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# Comparison of selenite (IV) and selenate (VI) effect on some oxidoreductive enzymes in soil contaminated with spent engine oil

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**ABSTRACT**

This paper assesses the impact of spent engine oil on activity of dehydrogenase, nitrate reductase, catalase and *o*-diphenol oxidase in sandy soil, and evaluates biostimulation with selenates in the restoration of homeostasis of soil with spent engine oil. The experiment was carried out on loamy sand samples with organic carbon content of 8.71 g/kg, with the following variable factors: dose of spent engine oil: 0, 2, 10, 50 g/kg dry matter (DM) of soil; selenate application: without selenate, selenite (IV) and selenate (VI) in the amount of 0.05 mmol/kg DM of soil; day of experiment: 1, 7, 14, 28, 56, 112. Obtained results showed that spent engine oil increased activity of dehydrogenase and catalase. Application of selenite (IV) and selenate (VI) to soil non-contaminated with spent engine oil stimulated activity of dehydrogenase and nitrate reductase and inhibited in *o*-diphenol oxidase. Among selenates tested regarding biostimulation of oxidoreductases in soil contained spent engine, selenate (VI) is more useful than selenite (IV).

**Keywords**: petroleum hydrocarbons; soil enzymes; selenium; remediation

Selenium (Se) is one of the most controversial trace elements in the world. It is chemically similar to sulfur (S), and as a result, plants and other organisms readily take up and metabolize Se via S transporters and pathways. Since replacement of S by Se in proteins and other S compounds disrupts the function of these molecules, Se is toxic at elevated levels to most organisms (Pilon-Smith and Quinn 2010). On the other hand, Se is an essential trace element for many organisms including mammals, many bacteria, and certain green algae (Fu et al. 2002). These organisms contain the selenoproteins, which invariably have antioxidant functions, including the scavenging of reactive oxygen species (Cartes et al. 2011). Higher plants do not appear to require Se, they readily take it up from their environment and incorporate it into organic compounds using S assimilation enzymes (Pilon-Smith and Quinn 2010). However many researchers showed a positive effect of Se in detoxication of reactive oxygen species and its positive impact on antioxidant enzymes in different plant species (Nowak et al. 2004, Cartes et al. 2011, Hermosillo-Cereceres et al. 2014).

Our previous studies also showed that the selenium application to soil contaminated with gasoline and diesel oil may limit influence of petroleum hydrocarbons on some soil oxidoreductive enzymes (Stręk and Telesiński 2015a) and on soil antioxidant capacity (Stręk and Telesiński 2015b). This could be important, because the petroleum compounds do not have active functional groups, and they are mainly degraded by microorganisms and oxidoreductive enzymes (Stręk and Telesiński 2015a).

Spent engine oil is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain (Achuba and Peretiemo-Clarke 2008). It is a mixture of several different chemicals, including low and high molecular weight (C15–C20) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products or heavy metal from engine wear (Wang et al.

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2000). Spent engine oil, when present in the soil, increases bulk density and decreases water holding capacity and aeration propensity, which creates unsatisfactory conditions for soil organisms (Nwite and Alu 2015).

The aim of the research was to assess soil oxidoreductase changes in soil contaminated with spent engine oil, and to compare selenite (IV) and selenate (VI) effect in limiting the impact of hydrocarbons on soil oxidoreductases.

## MATERIAL AND METHODS

The testing was performed on a soil material taken from the topsoil of Brunic Arenosol in Agricultural Experimental Station in Lipnik (53°24'N, 14°28'E), located in the West Pomeranian District, Poland. According to the classification of the United States Department of Agriculture, it was soil with a granulometric composition of loamy sand. The content of particular fractions, expressed in g/kg, was as follows: sand (0.05–2 mm) – 748.6; silt (0.002–0.05 mm) – 231.3; and clay (< 0.002 mm) – 20.1. The soil contained, in g/kg: $C_{org}$ – 8.71; $N_{tot}$ – 0.97. Its hydrolytic acidity was 9.9 $mmol_+$/kg, and the pH value in 1 mol/L KCl was 6.4. The soil was air-dried soil and sieved with a mesh size of 2 mm.

The experiments were carried out in triplicate under laboratory conditions, with the following variable factors: (A) dose of spent engine oil: 0, 2, 10, 50 g/kg dry matter (DM) of soil; (B) selenate application: without selenate, selenite (IV) and selenate (VI) in the amount of 0.05 mmol/kg DM of soil; (C) day of experiment: 1, 7, 14, 28, 56, 112. The 1-kg soil samples were adjusted to 60% maximum water holding capacity, and they were incubated in tightly closed glass containers at a temperature of 20°C.

