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Time-delayed effect of petroleum-derived products in soil and their bioremediation on plant – herbivore interaction

Milena Rusin[1], Janina Gospodarek[1]*, Aleksandra Nadgórska-Socha[2]

[1]Department of Microbiology and Biomonitoring, University of Agriculture, Kraków, Poland
[2]Department of Ecology, University of Silesia in Katowice, Poland

*Corresponding author's e-mail: rrjgospo@cyf-kr.edu.pl

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Abstract: The aim of the study was to determine the time-delayed (after three years from the moment of soil pollution) effect of petroleum-derived products (PDPs) (petrol, diesel fuel and used engine oil) on the interaction between selected host plant (broad bean) and a herbivorous insect closely related to it (*Sitona* spp.). We assessed the condition of the plant exposed to pollutants (i.e. its growth and chemical composition), then we evaluated the attractiveness of the plant for both larvae and adults of the insect. The evaluation covered also the effect of bioremediation by using ZB-01 biopreparation. The results showed that after 3 years from soil contamination, engine oil and diesel fuel limited the feeding of adult sitona weevils while petrol caused increase in the attractiveness of plants for these insects. The PDPs negatively affected the growth of plants. The changes in element content depended on the type of pollutant. The biopreparation ZB-01 eliminated or reduced the differences caused by the presence of PDPs in the soil regarding the chemical composition of the host plant, and limited feeding by both the larvae and adult individuals of sitona weevils. The negative relationships between the contents of both some macroelements (Mg, S) and heavy metals (Zn, Ni), and feeding of imago of Sitona were observed. The obtained results indicate that PDPs remain for a long time in the environment and adversely affect not only the organisms directly exposed to the pollution – plants growing on polluted soil but also further links of the trophic chain, i.e. herbivores.

List of abbreviations used at work:
PDPs – petroleum-derived products
EO – soil contaminated with engine oil,
DF – soil contaminated with diesel fuel,
P – soil contaminated with petrol,
C – control soil,
0R – without bioremediation,
R – with bioremediation
ZB-01 – the name of the biopreparation used
TPH – total petroleum hydrocarbons

Introduction

The mechanization and urbanization of society has coincided with a significant increase in the use of petroleum-derived products (PDPs). The extraction, industrial processing and transport, as well as PDPs use have resulted in the ever-increasing pollution of the natural environment. It is an extremely important issue because of the high toxicities of these compounds, the high rate of their dispersion, as well as their specific complexity. PDPs adversely affect all elements of the natural environment: air, water, soil, and, living organisms. They constitute potential threats to the life of humans and animals. These compounds are often fairly immobile and can remain in the environment for a long time (Rosik-Dulewska et al. 2012, Wu et al. 2021).

PDPs entering the soil disturb its air-water regimes, modify physicochemical properties, disturb biological equilibrium, as well as have an adverse impact on the growth of plants (Grifoni et al. 2020). Bioremediation is one of the most effective ways to remove PDPs from the soil. This method is an economically and environmentally-friendly procedure based on the metabolic capacities of microorganisms to degrade contaminants biochemically (Zawierucha et al. 2014, Burghal et al. 2015, Mauricio-Gutierrez et al. 2020, Lizbeth et al. 2020).

The effects of PDPs on the growth and development of plants and soil organisms exposed directly to the contact with pollutants are relatively well known (Pennings et al. 2014, Grifoni et al. 2020), but there is very scarce information about indirect effects of these compounds from polluted soil through plants to the subsequent links of trophic chain i.e. phytophagous insects. The results of studies on the impact of different oil concentrations on herbivory invertebrates (grass shrimp *Palaemonete spugio* and amphipods *Gammarus mucronatus*) of water plant (*Ruppia maritima*) suggest a significant and indirect influence of pollutants (Martin and Swenson 2018). Changes in the composition of the host plant due to oil-exposure affected the food preferences of herbivores as well as the amount of food they eat. However, no similar data are available on the effects of PDPs on terrestrial herbivores, which is the purpose of this experiment. Because of their diversity, easy collection and breeding, great fertility, and the short period of development, invertebrates are useful element for the determination of the effects of contamination on the environment. Our earlier research confirmed that PDPs have adverse effect on the developmental parameters in black bean aphid (*Aphis fabae* Scop.), resulting in the decrease of its fecundity, shortening of its average life span, and lowering of the population intrinsic growth rate (Rusin et al. 2017). Still, we do not know how PDPs could influence food preferences of herbivores and the amount of eaten food. Moreover, most of the available literature provide information on the effect of petroleum products on the natural environment immediately after the emergence of contamination or after a short time (Grifoni et al. 2020, Martin and Swenson 2018). The data on the subsequent (time-delayed) effects of the PDPs are still scarce. Especially rare are experiments involving realistic environmental conditions i.e., in the field (Sylvain et al. 2019). Finally, the effect of bioremediation on proposed soil-plant-herbivore system is still unrecognized.

Sitona weevils (*Sitona* spp., Coleoptera, Curculionidae) are common herbivorous insects that feed on the Fabaceae family plants. They damage cotyledons still in the soil before they emerge on the ground surface. They gnaw out semicircular-shape feeds fragments on leaf edges, markedly reducing the assimilation surface of leaves, which is particularly harmful at the early stage of plant development. The larvae of the insect feed on root nodules, thus reducing the quantity of nitrogen fixed by plants, as well as disturbing their water management, which leads to deteriorated growth and development (Hanavan and Bosque-Pérez 2012). The broad bean (*Vicia faba* L.) has been already used as a model plant in studies on the effect of soil contaminants (including PDPs) on the growth and development of plants (Malallah et al. 1996).

The aim of the study was to determine the time-delayed (i.e. after three years from contamination) effect of PDPs, such as petrol, diesel fuel, and spent engine oil on the interaction between host plant (broad bean) and a herbivore insect closely related to it (*Sitona* spp.). The effect of PDPs on the growth of broad bean and on the content of selected nutrients in plant organs was also investigated. Furthermore, the effects of the bioremediation process (by adding biopreparation ZB-01) on the abovementioned features were also determined. ZB-01 biopreparation is a microbial preparation which has been widely used to initiate and stimulate biodegradation of petroleum-derived substances in contaminated waters and soils. We chose it because its usefulness in decomposition of petroleum-derived contaminants has been already confirmed by many studies (Petryszak et al. 2008, Kaszycki et al. 2010, Kaszycki et al. 2011, Kaszycki et al. 2015). Finally, we analyzed the relationships between *Sitona* spp. feeding, soil contamination with PDPs, and broad bean chemical composition (macroelements and heavy metals). By this new multi-trophic approach we sought to determine whether soil contamination by PDPs may affect organisms indirectly exposed to pollution and the same way further links of the food chain, as well as evaluate the usefulness of bioremediation initiated by especially prepared microbial biopreparation in restoring balance in the soil-plant-herbivore system.

