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Assessment of selenium compounds use in limitation of petroleum impact on antioxidant capacity in sandy soil

Ocena wykorzystania związków selenu w ograniczeniu oddziaływania substancji ropopochodnych na pojemność antyoksydacyjną gleby lekkiej

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Abstract
The aim of this study is to assess the selenium (on two oxidation states +IV and +VI) effect on antioxidant capacity in light soil contaminated with gasoline and diesel fuel. The soil used in experiment is characterised by granulometric composition of loamy sand with organic carbon content of 8.7 g·kg^{-1}. Different combinations of gasoline or diesel fuel in dosage 1 or 5%, and H_2SeO_3 and H_2SeO_4 in dosage 0.05 mmol Se·kg^{-1} were added into soil. In the period of 35 days at weekly intervals, antioxidant capacity of the soil in methanol and alkali extracts was determined. Soil contamination with petroleum caused the decrease in antioxidant capacity. Selenium application increased in antioxidant capacity in extracts. In the case of uncontaminated soil, this effect was higher for selenium on the oxidation state +VI, and in soil that contained petroleum for selenium on the oxidation state +IV.

Streszczenie
Celem podjętych badań było określenie wpływu selenu (na dwóch stopniach utlenienia: +IV i +VI) na pojemność antyoksydacyjną gleby lekkiej zanieczyszczonej benzyną oraz olejem napędowym. Zastosowana w doświadczeniu gleba charakteryzowała się składem granulometrycznym piasku gliniastego oraz zawartością węgla organicznego 8,7 g·kg^{-1}. Do części ziemistych wprowadzono w różnych kombinacjach benzynę lub olej napędowy w ilości 1 i 5% oraz H_2SeO_3 i H_2SeO_4 w ilości 0,05 mmol Se·kg^{-1}. W okresie 35 dni w odstępach tygodniowych oznaczono pojemność antyoksydacyjną gleby w ekstraktach metanolowych oraz alkalicznych. Zanieczyszczenie gleby substancjami ropopochodnymi wpłynęło na zmniejszenie pojemności antyoksydacyjnej gleby. Natomiast wprowadzenie selenu spowodowało wzrost pojemności antyoksydacyjnej ekstraktów – w przypadku gleby niezawierającej substancji ropopochodnych efekt ten w większym stopniu uwidocznił się dla selenu na stopniu utlenienia +VI, a w przypadku gleby zanieczyszczonej benzyną dla selenu na stopniu utlenienia +IV.

1. INTRODUCTION

Selenium (Se) is an essential element for human and animal metabolism. Its antioxidant properties, comparable to those of vitamin E, are widely known [Nowak *et al.* 2004]. The concentration of Sein plants and animals is correlated with the concentration of this microelement in soil and its bioavailability [Hartikainen 2005]. In soil, Se occurs principally in insoluble elemental and selenide forms; however, in oxidising environments such as aerobic soils, Se is converted to soluble selenate (IV) and (VI) forms [Borowska *et al.* 2013].

Se content in soils is most often in the range of 0.1 to 2 mg·kg^{-1}, and its average content is 0.33 mg·kg^{-1} [White *et al.* 2004]. In Polish soils, the content of this element is lower than the global average [Skoczyliński and Patorczyk-Pytlik 2006, Borowska *et al.* 2007].

In Poland, as in most European countries, Se is not used in basic fertilisation. However, White *et al.* [2004] reported, that some fertilisers may be the source of Se. Stręk and Telesiński [2014] have shown that application of Se to the basic fertilisation stimulates the activity of o-diphenol oxidase in soil. This enzyme, and other polyphenol oxidases, catalyses the oxidation of phenolic compounds using oxygen as an electron acceptor [Bach *et al.* 2013].

Phenolic compounds have high antioxidant activity and they may play a protective role in relation to soil organic matter [Rimmer and Abbott 2011]. As reported by Zibilske and Bradford [2007], the accumulation of soil organic matter could be stimulated by higher phenol content, which could slow C mineralisation. Numerous studies have shown that the assessment of the soil antioxidant capacity is a very good indicator of resistance of organic matter to oxidation [Rimmer 2006, Rimmer and Abbott 2011, Rimmer *et al.* 2013, Cardelli *et al.* 2014].