The properties of spent engine oil are presented in Table 1.

During the experiment, the activity of oxidoreductases was measured six times (on day 1, 7, 14, 28, 56 and 112) in the soil samples from each repetition in 3 subsequent replications: the dehydrogenase activity was determined using the method described by Thalmann (1968), the *o*-diphenol oxidase activity was measured according to procedure of Perucci et al. (2000), the nitrate reductase was determined as described by Abdelmagid and Tabatabai (1987), catalase activity was measured according to Johnson and Temple (1964) method.

Table 1. Properties of spent engine oil

| Viscosity at 40° | Specific gravity | Total acid number | Content of aliphatic hydrocarbons (%) |
|---|---|---|---|
| 33.21 | 0.85 | 0.67 | 88.76 |

Based on the activity of assayed soil oxidoreductases, indices of the spent engine oil and selenate effects were calculated using the following formulas (Kaczyńska et al. 2015):

$$IF_{EO} = \frac{A_{EO}}{A_0}$$

$$IF_{Se} = \frac{A_{Se}}{A_{EO}}$$

Where: $IF_{EO}$ – index of the spent engine oil effect; $IF_{Se}$ – index of selenate effect; $A_{EO}$ – activity of oxidoreductases in the soil contaminated with the spent engine oil; $A_0$ – activity of oxidoreductase in the non-contaminated soil; $A_{Se}$ – activity of oxidoreductases in the soil treated with selenate.

If IF = 1, there is no influence of the tested factor on oxidoreductases; IF < 1, there is inhibition of the oxidoreductases activity by the tested factor and IF > 1, there is stimulation of the oxidoreductases activity by the tested factor.

The results of the studies were determined statistically using a statistical software package Statistica v. 12.0 (Statsoft, Inc., Krakow, Poland). Based on the analysis of the effect measure $\eta^2$ by variance analysis – ANOVA – the percentage shares of all variable factors affecting the activity of oxidoreductases were defined. Homogeneous groups were calculated using the Tukey's test with $P < 0.05$. The diversified influence of the spent engine oil on the activity of oxidoreductases was illustrated using the index of the spent engine oil effect and that of influence of selenate – using the index of selenate effect.

## RESULTS AND DISCUSSION

The activities of oxidoreductases in soil non-contaminated with spent engine oil were 2.72–4.46 mg TPF (triphenylformazan)/kg DM/16 h, 0.11–0.19 mg $NO_2^-$-N/kg DM/24 h, 1.75–2.71 mmol $H_2O_2$/kg DM/min and 2.78–3.22 mmol oxidated catechol//kg DM/10 min,

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Table 2. Activities of oxidoreductases in soil not contaminated with spent engine oil

| | Selenate application | Incubation time (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 28 | 56 | 112 |
| Dehydrogenase (mg TPF/kg DM/16 h) | 0 | 4.46$^{a}$ | 4.01$^{a}$ | 3.75$^{a}$ | 3.36$^{a}$ | 2.72$^{a}$ | 2.81$^{a}$ |
| | selenite (IV) | 3.36$^{b}$ | 2.78$^{b}$ | 2.91$^{b}$ | 3.17$^{b}$ | 2.46$^{b}$ | 2.26$^{b}$ |
| | selenate (VI) | 4.33$^{a}$ | 2.97$^{b}$ | 3.10$^{b}$ | 3.17$^{b}$ | 2.45$^{b}$ | 2.39$^{b}$ |
| Nitrate reductase (mg $NO_2^-$-N/kg DM/24 h) | 0 | 0.19$^{a}$ | 0.13$^{a}$ | 0.15$^{a}$ | 0.15$^{a}$ | 0.13$^{a}$ | 0.11$^{a}$ |
| | selenite (IV) | 0.17$^{b}$ | 0.11$^{b}$ | 0.13$^{b}$ | 0.10$^{b}$ | 0.07$^{b}$ | 0.07$^{b}$ |
| | selenate (VI) | 0.16$^{b}$ | 0.10$^{b}$ | 0.11$^{c}$ | 0.10$^{b}$ | 0.07$^{b}$ | 0.08$^{b}$ |
| Catalase (mmol $H_2O_2$/kg DM/min) | 0 | 2.71$^{a}$ | 2.30$^{a}$ | 2.53$^{b}$ | 1.75$^{b}$ | 2.25$^{b}$ | 2.15$^{b}$ |
| | selenite (IV) | 2.76$^{a}$ | 2.40$^{a}$ | 2.78$^{a}$ | 1.89$^{a}$ | 2.55$^{a}$ | 2.40$^{a}$ |
| | selenate (VI) | 2.19$^{b}$ | 2.01$^{b}$ | 1.57$^{c}$ | 1.60$^{c}$ | 1.92$^{c}$ | 2.04$^{b}$ |
| *o*-diphenol oxidase (mmol oxidated catechol/kg DM/10 min) | 0 | 2.80$^{b}$ | 2.94$^{c}$ | 3.22$^{c}$ | 3.05$^{c}$ | 2.78$^{c}$ | 2.88$^{b}$ |
| | selenite (IV) | 3.05$^{a}$ | 3.31$^{b}$ | 3.33$^{b}$ | 3.21$^{b}$ | 2.64$^{b}$ | 2.93$^{b}$ |
| | selenate (VI) | 2.89$^{b}$ | 3.81$^{a}$ | 3.44$^{a}$ | 4.36$^{a}$ | 3.08$^{a}$ | 3.06$^{a}$ |