Materials and methods

Experimental setup

The field experiment was conducted in 2013 at the Experimental Station of the University of Agriculture in Krakow, situated in Mydlniki near Krakow (Poland; 50.0815°N, 19.84730°E). In November 2009, indigenous soil (loamy sand; $pH_{(KCl)} = 6.45$; $pH_{(H2O)} = 7.12$; Carbon $_{total}$ = 10.4 g kg^{-1}; nitrogen = 0.90 g kg^{-1}; C:N = 11.6; $CaCO_3$ = 1.7 g kg^{-1}; available phosphorus = 7.14 mg 100 g^{-1}; available magnesium = 5.64 mg 100 g^{-1}; available potassium = 14.25 mg 100 g^{-1}) was placed in 32 special containers of 1 m^3 volume (1 m × 1 m × 1 m), retaining the natural arrangement of layers. We used plastic containers certified for the storage of such substances as oils, petroleum products and solvents. The containers were double-bottomed. The upper bottom, propped on supports, was perforated to enable the possible effluence of water (with possible contaminants). Before filling them with soil, the containers were additionally lined with a nonwoven geotextile to prevent soil leakage through the bottom perforation. A tank was installed on the side of each container below its bottom (connected with the container bottom) to collect possible effluent from the soil inside the container. The tank was provided with a plastic duct running towards the surface, which enabled excess water to be pumped out. This procedure was adopted to avoid environmental pollution with effluents from the contaminated soil. All containers were also supplied with perforated plastic tubes (placed at approx. 30 cm distances, 4 per container) to provide adequate soil aeration in a gravity system, which is a prerequisite for bioremediation to proceed correctly. The containers were sunk in the ground so that their upper edge was at the same level as the surface of the soil. The soil in the containers was left for eight months without any intervention in order to regain its natural biological functions. In June 2010, the soil surface was artificially contaminated with petrol (BP Unleaded 95) (P), spent engine oil (PLATINUM Classic Semisynthetic 10W-40, used for one year in a petrol engine) (EO), and diesel fuel (BP Diesel Fuel) (DF) in a quantity of 6000 mg of each PDP per 1 kg of dry mass, by pouring it on the soil. The PDPs were used separately. After one week, and then after one year starting from the moment of contamination by PDPs, half of the number of containers were subjected to the bioremediation process by adding biopreparation ZB-01. ZB-01 was specially produced for this experiment in Biochemistry Department of the University of Agriculture in Kraków and contained selected prokaryotic organisms (*Stenotrophomonas*, *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, *Ochrobactrum*, *Comamonas*, *Burkholderia*, *Corynebacterium*, and *Oligella*), which were isolated over years from sites heavily polluted with organic compounds (Kaszycki et al. 2001). The activity of this biopreparation in degradation of petroleum contaminants was confirmed also by our earlier investigations (Gospodarek et al. 2016). The treatment was performed by sprinkling, while maintaining 60% sorption moisture of the soil. Before the ZB-01 application, the soil surface in the containers intended for this treatment was treated with a compound fertilizer (Azofoska; 13.6% N, 2.8% P and 15.8% K) at a dose of 100 g per container. The non-contaminated soil, placed in identical containers, constituted the control treatment. ZB-01 contains strains of bacteria naturally occurring in the environment and specializing in the breakdown of organic pollutants. Their population decreases as petroleum products decompose, allowing to develop typical soil microflora (Kaszycki et al. 2011). However, to check if there are any effects that may arise from the circulation of ZB-01 in the soil, also the unpolluted soil was treated by ZB-01. The experiment was set in four repetitions in line with the randomized blocks method.

In three subsequent years, the soil in the containers was left without any intervention to enable natural plant succession. The 30 seeds per container (in accordance with the sowing standard) of the Windsor White variety of broad bean were sown in the containers at the beginning of April 2013, after earlier preparation of the soil (i.e. loosening and fertilizing). A pre-sowing soil fertilization with NPK fertilizer ('polifoska') was applied providing 2.88 g N, 8.64 g P_2O_5, 8.64 g K_2O, and 3.24 g SO_3 per container. After seedling emergence, the plants were thinned out to have the same number of plants in each container, i.e., 25.

Sitona spp. feeding

The evaluation of attractiveness of tested plants for herbivores was measured by analyzing feeding intensity by adult sitona weevils. It was conducted four times, once a week, beginning from the time when the first damage to germinating plants was noted. The analysis was conducted by measuring the loss of leaf surface (by relating the area of the feeds expressed in mm^2, to the total area of the leaf), the eaten away areas of the leaf, and by counting the damaged and undamaged leaves. The damage to leaves made by weevils was assessed with the use of graph paper to calculate the area of leaves and the damaged areas on all leaves from 10 plants randomly selected from each container. The presented results include mean values from four observations.

In order to determine larvae feeding, the underground parts of plants were analyzed. The roots of plants were removed from the soil and after thorough washing, the total number of root nodules was determined, as well as the number of nodules with traces of foraging. This analysis was completed once, in the last ten days of June 2013.

Growth and chemical composition of plants

The assessment of plant growth was performed at the stage of processing maturity (milk-ripe) of broad bean seeds. Six plants were collected at random from each container and then the following parameters were determined: the average length of shoot and number of leaves on shoot, the fresh biomass of vegetative and generative parts.

The plant material used to determine the nutrient contents (iron (Fe), calcium (Ca), magnesium (Mg)) was collected at the flowering stage, and the leaves used for the purpose of analysis were even-aged. The material was then cleaned of surface contamination such as honey-dew, washed in tap water and then in distilled water, and dried at 105°C. A portion of 0.25 g dried plant material was digested with 5 mL of HNO_3 at 120°C and then diluted to 10 mL with deionized water. Next, the metal contents were measured using flame absorption spectrometry (Thermo Scientific iCE 3500). The quality of the analytical procedure was checked using a reference material (Certified Reference Material CTA-OTL-1 Oriental Tobacco Leaves) with the same quantities of samples.

