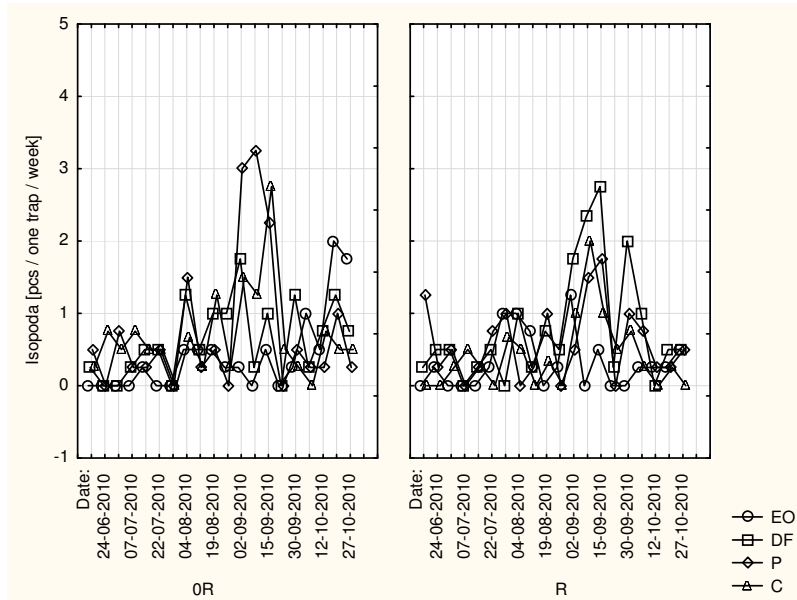


Fig. 1. Course of dynamics of Isopoda occurrence trapped using Barber's traps in 2010. EO - soil contaminated with used engine oil, DF - soil contaminated with diesel fuel, P - soil contaminated with petrol, C - unpolluted soil, OR - series without bioremediation, R - series with bioremediation

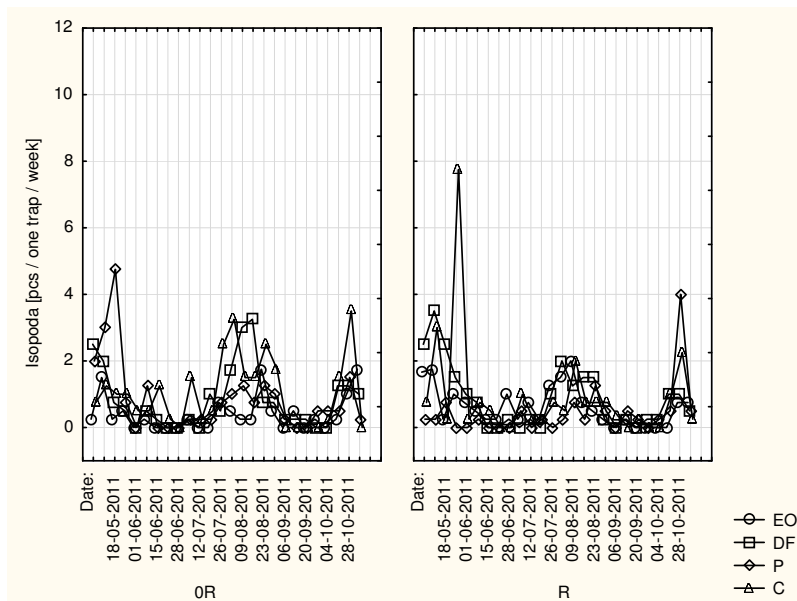


Fig. 2. Course of dynamics of Isopoda occurrence trapped using Barber's traps in 2011. The symbols as in Figure 1

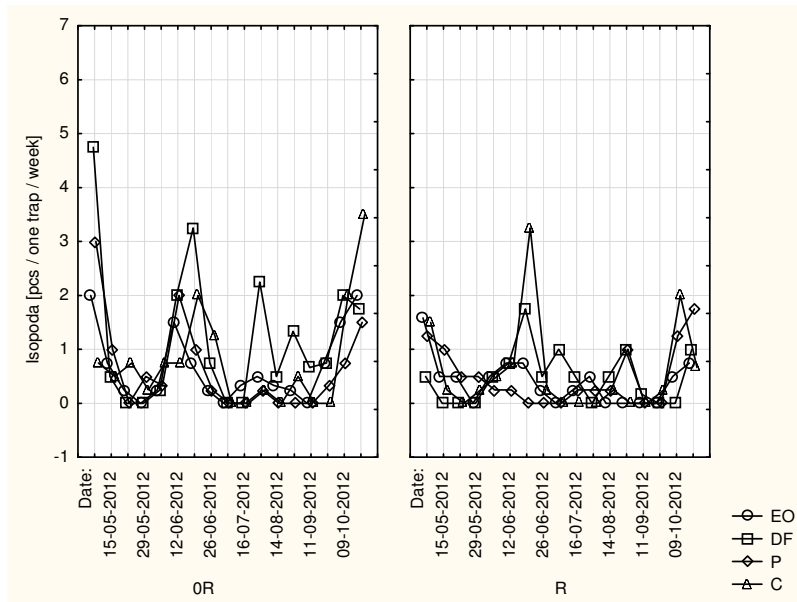


Fig. 3. Course of dynamics of Isopoda occurrence trapped using Barber's traps in 2012. The symbols as in Figure 1

