

Janina GOSPODAREK¹

**SURVIVAL RATE OF REDWORMS AND WOODLICE
IN SOIL CONTAMINATED WITH PETROL,
DIESEL OIL AND ENGINE OIL**

**PRZEŻYWALNOŚĆ DŹDŻOWNIC I PROSIONKÓW
PRZY SKAŻENIU GLEBY BENZYNA, OLEJEM NAPĘDOWYM
I OLEJEM SILNIKOWYM**

Abstract: The investigations aimed at an assessment of representatives of Isopoda and Lumbricidae sensitivity to soil pollution with oil derivatives from the perspective of their use as bioindicators. The laboratory experiment, conducted in 3 replications, comprised the following objects: soil contaminated with unleaded petrol; soil contaminated with diesel oil; soil contaminated with used engine oil; control. Doses of 3000 mg, 6000 mg and 10 000 mg of oil derivative per 1 kg soil d.m. were applied. The test animal most sensitive to soil pollution with petrol, diesel and engine oil were redworms. They revealed a rapid and clear response in the objects contaminated with these pollutants in comparison with the control. Woodlice proved the most sensitive to soil contamination with petrol and diesel oil. Used engine oil revealed the weakest effect on the tested invertebrate species among all three applied pollutants, whereas petrol had the most toxic effect.

Keywords: oil derivatives, soil pollution, redworms, woodlice

Representatives of terrestrial fauna, such as Isopoda, Lumbricidae are regarded as good bioindicators of the environmental contamination [1]. However, there are not many reports about oil derivatives influence on these invertebrates. Among others, survival rate and reproduction of *Eisenia foetida* redworm has been investigated with progressing bioremediation process of soil contaminated with oil derivatives [2] and its influence on properties of soil contaminated with these substances [3]. This species was also used to reveal the optimal indicators of sublethal doses of polycyclic aromatic hydrocarbons [4]. Redworms may positively contribute to bioremediation of soil contaminated with oil derivatives, although it depends on their species [5]. A number of papers have also addressed the issue of the ability of among others redworms or *Isopoda* representatives to metabolize polycyclic aromatic hydrocarbons (PAHs) [6–8].

¹ Department of Agricultural Environment Protection, Agricultural University of Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrjgospo@cyf-kr.edu.pl

Redworms were also used as indicators for an assessment of oil derivative contaminated soil quality [9].

The investigations aimed at an assessment of selected invertebrate groups (representatives of Isopoda, Lumbricidae) sensitivity to soil pollution with oil derivatives from the perspective of their use as bioindicators.

Material and methods

The laboratory experiment, conducted in 3 replications, comprised the following objects:

1. Soil contaminated with unleaded petrol;
2. Soil contaminated with diesel oil;
3. Soil contaminated with used engine oil;
4. Control.

The experiment was conducted on the representatives of redworms of *Lumbricus* genus and rough woodlouse (*Porcellio scaber*). Three contamination levels were used for redworms: 3000 mg of oil derivative per 1 kg soil d.m. (level I), 6000 mg of oil derivative per 1 kg of soil d.m. (level II) and 10 000 mg of oil derivative per 1 kg soil d.m. (level III). Doses of 3 000 mg and 10 000 mg of oil derivative per 1 kg soil d.m. were applied for rough woodlice (*Porcellio scaber*). The soil used for the experiment was degraded chernozem formed from loess, classified as the very good wheat complex and soil quality class. The soil was dried at 60 °C for 5 days in a dryer and then contaminated using a syringe with formerly calculated and precisely measured amount of oil derivatives.