To assess the relationships between plant composition and attractiveness of plants for herbivore we used also data of the contents of other macroelements (C, N, S determined in a Variomax CNS analyser) and heavy metals (Pb, Cd, Ni, Zn, Cu and Mn measured using flame absorption spectrometry), which in details (exact concentrations) were given in our previous paper (Rusin et al. 2015). Here we used them only to calculate N/S ratio, in Principal Component Analysis (PCA), and in multiple regression to obtain better understanding of possible reasons of the observed relationships between host plant and a herbivore.

Statistical analysis

The obtained results were analyzed, checked for normality (Shapiro-Wilk test with Lilliefors correction) and equality of variance (Levene's test). The significance of differences between the means was tested by one-factor variance analysis (STATISTICA 12.5 software), and the means were differentiated by Fisher's LSD test at $p<0.05$. Principal Component Analysis (STATISTICA 12.5 software) assessed the relationships between *Sitona* spp. feeding and PDPs contamination as well as between plant composition and PDPs contamination. Multiple regression equations were derived to determine which of the accumulated heavy metal and nutrient influenced sitona weevils feeding.

Results and discussion

The described experiment was planned as a long-lasting one. Because it acquired a large investment at the time of establishment (costs of containers, manual works related to setting up the experiment) it had a wide, comprehensive scope, which included the influence of PDPs and bioremediation on terrestrial, soil and phytophagous invertebrates, as well as on the condition of wild and cultivated plants. The results presented in this article are part of this wide project. To give the background for the obtained results we present below selected data (i.e. after two years from the moment of contamination) on the impact of PDPs and ZB-01 biopreparation on the total petroleum hydrocarbons (TPH) content in the soil. After 23 months from the date of pollution, TPH content in soil contaminated with EO was more than 10 times higher than in the control soil (16331.8 mg kg^{-1} and 1458.4 mg kg^{-1} respectively). In soil contaminated with DF this value was more than twice higher (3643.5 mg kg^{-1}), while in soil contaminated with P (2804.2 mg kg^{-1}) it was similar to the control soil (Gospodarek et al. 2016). Adding ZB-01 resulted in decrease in TPH content in EO and DF contaminated soils to the values 8957.2 mg kg^{-1} and 1767.4 mg kg^{-1}, respectively. The TPH content in P contaminated soil and in control soil after ZB-01 treatment increased (3807.5 mg kg^{-1} and 2006.0 mg kg^{-1}, respectively). However, the analysis which was performed systematically once a month during the first two years of the experiment showed that the TPH concentrations in P treatment and in control fluctuated between 1200 and 6000 mg kg^{-1}. This suggests that petrol contamination was eliminated via evaporation during the first months of the experiment, and that the recorded concentrations of organic compounds represent organic substances naturally occurring in soil (e.g. humic acids etc.) (Gospodarek et al. 2016).

Sitona spp. feeding

The contamination of soil by PDPs significantly affected the percentage of leaves damaged by adult sitona weevils (F=24.289, p<0.0001) (Fig. 1A). Compared to the control soil, DF contributed to a significant decrease in the percent of damaged leaves, whereas P affected this parameter very adversely, causing an increase up to 81.8%. The EO contamination did not have a significant effect. The biopreparation used most often decreased the percentage of damaged leaves, which was most noticeable in the EO contaminated soil. Both EO and DF caused a significant reduction of the areas of the leaf eaten away by adult sitona weevils (F=22.218, p<0.0001) (Fig. 1B). The P contamination, however, resulted in a significant increase in the analyzed parameter. In the EO and P treatments, ZB-01 applied contributed to a reduction in the area of feeds. In the case of control soil, the application of ZB-01 resulted in a significant increase of the area of leaf surface eaten by weevils.

The greatest loss of leaf blade area in result of feeding on them by sitona weevils occurred in plants growing on P contaminated soil, whereas the lowest in plants growing on DF contaminated soil (Fig. 1C). However, the difference compared with the control was significant only in the first case (F=20.585, p<0.0001). EO did not exert a significant effect on the parameter analyzed. In the soils contaminated by EO or P, the use of ZB-01 contributed to a significant reduction in the leaf blade losses (by ca. 50% and 25%, respectively). In the control treatment there was a reverse relationship which was also proved by statistical analysis. After applying the biopreparation, the loss of leaf blade in the control treatment increased by more than 25%.

All the PDPs applied in the experiment contributed to the reduction in numbers of root nodules developed by plants, compared with the control treatment (Fig. 2A). Significant differences were found in the treatments with soil contaminated by DF and EO. In EO treatment, the number of root nodules per plant was nearly three times lower than in plants growing on uncontaminated soil. The biopreparation applied has not exerted a significant effect on the parameter analyzed.

The highest number of root nodules with traces of foraging by the larvae of sitona weevils, compared with their total number per plant, was noted in plants growing on EO contaminated soil (35.4%, Fig. 2B). Significant differences compared with these in the control were also found in DF treatment. The biopreparation used in the majority of cases