Table 1  
Occurrence of Isopoda 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	Isopoda [pcs/trap/month]							
	Control		Petrol		Diesel fuel		Engine oil	
	OR	R	OR	R	OR	R	OR	R
1	2.25 a*	0.75 a	1.50 a	2.00 a	0.50 a	1.25 a	0.00 a	0.25 a
2	1.50 a	1.42 a	2.25 a	2.00 a	2.25 a	1.50 a	0.75 a	2.25 a
3	3.25 ab	1.33 a	3.75 ab	1.75 ab	4.25 b	3.25 ab	1.50 ab	2.25 ab
4	4.75 bc	4.25 ab	6.00 c	4.25 bc	2.50 ab	7.33 c	0.75 a	0.50 a
5	1.75 a	0.50 a	1.75 a	1.75 a	3.00 ab	2.00 a	5.25 b	1.25 a
11	4.50 a	12.00 a	10.50 a	1.25 a	5.75 a	11.00 a	2.50 a	5.42 a
12	2.00 a	1.25 a	1.25 a	0.25 a	0.75 a	1.00 a	0.25 a	2.00 a
13	4.75 a	2.25 a	1.50 a	0.75 a	1.75 a	1.25 a	1.00 a	2.50 a
14	10.50 b	4.75 ab	5.25 ab	3.00 a	9.50 ab	6.50 ab	3.25 a	5.00 ab
15	0.00 a	0.58 a	0.75 a	0.75 a	0.75 a	0.50 a	0.75 a	0.25 a
16	5.25 a	3.50 a	2.75 a	5.25 a	3.50 a	2.75 a	3.00 a	1.50 a
23	2.25 ab	2.00 ab	4.50 b	3.25 ab	5.25 b	0.50 a	3.00 ab	2.67 ab
24	4.75 b	4.75 b	3.58 ab	0.50 a	6.25 b	3.50 ab	2.75 ab	2.25 ab
25	0.25 a	0.00 a	0.25 a	0.50 ab	2.25 b	1.50 ab	0.83 ab	0.75 ab
26	0.50 ab	0.25 ab	0.00 a	1.25 ab	1.83 b	1.50 ab	0.58 ab	0.00 a
27	0.00 a	0.25 a	0.33 a	0.00 a	1.42 b	0.17 a	0.75 ab	0.00 a
28	5.50 b	2.67 ab	2.25 a	3.00 ab	3.75 ab	1.00 a	3.50 ab	1.25 a

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

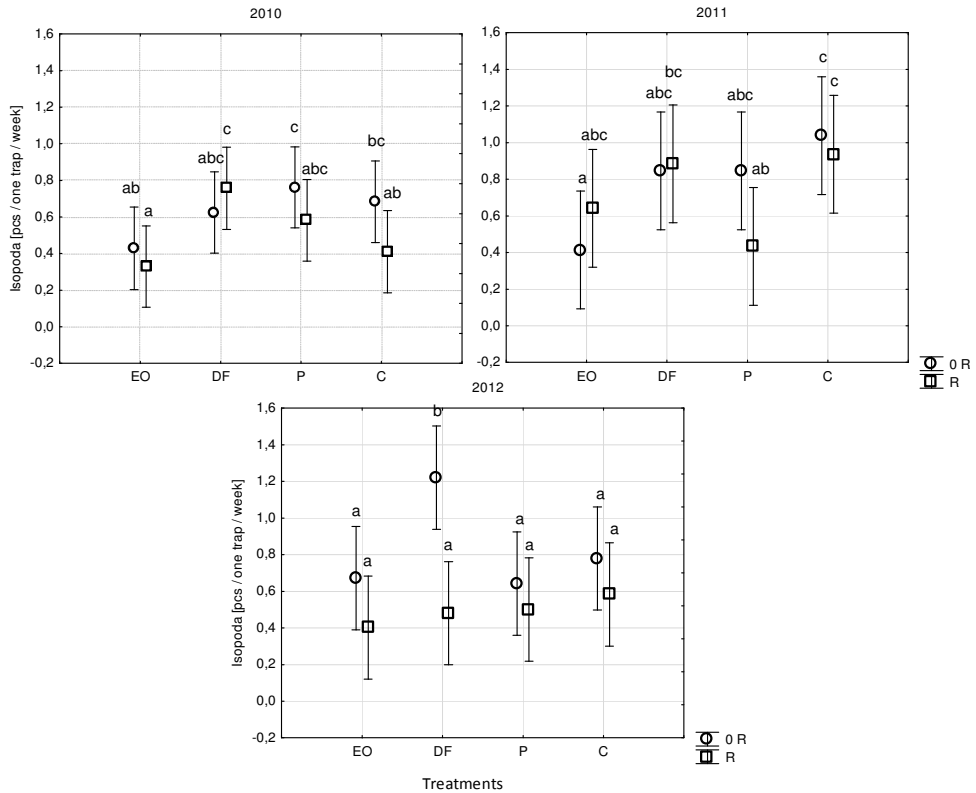


Fig. 4. Occurrence of Isopoda trapped using Barber's traps mean in the years 2010, 2011, 2012. The symbols as in Figure 1. Means marked with the same letters do not differ significantly according to NIR test at  $\alpha = 0.05$ ; factors contamination x remediation.  $\perp$  Mean  $\pm 0.95$  confidence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