Redworms used in the experiment were collected in Barwald Gorny village near Kalwaria Zebrzydowska. The experiment made use of redworm of similar length and well formed clitellum. The redworms were cultured in 500 cm³ containers. 10 redworms were put in each container with 400 g of prepared soil. Containers were covered with gauze for good ventilation. The redworms were cultured at 20 ± 2 °C. Every week 4 g of dry and crushed horse excreta was supplied as feed and of the specimens viability was checked regularly. Each week redworm weight was measured. Dead specimens were removed. If the soil in the containers dried, it was sprinkled with 20 cm³ of distilled water. Rough woodlice were collected on 19 and 20 June 2009 in Zawadka village near Wadowice. The woodlice were cultured in 100 cm³ containers and 6 specimens were placed per container. The woodlice were fed with small pieces of apple and carrot. Otherwise the culturing conditions were the same as for redworms.

Statistical analysis comprised one-way ANOVA. Means were differentiated using LSD Fisher's test. All computations were conducted using "Statistica 8.0" programme.

Results and discussion

Among the tested oil derivatives petrol proved the most toxic for redworms (Fig. 1). Already a day after the experiment outset mortality rate of these invertebrates in soil contaminated with 6000 and 10 000 mg of petrol · kg⁻¹ soil d.m. reached 100 %,

whereas over 80 % died in soil contaminated with 3 000 mg · kg⁻¹ soil d.m. On the following days of the experiment mortality rate was also increasing in the other objects containing soil contaminated with diesel and engine oil. On the fourth day of observa-

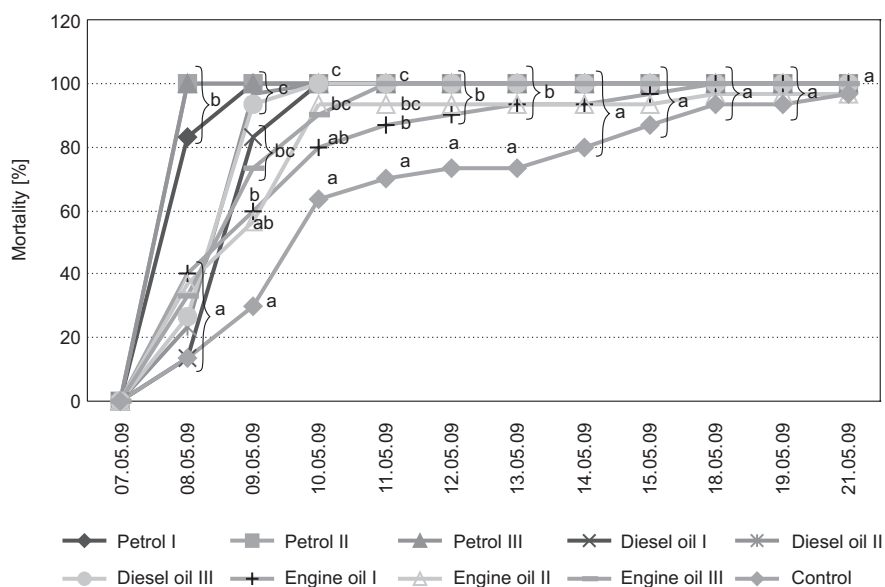


Fig. 1. Mortality rate of redworms [%] cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observation differ statistically at $p = 0.05$

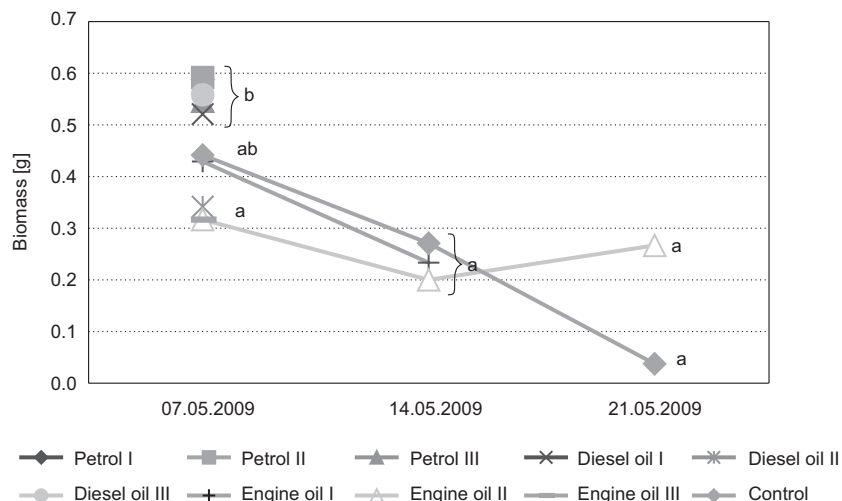


Fig. 2. Mean body weight of a single specimen of Lumbricidae cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observations differ statistically at $p = 0.05$