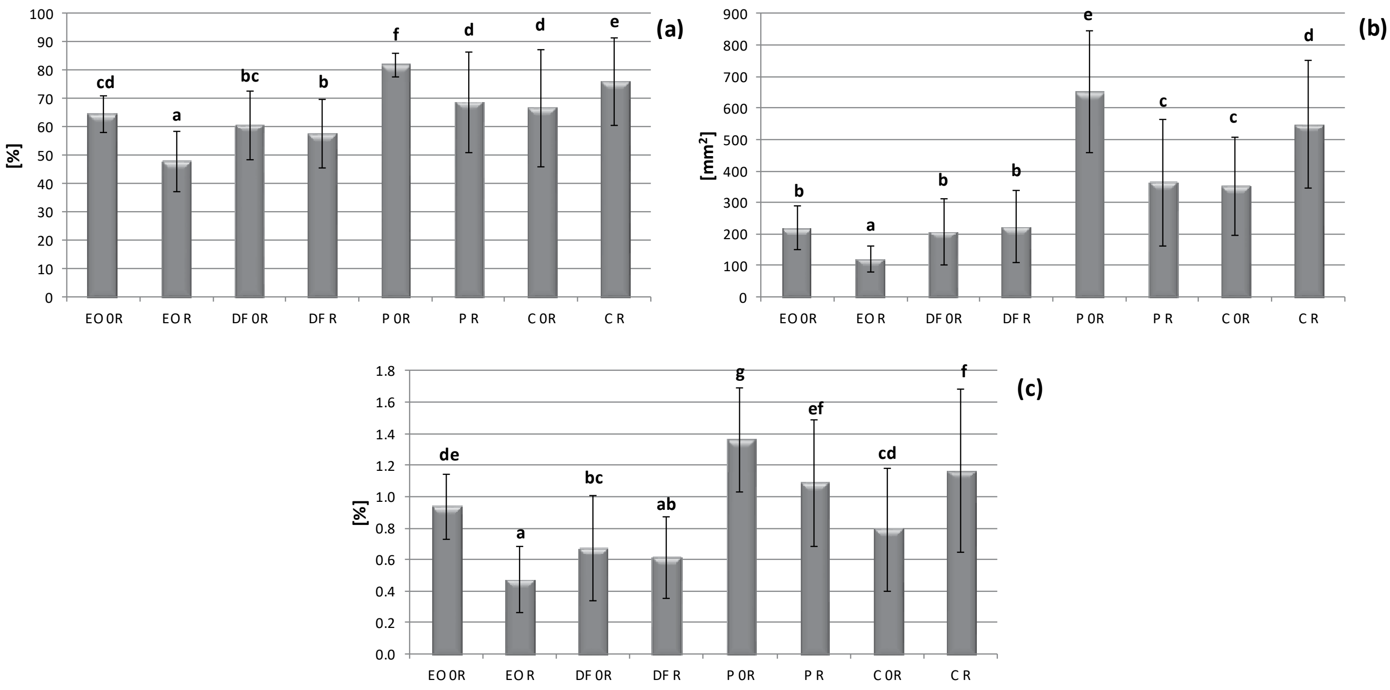

Fig. 1. The effect of PDPs on: (a) – the amount of leaves damaged by *Sitona* spp. [% of total number of leaves per plant], (b) – the eaten away areas of the leaf caused by *Sitona* spp. per 1 plant [mm^2], (c) – the loss of leaf blade caused by *Sitona* spp. [%]. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation. Values marked by different letters are statistically different (p<0.05).

contributed to the decrease in the number of damaged root nodules. However, statistically significant differences were noted only in the EO contaminated soil. In this treatment the drop in percentage of damaged root nodules almost doubled after applying the biopreparation.

Only a small number of studies have been done to date on the effect of PDPs on the phytophagous invertebrates (Rusin et al. 2017, Martin and Swenson 2018). Grass shrimp and amphipods consumed significantly less plant material (leaves of *Ruppia maritima*), which was exposed to high oil concentration in comparison to medium, low or none contamination (Martin and Swenson 2018). Analyzing the effects of soil pollutants on herbivores, the studies most often focused on the effect of heavy metals. However, PDPs can also contain in their composition significant amounts of heavy metals. Most studies on this topic indicated an adverse impact of heavy metals on feeding by phytophages (Jhee et al. 2006, Jiang et al. 2021). Such adverse effect was visible also in our investigations regarding to the feeding of adult stage.

The weaker feeding of adult *Sitona* spp. on plants growing on soils contaminated by PDPs could result from poor condition of plants, their weakened growth, reduced quantities of macroelements and, in result, the lower quality of food for herbivores (Ogboghodo et al. 2004). Literature data shows that phytophagous insects most often colonize healthy plants, which have an intensive green color, testifying to the high level of nitrogen content (Himanen et al. 2008). In plants growing on contaminated soils, there are frequent chloroses and chromatoses caused by a lowered content of chlorophyll (Wyszkowska et al. 2006) which may modify the pests' process of invading plants.

The larvae of sitona weevils live under the ground surface whereas the adult individuals lay eggs into the soil, and stay on its surface for a long time. This fact may support the notion that PDPs present in soil can affect the insects not only indirectly (via a host plant), but also directly. PDPs can contribute to the reduction of soil invertebrates (Pennings et al. 2014), however, in our research the percentage of root nodules damaged by larvae was high in contaminated soils. It can prove high resistance of this stadium to pollutants. The other explanation may be activating defensive mechanism to eliminate toxic effect of PDPs associated with the increased food requirement. Still, it is worth noticing that we analyzed the effect after three years from the moment of contamination.

Only sparse data is available in publications on the effect of the bioremediation process on invertebrates and on herbivores in particular. Most of these studies show positive influence of such activities, which in general agrees with our findings. Dorn and Salanitro (2000) proved that the bioremediation of soil contaminated by PDPs, using organic carbon, resulted in the reduced mortality of earthworms, and the effectiveness of this process depended on the duration of exposure, type of soil, and the type of contaminant. Applying a biopreparation containing prokaryotic microorganisms may result in increasing numbers of nematodes (Louati et al. 2015). Other authors studied the effect of the bioremediation process on aquatic organisms. Tsutsumi et al. (2000) found that the use of bacteria to purify waters from PDPs did not adversely affect the survivability of freshwater fish, whereas the addition of nutrients to contaminated sea waters resulted in the increase in numbers of nematodes and macroorganisms (Schratzberger et al. 2003). The only available studies related to terrestrial herbivores and remediation of oil polluted soil were conducted on 14 reclaimed oil well sites in northern Great Plains rangelands (2–33 yr since reclamation). They revealed successfully recovered abundances and biomass of arthropod herbivores and predators as a result of reclamation efforts, but also changes in community composition of one group of phytophagous insect, i.e. Orthoptera (Sylvain et al. 2019).