Soil organic matter and its stability is an important factor for the distribution of petroleum products. The bioavailability of hydrocarbons from petroleum depends on the interaction of these compounds with soil organic matter [Ghosh 2007]. Hydrocarbons and their degradation products (metabolites) interact with the soil organic matter with non-covalent bonds: sorption, donor-acceptor complex, hydrogen bonding, and covalent bonds: ester bonds, ether bonds and bonds between C–C [Richnow *et al*. 1994]. The petroleum compounds do not have active functional groups, and they could be incorporated into structure of organic matter after being converted by microorganisms and oxidoreductive enzymes [Oleszczuk 2004].

2. MATERIAL AND METHODS

The research was carried out, in the laboratory conditions, on soil collected from humus horizon (0–30 cm) of soil classified as Brunic Arenosol (WRB classification). The soil was characterised by the granulometric composition of loamy sand and organic carbon content of 8.7 $g \cdot kg^{-1}$. Soil was to air-dried and sieved through a sieve with mesh of 2 mm diameter. The soil material was divided into 0.5-kg samples. Se (IV or VI) in dosage 0.05 $mmol \cdot kg^{-1}$, as aqueous solutions of H_2SeO_3 and H_2SeO_4, and gasoline or diesel fuel in an amount of 1 or 5% of the weight were added to the soil samples in appropriate combinations. Samples were adjusted to 60% maximum water holding capacity, and they were incubated for 5 weeks in tightly closed glass containers at a temperature of 20°C.

The soil antioxidant capacity was determined on the methanol and alkali extracts [Rimmer and Smith 2009] according to the method of Re *et al*. [1999], on day 1, 7, 14, 21, 28 and 35. The measure of antioxidant capacity was based on the use of $ABTS^{\bullet+}$2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate), a stable coloured radical in aqueous solution. AOC was expressed as a decrease in absorbance of the solution of $ABTS^{\bullet+}$ after the addition of an antioxidant. The decrease in absorbance due to the activity of soil extract on antioxidant $ABTS^{\bullet+}$ radical was expressed as a percentage of initial absorbance [Cybul and Nowak 2008, Cardelli *et al*. 2014]. A calibration curve was made using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as reference substance. Soil antioxidant capacity was expressed in mmol TEAC (Trolox Equivalent Antioxidant Capacity) per gram dry mass of soil.

All analyses were done in three repetitions. A two-way analysis of variance (ANOVA) was used to examine the effect of petroleum and Se on soil antioxidant capacity. Where a significant difference was observed, a post-hoc test by Tukey HSD was used to determine significant differences at a level of $\alpha = 0.05$. All statistics were done using Statistica 10.0 (StatSoft).

To assess the effect of Se on the soil antioxidant capacity, obtained results were calculated as a percentage of control soil (without Se and hydrocarbons). Then differences between the antioxidant capacity of soil containing petroleum with Se, and antioxidant capacity of soil containing only petroleum were calculated. The positive differences indicated an increase, and negative – a decrease of antioxidant capacity after Se application.

3. RESULTS AND DISCUSSSION

During the experiment, the methanol-soluble antioxidant capacity (MeOH–TAEC) varied within range 0.025 - 0.150 $mmol \cdot g^{-1}$ dm. Alkali-soluble antioxidant capacity (NaOH–TEAC) had higher antioxidant capacity, with values ranging between 1.113 and 2.379 $mmol \cdot g^{-1}$ dm (Table 1). Cardelli *et al*. [2014] have shown a similar trend studying the urban soil. This is most likely due to the higher efficiency of polyphenols extracted with alkaline solutions [Rimmer and Smith 2009, Rimmer and Abbott 2011, Cardelli *et al*. 2012]. However, Schlichting *et al*. [2013] have reported, that the presence of polyphenol could only account for a very small percentage of the overall antioxidant capacity of soil extracts.

Soil treatment with 1% of gasoline caused the statistically significant changes of MeOH–TAEC on day 1 (decrease of 10.0%) and on day 21 (increase of 38.8%), and also the statistically significant changes of NaOH-TEAC on day 14 (increase of 8.0%) and on day 28 (decrease of 7.4%) in relation to the control soil. After application of 5% of gasoline, the statistically significant decrease was observed in the case of MeOH-TEAC on days 7 and 14 (12.8 and 22.4%), and NaOH-TEAC on days 7, 21 and 28 (10.4, 10.3 and 7.9%, respectively) (Fig. 2).

In soil contaminated with diesel fuel, the statistically significant decrease in MeOH-TEAC was noted for dosage of 1% on days 1, 7 and 14 (15.2–25.6%), and for dosage of 5% during almost the whole experiment (15.2–30.1%). In the case of NaOH–TEAC, statistically significant inhibition was found after application of 1% of diesel fuel on days 28 and 35 (19.1 and 10.2%), and after application of 5% of diesel fuel on days 7, 21 and 28 (18.4–32.8%).