tion live specimens were still registered, beside the control, in the objects where soil was contaminated with doses of 3 000 and 6 000 mg of engine oil per 1 kg of soil d.m. Due to very high mortality rate of redworms in most objects with contaminated soil, it

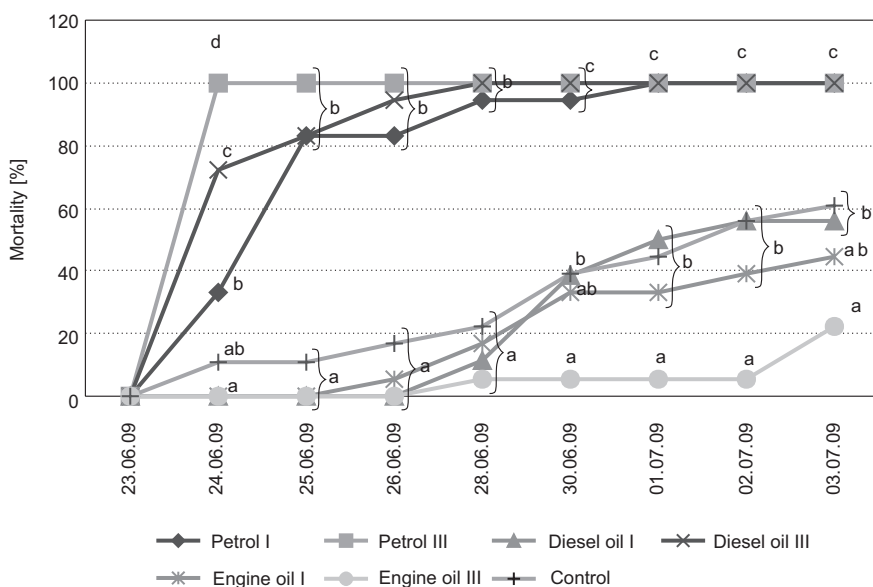


Fig. 3. Mortality rate of Isopoda [%] cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observation differ statistically at $p = 0.05$

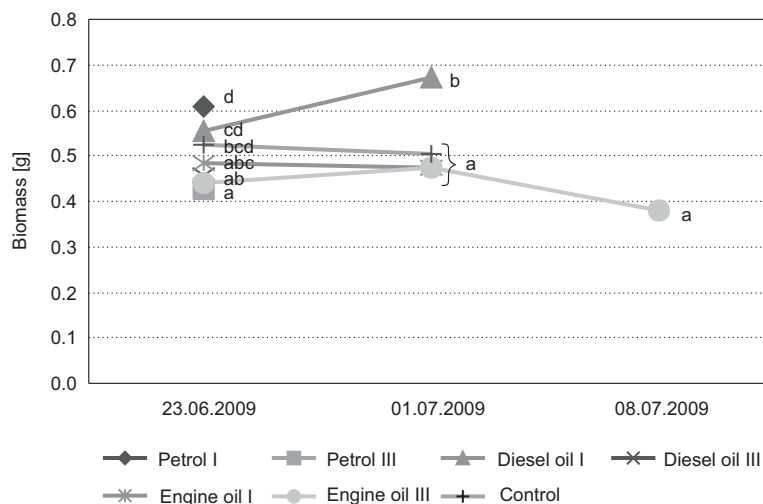


Fig. 4. Mean body weight of a single specimen of Isopoda cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observations differ statistically at $p = 0.05$

proved impossible to measure changes in body weight of live specimens after a week and two weeks from the experiment outset. In the objects where the measurement was possible an apparent decline in body weight was noted in all objects (also in the control) (Fig. 2). Investigations on the use of *Eisenia foetida* redworm for the quality assessment of soils contaminated with various kinds of crude oils (heavy, medium and light (API gravity 16–18.30 and 53) after remediation tests, demonstrated that the species was between 1.4 and 14 times more sensitive than microtox test and between 1.3 and > 77 times more sensitive than plant toxicity tests. Light oil in the silty low organic carbon soil proved the most toxic [9].