In the 2012 season (23-28 months after contamination) heightened Isopoda activity was observed in the soil contaminated with diesel oil (Figs. 3 and 4, Table 1). In this case bioremediation contributed to a decrease in the number of trapped Isopoda to the level similar to the control soil.

Research by Couceiro et al [8] demonstrated a very strong reduction of Isopoda representatives occurrence on the banks of a stream exposed to oil spill. In comparison with the banks of unimpacted stream, 19-fold less of this order representatives were found in the samples collected from the banks of polluted stream. Engine oil most negatively affected Isopoda occurrence, which is undoubtedly connected with the presence of hardly decomposable components, among others PAHs, whereas no significant effect of petrol on the analysed fauna was noted during almost whole period of investigations. This kind of pollutions becomes fast eliminated from the soil due to high content of volatile fractions. During the final period of research, *ie* after 2 years from the contamination, intensified activity of Isopoda was observed in conditions of soil contaminated with diesel oil. The reason might have been decreasing concentration of repellent substances proceeding in time

and possibly the appearance of some attractant substances. As has been demonstrated by research of Zimmer et al [9] one of the most common Isopoda representatives - *Porcellio scaber* is able to identify food by olfaction. The species recognizes the smell of metabolites produced by the microorganisms colonizing leaves. One of the attractants is a by-product of cellulolytic activity of these microorganisms. Santos et al [4] stated an apparent avoiding of soil polluted with high doses of dimethoate, glyphosate and spiroticlofen by *Porcellionides pruinosus*. However, at lower doses of spiroticlofen, even attractant effect was registered. The authors explained it by a possible attractant effect of some substances included in the content of formulations.

### Conclusions

1. Activity of Isopoda order representatives was reducing under the influence of soil pollution with engine oil - the result was discernible even after 14 months from the moment of contamination. Pollution with petrol generally did not affect the occurrence of Isopoda, whereas contamination with diesel oil even favoured their presence after two years from the contamination moment.
2. Supported bioremediation applied to the soil contaminated with diesel and engine oils was regulating, *ie* contributed to increase in the number of trapped specimens in places where the oil derivatives limited their occurrence (the object polluted with engine oil) or to diminish their activity where the soil pollution favoured the occurrence of crustaceans (the object where the soil was contaminated with diesel oil in the 2012 season). In result, the number of trapped Isopoda was similar as in the unpolluted object.

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## ODDZIAŁYWANIE ZANIECZYSZCZENIA GLEBY ROPOPOCHODNYMI NA WYSTĘPOWANIE ISOPODA

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**Abstrakt:** Celem pracy było zbadanie oddziaływania substancji ropopochodnych w trakcie procesu bioremediacji gleby na przebieg dynamiki występowania Isopoda. Utworzono następujące obiekty: kontrola - gleba niezanieczyszczona; gleba zanieczyszczona benzyną; gleba zanieczyszczona olejem napędowym oraz gleba zanieczyszczona zużytym olejem silnikowym (dawka: 6000 mg paliwa · kg<sup>-1</sup> s.m. gleby). Eksperyment został przeprowadzony w dwóch seriach: z naturalną i wspomaganą bioremediacją. Bezkręgowce były odławiane z użyciem pułapek Barbera. W okresie od czerwca do października 2010 roku oraz od maja do października 2011 i 2012 roku pułapki były opróżniane raz w tygodniu. Aktywność przedstawicieli rzędu Isopoda ulegała ograniczeniu pod wpływem zanieczyszczenia gleby olejem silnikowym - efekt ten widoczny był jeszcze po upływie 14 miesięcy od momentu skażenia. Zanieczyszczenie benzyną nie wpływało na ogół na występowanie równonogich, zaś skażenie gleby olejem napędowym po upływie 2 lat od momentu zanieczyszczenia wręcz sprzyjało ich obecności. Bioremediacja wspomaganą zastosowaną na glebę skażoną olejami silnikowym i napędowym oddziaływała regulująco, tj. przyczyniała się do zwiększenia liczby odławianych osobników tam, gdzie ropopochodne ograniczały ich występowanie (obiekt zanieczyszczony olejem silnikowym), bądź do zmniejszenia ich aktywności tam, gdzie zanieczyszczenie gleby sprzyjało występowaniu skorupiaków (obiekt z glebą zanieczyszczoną olejem napędowym w sezonie 2012 roku). W rezultacie łowność Isopoda kształtowała się podobnie jak w obiekcie niezanieczyszczonym.

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