The same letter means a homogenous group in the columns for an enzyme ($P < 0.05$); DM – dry matter; TPF – triphenylformazan

for dehydrogenase, nitrate reductase, catalase and *o*-diophenol oxidase, respectively (Table 2). Application of selenite (IV) caused a decrease in dehydrogenase and nitrate reductase activities in the whole experiment. The inhibitory effect of selenate (VI) was observed in activity of dehydrogenase (except day 1), nitrate reductase and catalase. It is similar with the results obtained by Nowak et al. (2002). They reported a negative effect of selenite (IV) on activity of dehydrogenase and

Table 3. Index of the spent engine oil effect ($IF_{EO}$) on the activity of soil oxidoreductases

| Dose engine oil (g/kg dry matter) | | Incubation time (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 28 | 56 | 112 |
| Dehydrogenase | 2 | 1.59$^{a}$ | 2.01$^{c}$ | 2.33$^{c}$ | 0.87$^{c}$ | 0.50$^{c}$ | 0.48$^{a}$ |
| | 10 | 1.06$^{c}$ | 3.39$^{b}$ | 3.78$^{a}$ | 2.17$^{b}$ | 4.02$^{b}$ | 0.64$^{a}$ |
| | 50 | 1.35$^{b}$ | 3.74$^{a}$ | 3.43$^{b}$ | 5.29$^{a}$ | 6.32$^{a}$ | 0.46$^{a}$ |
| Nitrate reductase | 2 | 1.06$^{b}$ | 1.06$^{a}$ | 1.00$^{a}$ | 0.61$^{b}$ | 0.59$^{b}$ | 0.66$^{a}$ |
| | 10 | 1.18$^{a}$ | 0.56$^{b}$ | 0.68$^{b}$ | 0.16$^{c}$ | 0.12$^{c}$ | 0.09$^{b}$ |
| | 50 | 0.88$^{c}$ | 1.13$^{a}$ | 1.11$^{a}$ | 1.57$^{a}$ | 1.45$^{a}$ | 0.54$^{a}$ |
| Catalase | 2 | 0.95$^{a}$ | 1.07$^{b}$ | 1.59$^{b}$ | 1.47$^{b}$ | 1.10$^{b}$ | 1.06$^{b}$ |
| | 10 | 1.00$^{a}$ | 1.53$^{a}$ | 1.93$^{a}$ | 2.39$^{a}$ | 1.25$^{a}$ | 1.36$^{a}$ |
| | 50 | 0.70$^{b}$ | 1.51$^{a}$ | 1.65$^{b}$ | 1.43$^{b}$ | 1.13$^{b}$ | 1.01$^{b}$ |
| *o*-diphenol oxidase | 2 | 0.99$^{a}$ | 0.90$^{a}$ | 0.91$^{a}$ | 0.92$^{a}$ | 0.86$^{a}$ | 0.99$^{a}$ |
| | 10 | 0.96$^{a}$ | 0.87$^{a}$ | 0.83$^{b}$ | 0.83$^{b}$ | 0.78$^{b}$ | 0.91$^{a}$ |
| | 50 | 0.96$^{a}$ | 0.79$^{b}$ | 0.72$^{c}$ | 0.70$^{c}$ | 0.70$^{c}$ | 0.71$^{b}$ |