Growth and the content of selected macrocomponents in plants

Broad bean plants growing on EO and DF contaminated soils were characterized by a significantly lower average length of shoots (by about 10 cm) and a lower mass of vegetative parts (by about 45 g) as compared to plants growing in control (Table 1). However, PDPs did not affect the average number of leaves on the shoots and the mass of generative parts. Biopreparation ZB-01 most often did not affect the analyzed morphological characteristics of plants and only shoots of plants growing on DF contaminated soil after using biopreparation were significantly longer (by more than 20 cm) than the shoots of plants growing

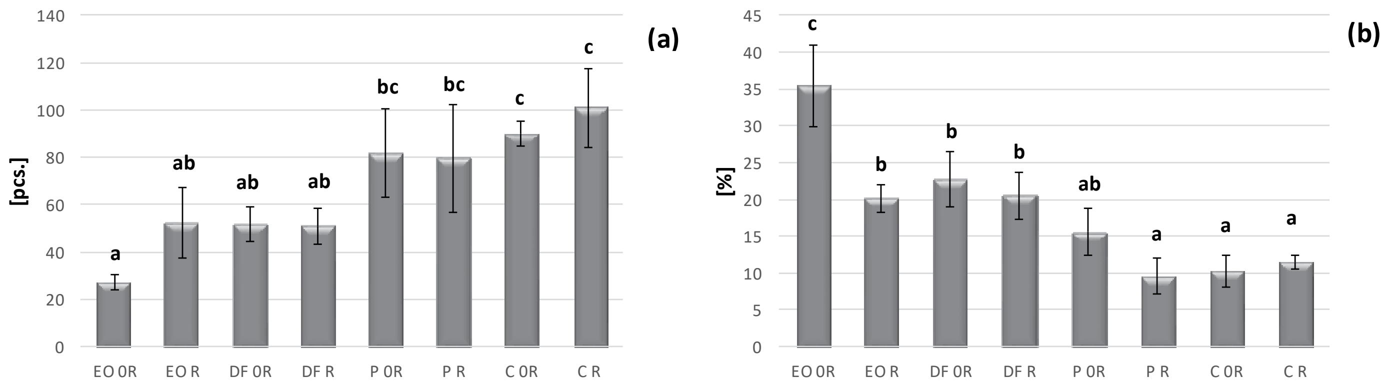

Fig. 2. The effect of PDPs on: (a) – the number of root nodules per 1 plant [pcs.], (b) – the amount of root nodules damaged by the larvae of *Sitona* spp. [% of total number of root nodules per 1 plant]. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation. Values marked by different letters are statistically different ($p<0.05$).

in soil without its use. These results indicate the sensitivity of broad bean plants to this type of soil contamination, and also confirm the durability of PDPs in the soil environment (their negative effect on plant growth may persist even for several years after contamination).

Calcium is a basic nutrient for plants. It performs a structural function, as it is a component of cell walls, activates enzymes, neutralizes organic acids, boosts resistance, reduces the phenomenon of plant lodging, and affects the growth and development of root systems (Hepler 2005). The contamination of soil with PDPs significantly affected the Ca content of broad bean leaves (F=341.84, p<0.00001) (Table 2). Among all the substances applied, DF contributed to the greatest decrease in the content of this element. In the leaves of plants growing on soil contaminated with this substance, there was over 10% less of this element than in plants growing on the control soil. P also contributed to a significant decrease in Ca content. Our previous research on the effect of different doses of PDPs on the composition of broad bean leaves (Rusin et al. 2017) also showed that soil contamination with DF and P at a dose of 9 g kg^{-1} causes a reduction in the content of Ca by 35% and 18% respectively. It should be emphasized, however, that the effect of PDPs on the content of this macronutrient may differ depending on the plant species and a dose of pollutant. Wyszkowski et al (2020) found that DF doses at the level of 5 and 10 ml kg^{-1} d.m. of soil do not significantly affect the content of Ca in the above-ground parts of oat, while the dose of 15 ml kg^{-1} caused a significant increase in its content. Current experiment has shown that EO caused a significant increase in Ca content in leaves that amounted to 4,427.4 mg kg^{-1}. In DF and P contaminated soils ZB-01 caused an increase in the content of analyzed nutrient, whereas in control and EO treatment it led to its decrease. Thus, the impact of ZB-01 in this case resulted in a reversal of trends caused by the impact of individual PDPs. A similar leveling effect in relation to Ca content was observed when sewage sludges were used for remediation of DF contaminated soils (Wyszkowski et al. 2020).

Magnesium is a principal component of chlorophyll as well as an activator of many enzymes involved in biochemical processes, e.g., respiration. It also regulates the pH level in plant cells, and is responsible for accumulating sugars in plants (Bose et al. 2010). Compared with the control treatment, DF and P contributed to a significant increase in Mg content in leaves of broad bean (F=21.785, p=0.00001). Still, EO had no significant effect on this nutrient. Gospodarek and Nadgórska-Socha (2016) in their pot experiment with the dose of 6 g kg^{-1} showed that PDPs generally cause a decrease in Mg content in broad bean leaves, except for DF. This pollutant contributed to a significant increase in the content of Mg by nearly 200 mg kg^{-1} compared to the control. In the present experiment, DF caused an increase by 65 mg kg^{-1}. However, Wyszkowski et al. (2020) found a lower Mg content in oats after applying 5, 10 or 15 ml of DF kg^{-1}d.m. of soil. The present experiment showed that applying ZB-01 contributed to an increase in Mg content in all treatments. However, significant differences were noted in plants growing on soils contaminated with DF and P. Only a few studies have been conducted to date on the effect of the remediation upon the Mg content in plants. Wyszkowski and Ziółkowska (2009) proved that the addition of compost to the contaminated soil led to the increase in the Mg content in spring rape, growing on soil contaminated with P and EO. A similar effect was obtained in oats leaves by adding sewadge sludges to DF contaminated soil (Wyszkowski et al. 2020). However, bentonite and calcium oxide added to soil contaminated with P and EO caused a reduction of Mg content in oats (Wyszkowski and Ziółkowska 2009).

Iron is an indispensable element for the normal growth and development of plants, primarily because of its role in metabolic processes (Thomine and Lanquar 2011). It functions as an electron carrier in respiration and photosynthesis, participates in the production and detoxification of oxygen radicals, as well as in the course of many chemical reactions (Buckhout and Schmidt 2010). The Fe content in broad bean leaves in our experiment ranged from 109.17 to 150.84 mg kg^{-1} of dry mass. Compared with the control, EO contributed to a significant increase of this nutrient content, whereas DF – to its decrease (F=11.249, p=0.0032). Petrol had no significant effect on Fe content. Different results were obtained in the studies conducted by Odjegba and Atebe (2007) who found that soil contamination with increasing doses of EO caused a proportional decrease in Fe content in *Amaranthus hybridus* L shoots. However, the authors conducted analyzes after 8 weeks from the soil contamination, while in this experiment

Table 1. The effect of PDPs on the selected morphological characteristics of broad bean. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation

Treatment	The average length of shoot [cm]	The average number of leaves on shoot [pcs.]	The fresh biomass of vegetative parts [g]	The fresh biomass of generative parts [g]
EO 0R	85.62 (±22.9)[a*]	72.78 (±16.6)[a]	151.99 (±30.0)[ab]	120.74 (±4.0)[ab]
EO R	93.64 (±13.8)[ab]	75.75 (±15.8)[ab]	149.69 (±5.2)[a]	140.06 (±8.1)[ab]
DF 0R	87.83 (±14.1)[a]	73.28 (±10.6)[a]	152.53 (±12.9)[ab]	108.75 (±6.2)[a]
DF R	108.24 (±11.0)[c]	84.49 (±21.5)[a]	188.44 (±30.2)[abc]	172.98 (±30.8)[ab]
P 0R	102.02 (±10.3)[bc]	106.72 (±17.2)[b]	218.60 (±28.4)[c]	193.40 (±34.0)[b]
P R	109.72 (±20.0)[c]	107.79 (±19.0)[b]	195.34 (±30.9)[bc]	163.38 (±30.2)[ab]
C 0R	108.55 (±17.6)[c]	93.41 (±11.4)[ab]	209.46 (±11.2)[c]	159.28 (±41.7)[ab]
C R	97.51 (±12.9)[abc]	84.72 (±16.2)[ab]	196.47 (±38.2)[c]	148.41 (±19.8)[ab]

* Values ± SE marked by different letters in columns are statistically different (p<0.05).

the subsequent effect of soil contamination was examined after 3 years. The biopreparation used in EO contaminated soil contributed to a significant decrease in Fe content by more than 25 mg kg^{-1} of dry mass, whereas in the soil contaminated by DF – to its increase by nearly 15 mg kg^{-1}, which brought Fe contents to values similar to that in the control. Thus, as in the case of Ca, it was possible to notice a positive (reducing changes caused by PDPs) effect of ZB-01.

The N/S ratio informs of the degree to which plants are supplied with sulphur and its value in vegetative plant organs should be around 15/1 (Jamal et al. 2010). In the conducted experiment, the value of this indicator was slightly higher than optimal. All PDPs caused a significant decrease in the N/S ratio in broad bean leaves (Table 2). This may be due to the reduction of nitrogen content in leaves under the influence of PDPs. Possible causes of such a phenomenon (also noted by other authors (Wyszkowski and Ziółkowska 2009, Wyszkowski et al. 2020)) include an increase in the activity of microorganisms in contaminated soil, which causes their greater demand for nitrogen. Moreover, in soil contaminated with PDPs, denitrification bacteria develop more intensively at the expense of nitrifying bacteria, which, in turn, leads to a reduction in the amount of available nitrogen (John et al. 2010). DF and P in present experiment led to a decrease in the Ca/Mg ratio by 0.57 and 0.38 respectively. It resulted from the reduction of the Ca content with a simultaneous increase in the Mg content in plants from these treatments. A reverse relation was noted in the case of EO, which caused a significant increase in the Ca/Mg ratio by 0.18 compared to the control. This, in turn, resulted from the increased Ca content with no significant changes in the Mg content in EO contaminated plants. The used ZB-01 biopreparation generally caused a significant decrease in the value of the analyzed indicators, both after applying it to the soil contaminated with PDPs and to the control soil (Table 2).

Relationships between Sitona spp. feeding, soil contamination with PDPs and broad bean chemical composition

PCA of *Sitona* spp. feeding and soil contamination with PDPs showed that the 1st and 2nd ordination axes explain together 44.79 percentage of variation, dividing the samples into three sets (set one: EO R, DF R, DF 0R; set two: EO 0R; set three: C R, C0R, P R) (Fig. 3). Damages of leaves were negatively correlated with the second ordination axis, whereas the number of nodules and damaged nodules were correlated with the first ordination axis. Those last parameters were negatively correlated. The PCA confirmed negative influence of oils (DF and EO) on feeding and the production of root nodules by plants, while larval feeding was favored by these pollutants.

PCA of macroelements and trace element levels in *V. faba* leaves allowed to identify two main ordination axes, explaining in total 53.23% of the variability of the analyzed samples (Fig. 4). The C, N, and S contents were the most negatively correlated with the first ordination axis, whereas the Mn and Fe contents – with the second ordination axis. The Cd content was positively correlated with the first axis.

Multiple regression revealed that Zn, Mg, Ni and S had negative effects on feeding of adult individuals while positive effect was exerted by N (Table 3). The percent of damaged nodules was negatively correlated with N, and positively with Mg, Ni, and C (Table 3).

The difference in the particular nutrient level in host plants will play a vital role in attractiveness of a plant for herbivores. In the present experiment, plants growing on EO contaminated soil, both with and without biopreparation, were characterized by lower N content. Moreover, plants from EO treatment without using biopreparation also contained more of some heavy metals such as Pb, Zn or Mn and were characterized by a higher content of Ca and Fe (Fig. 4). Plants from the DF contaminated soil contained more Ni, Pb, Cu, and Mn as well as Mg in the leaves. Among these elements, especially N content is regarded as an indicator of plant quality and also one of the most important performance limiting factors of herbivores (Lu et al. 2005). Its effect on sitona weevils feeding was confirmed by multiple regression. This macronutrient may not affect insect biology directly but change the host-plant morphology, physiology, and biochemistry, which can improve nutritional conditions for herbivores. Nitrogen is a major kind of herbivore feeding stimulant and plays a key role in preference patterns as well as in modifying and reducing host resistance to herbivores (Rashid et al. 2016, Martin and Swenson 2018). The lower N content in plants growing on soil contaminated with EO and DF could indirectly affect the reduction of sitona weevils feeding. In addition, plants containing smaller amounts of N produce significant quantities of secondary metabolites that are toxic and repellent to pests (Lou and Baldwin 2004).

Table 2. The effect of PDPs on the content of calcium, magnesium, iron [mg kg^{-1}] and on the ratio of nutrients in broad bean's leaves. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation.