Osuji and Nwoje [2007] reported that the soil contamination with petroleum reduces the stability of organic matter. This may result from a decrease in the activity of oxidoreductive enzymes involved in mineralisation of organic substances [Marchut-Mikołajczyk *et al.* 2013]. Moreover, the presence of petroleum substances, may limit the amount of oxygen in the soil by clogging pores and spaces between soil aggregates [Gmitrzuk 2011]. Depletion of oxygen reserve in the soil solution is caused by reduction of oxygen connections. This is connected with the electron acquisitions into the soil environment [Ostrowski 2004]. Excessive production of reactive oxygen species could be the effect of this process.

Se is one of the elements with high antioxidant properties [Nowak *et al*. 2004]. After application of Se to soil, the increase in MeOH–TEAC and NaOH–TEAC was observed almost in all days of measurements. The effect of Se (VI) was higher than that of Se (IV). The statistically significant increase in MeOH–TEAC after treatment with Se (IV) and Se (VI) occurred 14.1–20.4% and 15.2–26.3%. The stimulation of NaOH-TEAC in soil treated with Se (IV) varied in a range between 11.9 and 31.1%, and with Se (VI) between 18.1 and 58.3%, respectively (Figs. 1, 2). Increased antioxidant capacity of the soil after the addition of Se could be caused by the synthesis selenomethionine and selenocysteine, by soil microorganisms. These amino acids are incorporated in the enzymatic proteins, which contain Se atoms in place of determining the catalytic properties of the enzyme [Stolz *et al*. 2006]. Some Se-related enzymes involved in the detoxification of peroxides, are formed by the action of reactive oxygen species [Cabiscol *et al*. 2000, Allocati *et al*. 2009].

After application of Se to soil containing petroleum substances, the increase in antioxidant capacity in relation to the changes caused by gasoline or diesel fuel, was generally observed. In contrast to soil without petroleum, the calculated differences were mainly higher for Se (IV) than Se (VI). This effect was especially

Table 1. Methanol-soluble and alkali-soluble antioxidant capacity in soil treated with petroleum and Se [mmol TEAC·g^{-1} dm]

Petroleum	Se addition	Day of experiment					
		1	7	14	21	28	35
Methanol-soluble antioxidant capacity (MeOH-TEAC)							
Control	-	0.040c	0.125cd	0.125b	0.103fg	0.099cde	0.125cde
	Se (+IV)	0.046a	0.119d	0.135ab	0.124cd	0.113bc	0.142ab
	Se (+VI)	0.033e	0.136abc	0.145a	0.130c	0.125ab	0.144a
1% of gasoline	-	0.036d	0.132bcd	0.136ab	0.143ab	0.100cde	0.127cde
	Se (+IV)	0.035de	0.149a	0.145a	0.150a	0.132a	0.135abc
	Se (+VI)	0.036d	0.135abc	0.133ab	0.149a	0.113bc	0.130bcd
1% of diesel fuel	-	0.033e	0.106ef	0.093c	0.102fg	0.099cde	0.115def
	Se (+IV)	0.036d	0.128cd	0.102c	0.122cde	0.110bc	0.131abcd
	Se (+VI)	0.044ab	0.128cd	0.101c	0.112ef	0.105cd	0.127cde
5% of gasoline	-	0.038cd	0.109ef	0.097c	0.103fg	0.088e	0.120de
	Se (+IV)	0.040c	0.143ab	0.125b	0.132bc	0.133a	0.136abc
	Se (+VI)	0.027h	0.133bcd	0.103c	0.113def	0.125ab	0.123cde
5% of diesel fuel	-	0.030f	0.104f	0.088c	0.072h	0.089e	0.106f
	Se (+IV)	0.025h	0.096f	0.096c	0.099g	0.092de	0.105f
	Se (+VI)	0.043b	0.106ef	0.092c	0.085g	0.089e	0.100f
Alkali-soluble antioxidant capacity (NaOH-TEAC)							
Control	-	1.503def	1.716e	1.626d	1.596cd	1.657d	1.732de
	Se (+IV)	1.971b	2.139b	2.002b	1.822b	1.854c	2.065ab
	Se (+VI)	2.379a	2.363a	2.225a	2.002a	1.957b	2.117a
1% of gasoline	-	1.647cde	1.823de	1.756c	1.622c	1.537e	1.856cd
	Se (+IV)	1.704bc	1.940cd	2.002b	1.795b	2.126a	1.965abc
	Se (+VI)	1.668cde	1.804de	1.822bc	1.701bc	1.852c	1.933bc
1% of diesel fuel	-	1.398ef	1.781e	1.633cd	1.465de	1.340g	1.556f
	Se (+IV)	1.511def	1.756e	1.722c	1.826b	1.693d	1.798d
	Se (+VI)	1.541cdef	1.772e	1.763c	1.803b	1.672d	1.796d
5% of gasoline	-	1.671cd	1.538f	1.496de	1.432e	1.526ef	1.655def
	Se (+IV)	1.829bc	2.007bc	1.633cd	1.825b	2.000b	1.695def
	Se (+VI)	1.526def	1.976c	1.605d	1.792b	1.552e	1.655def
5% of diesel fuel	-	1.299f	1.700e	1.326e	1.254f	1.113h	1.556f
	Se (+IV)	1.470def	1.706e	1.336e	1.246f	1.426f	1.565ef
	Se (+VI)	1.541cdef	1.696e	1.355e	1.285f	1.333g	1.560f