The same letter means a homogenous group in the columns for an enzyme ($P < 0.05$)

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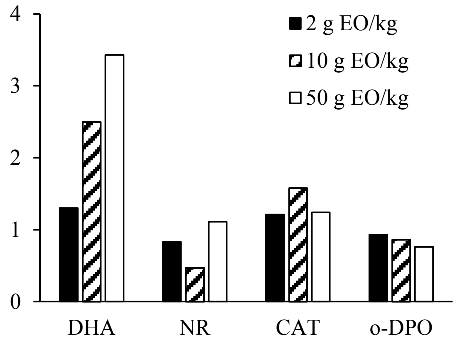


Figure 1. Mean index of the influence of spent engine oil ($IF_{EO}$) on the activity of dehydrogenase (DHA), nitrate reductase (NR), catalase (CAT) and *o*-diphenol oxidase (*o*-DPO) in soil; EO – engine oil

nitrate reductase. According to Hu et al. (2013) catalase activity was inhibited in soil treated with selenium, while Nowak et al. (2004) showed that selenium in low doses increased in catalase activity. In our study, catalase activity was increased from day 14 to day 112 after the treatment with selenite (IV). On almost all test days, activity of *o*-diphenol oxidase was significantly stimulated in soil treated with selenite (IV) (except day 112) and selenate (VI) (except day 1).

Spent engine oil (EO) at all doses caused mainly an increase in dehydrogenase and catalase activities, and a decrease in *o*-dipenol oxidase activity in soil. Effect of EO on nitrate reductase was unclear. Changes of all oxidoreductase activities depended on spent engine oil dose and day of experiment (Table 3).

Summarizing the evaluation of the influence of spent engine oil on soil oxidoreductases, one may ascertain that spent engine oil stimulated the activity of dehydrogenase and catalase (Figure 1). The mean index of the effect of spent engine oil ($IF_{EO}$) on activity of dehydrogenase increased, together with increase in dose of EO, while the highest value of mean $IF_{EO}$ for catalase activity was observed after treatment dose of 10 mg/kg. Many authors reported that addition of spent engine oil to soil was stimulatory to the activity of dehydrogenase, but inhibitory to the activity of catalase (Achuba and Peretiemo-Clarke 2008, Achuba and Okoh 2014, Ramadass et al. 2015). Kaczyńska et al. (2015) showed that soil dehydrogenase is a good indicator of contamination of the environment with petroleum products. The increase of dehydrogenase activity could be due to the involvement of specific microorganisms in the metabolism of polyaromatic hydrocarbons, which

Table 4. Index of the selenite (IV) effects on the activity of soil oxidoreductases in soil contaminated with spent engine oil

| Dose engine oil (g/kg dry matter) | | Incubation time (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 28 | 56 | 112 |
| Dehydrogenase | 2 | $0.54^{c}$ | $0.70^{b}$ | $0.83^{b}$ | $1.09^{a}$ | $1.04^{a}$ | $1.26^{a}$ |
| | 10 | $0.96^{a}$ | $0.84^{a}$ | $0.97^{a}$ | $0.50^{b}$ | $0.56^{c}$ | $0.71^{b}$ |
| | 50 | $0.72^{b}$ | $0.55^{c}$ | $0.93^{a}$ | $0.42^{b}$ | $0.80^{b}$ | $0.50^{c}$ |
| Nitrate reductase | 2 | $0.91^{a}$ | $0.90^{b}$ | $0.79^{b}$ | $0.97^{b}$ | $0.88^{b}$ | $0.97^{b}$ |
| | 10 | $0.87^{b}$ | $1.35^{a}$ | $0.78^{b}$ | $0.57^{c}$ | $1.53^{a}$ | $2.10^{a}$ |
| | 50 | $0.94^{a}$ | $0.75^{c}$ | $0.92^{a}$ | $0.92^{a}$ | $0.66^{c}$ | $0.52^{c}$ |
| Catalase | 2 | $1.02^{a}$ | $1.04^{a}$ | $1.10^{a}$ | $1.08^{a}$ | $1.13^{a}$ | $1.12^{b}$ |
| | 10 | $0.82^{c}$ | $0.92^{b}$ | $0.78^{c}$ | $0.70^{b}$ | $0.98^{b}$ | $0.98^{c}$ |
| | 50 | $0.93^{b}$ | $0.94^{b}$ | $0.90^{b}$ | $1.08^{a}$ | $1.09^{a}$ | $1.28^{a}$ |
| *o*-diphenol oxidase | 2 | $1.09^{a}$ | $1.13^{a}$ | $1.03^{a}$ | $1.05^{a}$ | $1.06^{b}$ | $1.02^{a}$ |
| | 10 | $1.06^{a}$ | $1.01^{b}$ | $1.05^{a}$ | $1.09^{a}$ | $1.02^{b}$ | $1.03^{a}$ |
| | 50 | $1.09^{a}$ | $1.05^{b}$ | $1.06^{a}$ | $1.09^{a}$ | $1.12^{a}$ | $1.08^{a}$ |