The tested species of Isopoda order, *ie* rough woodlouse showed weaker sensitivity to the applied substances (Fig. 3). Only the highest dose of petrol caused a 100 % mortality of these animals already after one day from the soil contamination. Relatively high mortality rate was observed among woodlice in the object where the soil was contaminated with the highest dose of engine oil. On the second day of observations mortality rate of woodlice increased also in the second object contaminated with petrol (a dose of 3000 mg · kg⁻¹ d.m.), where it reached over 80 %. At the same time woodlice mortality rate in the objects with soil contaminated with both doses of engine oil and diesel oil in the lower dose did not exceed 20 %, similar as in the control. During the whole period of the experiment the lowest percentage of dead woodlice (significantly lower than in the control) was registered in the object where soil was contaminated with the higher dose of engine oil. No significant changes in the weight of live specimens were observed during culturing in most objects (Fig. 4). Only in the object with soil contaminated with diesel oil in a dose of 3000 mg · kg⁻¹ soil d.m. an increase in *Porcelio* weight was noted a week after the experiment outset. Research conducted under field conditions by the Author on Isopoda occurrence in soil polluted with oil derivatives (petrol, diesel and engine oil dosed 2 dm³ · m⁻²) did not demonstrate any marked differences in these invertebrate number caught into pitfall traps, as dependant on the pollutant substance [10], however they were caught sporadically. There were no clear dependencies either between Isopoda presence and soil pollution with heavy metals (Cd, Zn and Pb) in case when weakly polluted soils were analyzed. On the other hand, no Isopoda were caught in strongly polluted soils [11].

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Conclusions

1. The test animal more sensitive to soil pollution with petrol, diesel and engine oil were redworms. They revealed a rapid and clear response in the objects contaminated with these pollutants in comparison with the control.
2. Woodlice proved the most sensitive to soil contamination with petrol and diesel oil.

3. Used engine oil revealed the weakest effect on the tested invertebrate species among all three applied pollutants, whereas petrol had the most toxic effect.

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Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem badań była ocena wrażliwości wybranych grup bezkręgowców (przedstawicieli *Isopoda*, *Lumbricidae*) na zanieczyszczenia gleby substancjami ropopochodnymi pod kątem możliwości ich wykorzystania jako biowskaźników. Doświadczenie laboratoryjne przeprowadzono w 3 powtórzeniach i obejmowało ono następujące obiekty: gleba skażona benzyną bezołowiową, gleba skażona olejem napędowym, gleba skażona przepracowanym olejem silnikowym, kontrola. Zastosowano dawki 3000, 6000 i 10 000 mg substancji ropopochodnej na kg s.m. gleby. Zwierzęciem testowym najbardziej wrażliwym na zanieczyszczenia gleby benzyną oraz olejami (napędowym i silnikowym) były dżdżownice. Cechowała je szybka i wyraźna odpowiedź w obiektach skażonych tymi polutantami w stosunku do obiektów kontrolnych. Stonogi okazały się najbardziej wrażliwe na skażenie gleby benzyną i olejem napędowym. Najślabszym działaniem na testowane gatunki bezkręgowców spośród wszystkich trzech zastosowanych polutantów charakteryzował się zużyty olej silnikowy, natomiast najbardziej toksycznie oddziaływała benzyna.

Słowa kluczowe: ropopochodne, skażenie gleby, dżdżownice, równonogie