Treatment	Ca	Mg	Fe	N/S	Ca/Mg
EO 0R	4427.36 (±8.1) ^{e*}	1201.59 (±41.6) ^{a}	150.84 (±14.9) ^{d}	19.17 (±3.2) ^{bc}	3.69 (±0.9) ^{f}
EO R	2966.23 (±82.0) ^{a}	1218.00 (±37.1) ^{ab}	125.35 (±4.8) ^{bc}	17.76 (±2.8) ^{a}	2.44 (±0.3) ^{a}
DF 0R	3778.86 (±99.3) ^{b}	1284.10 (±37.3) ^{cd}	109.17 (±3.3) ^{a}	19.46 (±1.3) ^{bc}	2.94 (±0.7) ^{c}
DF R	4001.28 (±39.6) ^{c}	1510.89 (±14.0) ^{f}	124.10 (±4.0) ^{b}	20.35 (±3.4) ^{c}	2.65 (±0.5) ^{b}
P 0R	4075.05 (±41.5) ^{c}	1303.64 (±18.6) ^{d}	135.74 (±2.6) ^{c}	20.29 (±3.1) ^{c}	3.13 (±0.4) ^{d}
P R	4365.04 (±20.2) ^{de}	1357.10 (±10.5) ^{e}	127.97 (±7.1) ^{bc}	20.11 (±2.7) ^{bc}	3.22 (±0.9) ^{d}
C 0R	4275.00 (±47.0) ^{d}	1218.17 (±0.3) ^{ab}	126.54 (±0.7) ^{bc}	20.50 (±1.9) ^{d}	3.51 (±1.1) ^{e}
C R	3993.34 (±22.2) ^{c}	1253.92 (±5.4) ^{bc}	132.5 1 (±1.4) ^{bc}	19.07 (±2.3) ^{b}	3.18 (±0.8) ^{d}

* Values ± SE marked by different letters in columns are statistically different ($p<0.05$).

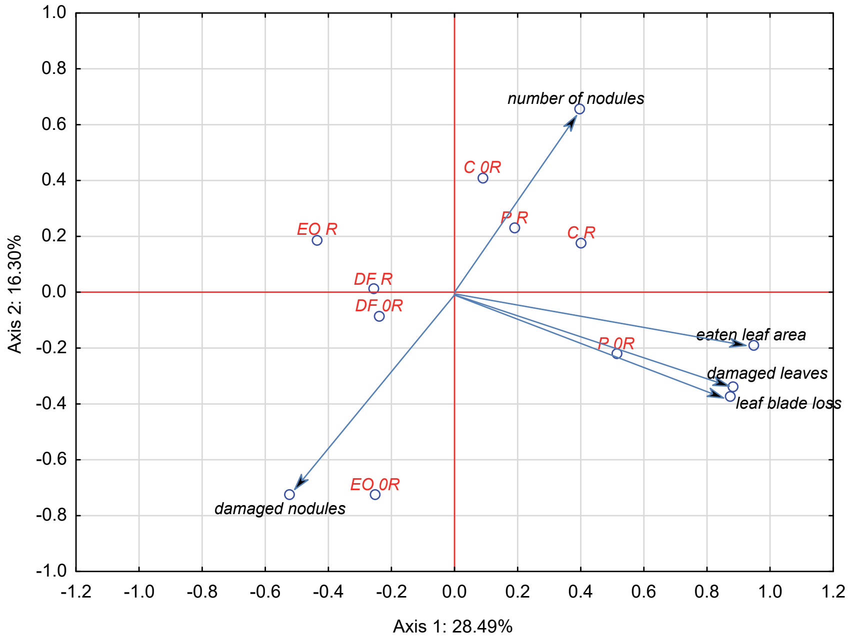

Fig. 3. Principal component analysis of *Sitona* spp. feeding, and soil contamination with PDPs. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation.

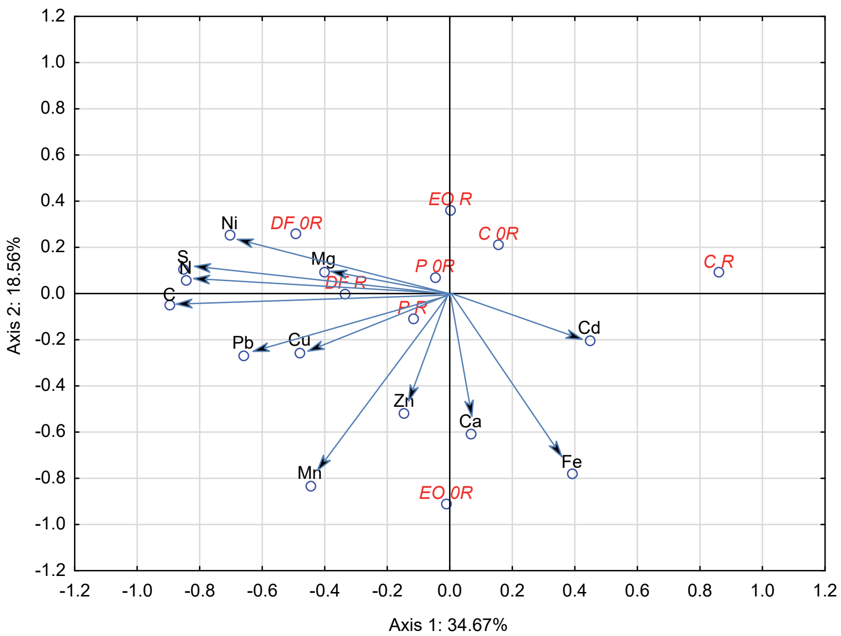

Fig. 4. Principal component analysis of element levels in *V. faba* leaves, and soil contamination with PDPs. EO – soil contaminated with engine oil, DF – soil contaminated with diesel fuel, P – soil contaminated with petrol, C – control soil, 0R – without bioremediation, R – with bioremediation.