The same letter means a homogenous group in the columns for an enzyme ($P < 0.05$)

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Table 5. Index of the selenate (VI) effects on the activity of soil oxidoreductases in soil contaminated with spent engine oil

| Dose engine oil (g/kg dry matter) | | Incubation time (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 28 | 56 | 112 |
| Dehydrogenase | 2 | 0.76[b] | 0.86[b] | 0.99[b] | 1.98[a] | 2.95[a] | 4.04[a] |
| | 10 | 1.25[a] | 1.33[a] | 1.40[a] | 1.95[a] | 1.54[b] | 2.39[c] |
| | 50 | 0.66[b] | 1.20[a] | 1.42[a] | 1.26[b] | 1.25[c] | 3.76[b] |
| Nitrate reductase | 2 | 0.88[c] | 0.75[c] | 0.74[c] | 0.58[c] | 0.43[c] | 0.80[c] |
| | 10 | 1.22[a] | 1.76[a] | 1.46[a] | 4.17[a] | 7.07[a] | 7.30[a] |
| | 50 | 1.08[b] | 1.03[b] | 1.08[b] | 1.03[b] | 1.07[b] | 3.54[b] |
| Catalase | 2 | 0.81[b] | 0.87[a] | 0.62[b] | 0.91[b] | 0.85[b] | 0.95[b] |
| | 10 | 0.65[c] | 0.82[a] | 0.74[a] | 0.65[c] | 0.90[b] | 0.73[c] |
| | 50 | 0.91[a] | 0.51[b] | 0.59[b] | 1.35[a] | 1.22[a] | 1.32[a] |
| *o*-diphenol oxidase | 2 | 1.03[b] | 1.30[a] | 1.07[b] | 1.43[a] | 1.11[b] | 1.06[b] |
| | 10 | 1.33[a] | 1.07[c] | 1.12[b] | 1.33[b] | 1.05[b] | 1.08[b] |
| | 50 | 1.28[a] | 1.14[b] | 1.24[a] | 1.24[c] | 1.24[a] | 1.21[a] |

The same letter means a homogenous group in the columns for an enzyme ($P < 0.05$)

could serve as a carbon source for the microbial growth (Margesin et al. 2000). Oxidoreductases play an important role in energy transformation in the respiration chain and participate in the synthesis of soil humics and in the soil formation process (Rao et al. 2010). Polyphenol oxidases, including *o*-diphenol oxidase, mediate biogeochemical processes in soils, including microbial acquisition of carbon and nitrogen, lignin degradation, carbon mineralization and sequestration, and dissolved organic carbon export (Bach et al. 2013). In our study the mean index of spent engine oil for activity of *o*-diphenol oxidase was decreased, while Li et al. (2007) noted the increase in polyphenol oxidase in soil treated with different petroleum hydrocarbons. Mean $IF_{EO}$ for nitrate reductase was lower than 1 only for doses of 2 and 10 g/kg (0.83 and 0.47, respectively), which indicates a negative effect of spent engine oil on this enzyme. Ramadass et al. (2015) reported a decreased effect of spent engine oil in soil nitrification.

Effect of selenates on oxidoreductase activities in soil contaminated with spent engine oil proved diversified, and depended on enzyme, incubation