Values denoted by the same letters within one column for kinds of extracts defines homogenous groups calculated using Tukey post-hoc HSD test

seen in soil contaminated with gasoline. After addition of Se (IV) to soil with gasoline, in dosage of 1%, stimulation of MeOH–TEAC and NaOH–TEAC reached to 32.3 and 35.5%, respectively (Fig. 1, Fig. 2); and in dosage of 5%, reached to 45.5 and 28.6%, respectively. After application of Se (VI) to the soil containing 1% of gasoline, statistically significant increase was observed only in NaOH–TEAC on day 28 (19.0%). In soil contaminated with 5% of gasoline, statistically significant changes after addition of Se (VI) were observed in the case of MeOH–TAEC on days 1 (decrease of 27.5%), 7 (increase of 19.2%) and 28 (increase of 37.4%), and NaOH–TAEC on days 7 and 21 (increase of 25.5 and 22.6%) (Fig. 2).

After application of Se to soil containing 1% of diesel fuel, the statistically significant increase in MeOH–TAEC were found for Se (IV) on days 7, 21 (17.6 and 19.4%, respectively), and Se (VI) on day 7 (17.6%). However, statistically significant increase in NaOH–TAEC, after application of Se on two oxidation states to soil contaminated with 1% of diesel fuel was observed on days 21, 28 and 35. This stimulation of NaOH–TAEC is very similar for Se (IV) and (VI) varied in a range 14.0 - 22.6%, and 13.9 and 21.2% , respectively. MeOH–TAEC and NaOH–TAEC in soil treated with Se and 5% of diesel fuel almost in whole experiment did not differ significantly in relation to soil with the addition of only 5% of diesel fuel.