Table 3. Multiple regression equations ($p < 0.05$)

Trait	R^2
Damaged leaves = 219.76 — 0.58 (Zn) – 0.28(Mg) – 0.49 (Ni) – 2.81 (S) + 1.70 (N)	0.96
Eaten leaf area = 3511.78 – 0.66 (Zn) – 0.47 (Ni) – 3.11 (S) + 1.86 (N)	0.95
Leaf blade loss = 6.08 – 0.68 (Zn) – 0.42 (Mg) – 0.51 (Ni) + 0.79 (Mn) – 2.68 (S) + 2.04 (N)	0.96
Number of nodules = 424.07 – 0.16 (Zn) – 0.25 (Mg) – 0.12 (Fe) – 0.26 (Ni) – 1.02 (S) + 2.98 (N) – 2.28 (C)	0.99
Damaged nodules = —95.54 + 0.27 (Mg) + 0.14 (Ni) – 4.04 (N) + 3.84 (C)	0.98

It should be emphasized that the present experiment was conducted in field conditions that allowed sitona weevils to unlimited choice of plants as a food source. More feeds on non-polluted plants with higher nitrogen content could have been the result of their greater attractiveness for adult *Sitona* spp. at the moment of host-plant searching. In an experiment with water invertebrates, which were offered a choice between plants with lower and higher share of nitrogen (in comparison to carbon) they chose more often the latter one (Martin and Swenson 2018). Unlike in our experiment, these were plants exposed to oil pollution. Thus, the reaction to nitrogen content was the same as in our experiment, except that the host plant (in this case *Ruppia maritima*) reacted to the presence of oil pollution with an increased nitrogen share in comparison to carbon. In our experiment C/N ratio remained similar in polluted and control treatments. This suggests plant species--specific response. Reported in the present experiment increase of some heavy metal contents and changes in S and Mg level could also affect the limitation of sitona weevils feeding in EO and DF treatments.

The high attractiveness of plants growing on soil contaminated with P for the adult sitona weevils is worth noticing. The chemical composition of plant from this treatment was characterized by a low content of Ni, Zn, Cd but elevated content of Pb and Mg and differed with control plants although the analysis of TPH in this soil did not reveal a significant difference comparing to the control soil. The N/S ratio in this treatment was higher than in EO and DF, which again suggests a beneficial effect of higher content of nitrogen in host-plant. Moreover, Pb content was in P-exposed plants only two times higher than in control plants while in EO and DF treatments these values were almost three and four times higher, respectively. Furthermore, lack of differences in TPH content in the soil, with significant differences found at the same time in plant and herbivore responses, confirms that it is not sufficient to rely solely on soil analysis in the PDPs environmental impact assessment.

The results obtained could also be modified with additional factors that cannot be avoided under field conditions, e.g., predators response. Sitona weevils do not have specific predators, however, they can be eaten by polyphagous species. Thus, future efforts need to verify possible influence of PDPs and bioremediation on further trophic chain links, i.e., predators and parasitoids.

Summarizing, our results showed that the influence of PDPs on the environment is long-lasting and these pollutants may affect also further links of the trophic chain, i.e., herbivores, in various ways. At the same time, it is worth noticing that bioremediation not only accelerates soil cleaning but also improves the nutrient balance in plants disturbed by the presence of PDPs thus reducing some effects on herbivores (*Sitona* spp. larvae feeding). As far as we know this is the first experiment in which indirect effect of PDPs in soil and their bioremediation on host plant and a herbivore was considered.

Conclusions

1. After 3 years from soil contamination, engine oil and diesel oil limited feeding of adult sitona weevils while petrol caused increase in the attractiveness of plants for these insects. In addition, oils caused a reduction in the number of root nodules developed by plants and also contributed to more intensive damage of nodules by sitona weevils larvae, which indirectly may indicate the resistance of larvae to the presence of PDPs in their living environment.
2. The PDPs negatively affected the growth of vegetative parts of broad bean. Diesel oil contributed to the decrease in Ca and Fe content in the leaves of broad bean plants but resulted in an increase in Mg content. Engine oil contamination led to the increase in Ca and Fe content, whereas petrol caused the increase in Mg but a decrease in Ca content. The negative relationships between the contents of both macroelements (Mg, S) and heavy metals (Zn, Ni) as well as feeding of adult individuals of *Sitona* spp. were observed. Changes in the chemical composition of plants might contribute to the deterioration of the nutritional quality of the host plant, and thus cause a decrease in their attractiveness for the adult stage of sitona weevils.
3. In general, the biopreparation ZB-01 eliminated or reduced the differences caused by the presence of PDPs in the soil regarding the chemical composition of the host plant, and limited feeding of both the larvae and adult individuals of sitona weevils on plants exposed to pollution.
4. The obtained results indicate that PDPs remain for a long time in the environment and adversely affect not only the organisms directly exposed to the pollution, i.e., plants growing on polluted soil, but also further links of the trophic chain, i.e., herbivores. A proposed new multi-trophic approach (evaluation of soil, plant and herbivore) may be helpful in developing a more comprehensive understanding of both PDPs and bioremediation impact on ecosystems.

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Następczy wpływ produktów ropopochodnych w glebie i ich bioremediacji na interakcję roślina – roślinożerca

Streszczenie: Celem badań było określenie następczego (tj. po trzech latach od momentu zanieczyszczenia gleby) wpływu produktów ropopochodnych (benzyny, oleju napędowego i zużytego oleju silnikowego) na interakcję między wybraną rośliną (bób *Vicia faba* L.) i blisko z nią związanym owadem roślinożernym (oprzędzik – *Sitona* spp.). Oceniono stan rośliny narażonej na działanie poszczególnych zanieczyszczeń (tzn. jej wzrost i skład chemiczny), a następnie określono atrakcyjność rośliny zarówno dla larw, jak i postaci imaginalnych oprzędzików. Ocenie poddano również wpływ bioremediacji z użyciem biopreparatu ZB-01 na wymienione parametry. Stwierdzono, że po 3 latach od zanieczyszczenia gleby olej silnikowy i olej napędowy ograniczały żerowanie dorosłych oprzędzików, natomiast benzyna przeciwnie – spowodowała wzrost atrakcyjności roślin dla tych owadów. Ropopochodne negatywnie wpłynęły na wzrost roślin bobu, natomiast zawartość pierwiastków w roślinach była zróżnicowana i zależała od rodzaju zanieczyszczenia, na które były narażone. Biopreparat ZB-01 zniwelował lub wyraźnie zmniejszył różnice spowodowane obecnością ropopochodnych w glebie w odniesieniu do składu chemicznego roślin oraz ograniczył żerowanie zarówno larw, jak i dorosłych postaci *Sitona* spp. Zaobserwowano negatywne zależności pomiędzy zawartością zarówno niektórych makroelementów (Mg, S), jak i metali ciężkich (Zn, Ni) a żerowaniem imago oprzędzików. Uzyskane wyniki potwierdzają, że ropopochodne wykazują długotrwałe negatywne oddziaływanie na środowisko i wskazują, że mogą one niekorzystnie wpływać nie tylko na organizmy bezpośrednio narażone na zanieczyszczenia – rośliny rosnące na zanieczyszczonej glebie, ale także na dalsze ogniwa łańcucha troficznego, czyli roślinożerców.