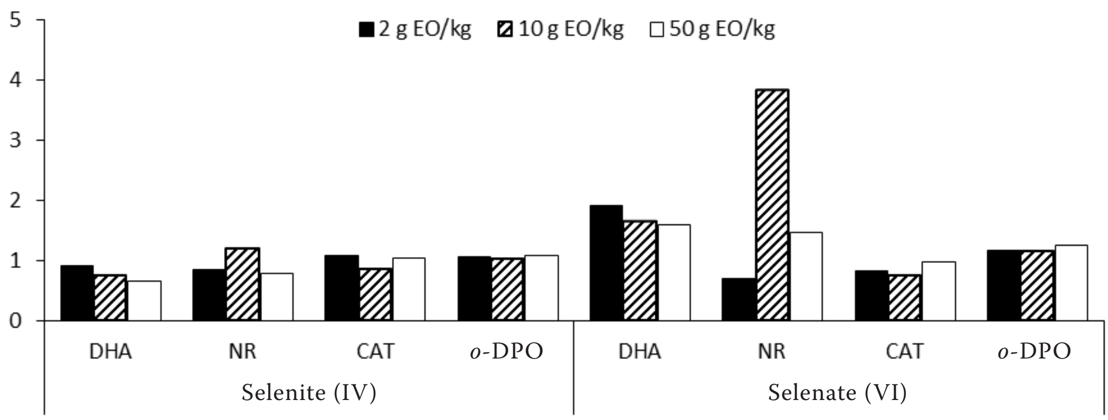


Figure 2. Mean index of the influence of selenate ($IF_{Se}$) on the activity of dehydrogenase (DHA); nitrate reductase (NR); catalase (CAT) and *o*-diphenol oxidase (*o*-DPO) in soil contaminated with spent engine oil (EO)

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Table 6. Participation of variable factors in the formation of oxidoreductase activities (%)

| | DHA | NR | CAT | *o*-DPO |
|---|---|---|---|---|
| EO dosage (A) | 42.43 | 16.65 | 24.40 | 34.91 |
| Selenate (B) | 2.20 | 2.70 | 10.56 | 11.32 |
| Day (C) | 24.79 | 40.22 | 18.11 | 20.36 |
| A × B | 1.81 | 5.06 | 1.23 | 1.46 |
| A × C | 20.95 | 22.25 | 14.93 | 11.34 |
| B × C | 0.91 | 3.17 | 14.68 | 6.38 |
| A × B × C | 3.18 | 7.36 | 8.27 | 9.30 |
| Error | 3.73 | 2.58 | 7.81 | 4.94 |

DHA – dehydrogenase; NR – nitrate reductase; CAT – catalase; *o*-DPO – *o*-diphenol oxidase; EO – engine oil

time, EO dose and form of selenate. On the most days of experiment the selenite (IV) application affected dehydrogenase, nitrate reductase, catalase negative, but *o*-diphenol oxidase favourably (Table 4). However after the treatment with selenate (VI), an increase in dehydrogenase, nitrate reductase, *o*-diphenol oxidase and a decrease in catalase activity was mainly observed (Table 5).

Mean values of index of selenite (IV) effect ($IF_{Se}$) in soil contaminated with spent engine oil above 1 were observed in the activity of nitrate reductase for EO dose of 10 g/kg DM (1.20), catalase for EO doses of 2 g/kg DM (1.06) and 50 g/kg DM (1.04), and *o*-diphenol oxidase for all doses of EO (1.04–1.08). In soil treated with selenate (VI) mean values of $IF_{Se}$ higher than 1 were noted in the activity of dehydrogenase (1.59–1.93) and *o*-diphenol oxidase (1.16–1.25) for all doses of EO, and in the activity of nitrate reductase for EO doses of 10 g/kg DM (3.83) and 50 g/kg DM (1.47) (Figure 2). $IF_{Se} > 1$ indicated the stimulation of the oxidoreductases activity by the selenate application. Selenium addition to soil contaminated with gasoline, eliminated also the impact of gasoline on soil dehydrogenase, peroxidase and antioxidant capacity (Stręk and Telesiński 2015a,b).

The data presented in Table 6 indicated unequivocally that the activity of oxidoreductases varied over time. Also, it depended on the spent engine oil dosage. The share of this factor in the formation of dehydrogenase activity ranged from 16.65% (NR) to 42.43% (DHA). The biostimulation of the soil with selenates affected all oxidoreductases significantly. The share of this factor in the formation of the activity ranged from 2.20% (DHA) to 11.32% (*o*-DPO). Also, the incubation time of the soil affected these enzymes significantly. This independent variable determined the activity of dehydrogenases in the range from 18.11% (CAT) to 40.22% (NR).

In conclusion, the spent engine oil and selenates affect the soil oxidoreductases in various ways. Spent engine oil stimulated activity of dehydrogenase and catalase. Application of selenite (IV) and selenate (VI) to soil non-contaminated with spent engine oil decreased the activity of dehydrogenase and nitrate reductase and increased in *o*-diphenol oxidase. Among selenates tested regarding biostimulation of oxidoreductases in soils containing spent engine oil, selenate (VI) is more useful than selenite (IV).

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