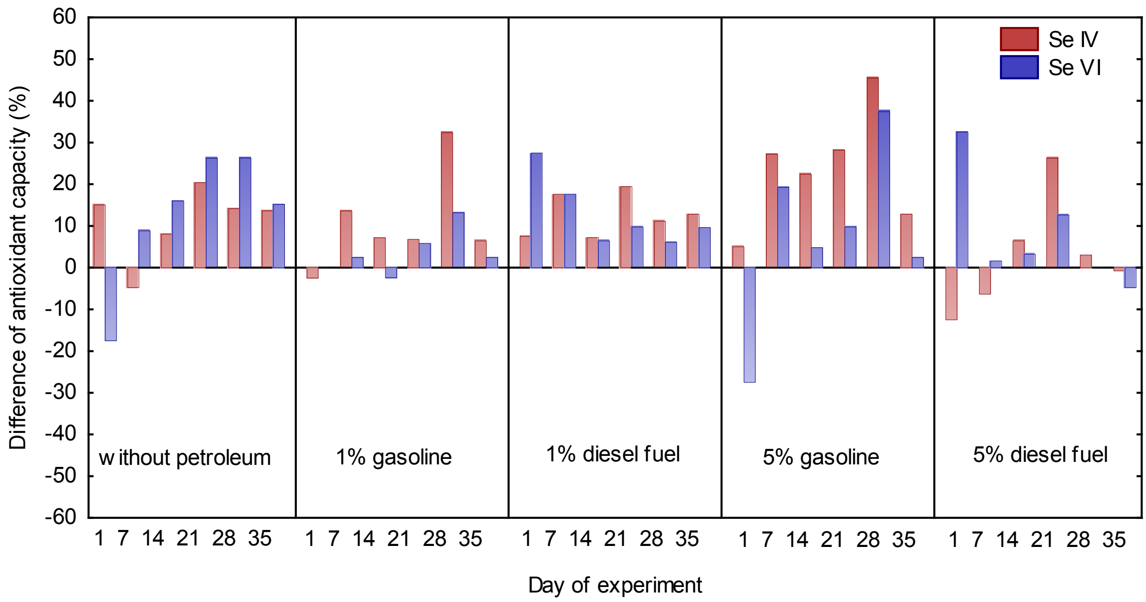

Fig. 1. Percentage changes between antioxidant capacity in methanol extracts from soil treated with Se and petroleum, and with petroleum

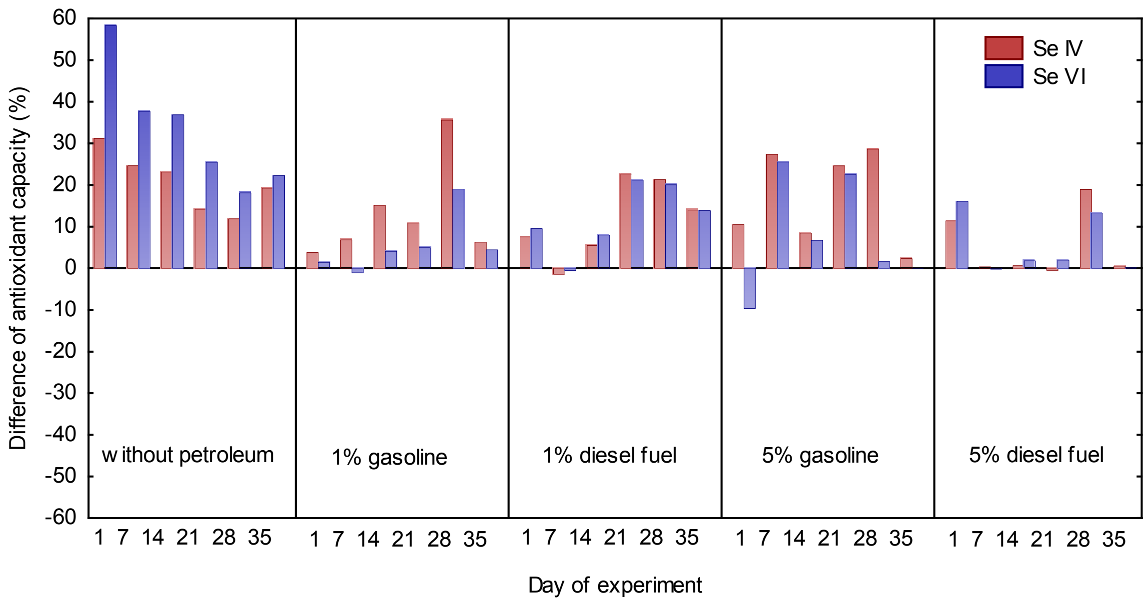

Fig. 2. Percentage changes between antioxidant capacity in alkali extracts from soil treated with Se and petroleum, and with petroleum

Many authors have shown the stimulatory activity of the Se compounds on the antioxidant properties of plant and animal cells [Tapiero *et al*. 2003, Telesiński *et al*. 2009, Cartes *et al*. 2010, Giacosa *et al*. 2014]. There have also been reports of the positive impact of Se ions on the soil enzyme activities [Nowak et al. 2004, Borowska *et al*. 2013, Stręk and Telesiński 2014]. Stręk and Telesiński [2015] have demonstrated that use of Se limited negative effects of gasoline on dehydrogenase and peroxidase activities in soil. Cardelli *et al.* [2014] have shown that there was a significant positive correlation between antioxidant capacity and the enzymatic activity in soil.

4. CONCLUSION

Contamination with petroleum substances caused inhibition of soil antioxidant capacity, which could result in increased organic matter decomposition. The application of Se increased the antioxidant capacity of methanol and alkali extracts from soil. In the case of soil containing no petroleum substances, this effect was higher for Se on the oxidation state +VI. In contrast, in soil contaminated with gasoline, the effect was higher for Se on the oxidation state +IV. Se fertilisation, especially in the form of selenate (IV) ions may be a factor limiting the impact of gasoline contamination on the organic matter stability.

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