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EFFECT OF CALCIUM PEROXIDE ON OXIDOREDUCTASE ACTIVITIES IN SOIL CONTAMINATED WITH GASOLINE AND DIESEL OIL

WPŁYW NADTLENKU WAPNIA NA AKTYWNOŚĆ OKSYDOREDUKTAZ W GLEBIE ZANIECZYSZCZONEJ BENZYNĄ I OLEJEM NAPĘDOWYM

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Streszczenie. W badaniach określono oddziaływanie nadtlenku wapnia na aktywność wybranych enzymów oksydoredukcyjnych: dehydrogenaz, oksydazy *o*-difenolowej oraz katalazy w glebie zanieczyszczonej benzyną i olejem napędowym. Doświadczenie laboratoryjne przeprowadzono na próbkach gleb pobranych z poziomu ornopróchnicznego (0–30 cm) ziem rdzawych typowych (piasek gliniasty, C_{org} 8,7 g · kg^{-1}). Do części ziemistych pobranego materiału glebowego wprowadzono w różnych kombinacjach benzynę lub olej napędowy (w ilości 0 i 50 g · kg^{-1}) oraz nadtlenek wapnia (w ilości 0, 100 i 200 mg · kg^{-1}). Próbki doprowadzono do 60% maksymalnej pojemności wodnej i przechowywano w szczelnie zamkniętych szklanych pojemnikach, w temperaturze 20°C. We wszystkich kombinacjach oznaczono w 1., 7., 14. i 28. dniu doświadczenia aktywność dehydrogenaz, oksydazy *o*-difenolowej oraz katalazy. Wprowadzenie do gleby benzyny spowodowało inhibicję aktywności oznaczanych enzymów, natomiast aplikacja oleju napędowego stymulowała aktywność dehydrogenaz. Oddziaływanie nadtlenku wapnia zmieniało się w trakcie doświadczenia i zależało od właściwości enzymu, czasu, rodzaju substancji ropopochodnych oraz dawki CaO_2. Aplikacja CaO_2 do gleby zanieczyszczonej substancjami ropopochodnymi spowodowała głównie stymulację aktywności dehydrogenaz i katalazy.

Key words: calcium peroxide, catalase, dehydrogenases, phosphatases, soil.
Słowa kluczowe: dehydrogenazy, fosfatazy, gleba, katalaza, nadtlenek wapnia.

INTRODUCTION

Petroleum products utilized to power combustion engines are commonly used worldwide. Increasing scale of consumption of the high-energy organic compounds causes a proportional increase in threat of environmental pollution. The energy contained in chemical bonds allows the transport and storage without creating too many problems (Jain et al. 2011). However, when it comes to accidental leakage the major part of the mixture is depositing in the soil. In small quantities this is not a problem because the soil has natural ability to self-purification of organic compounds. The process of biodegradation involves the oxidation of the chemical bonds in aromatic and aliphatic chains, using specific enzymes produced by microorganisms. The problem may be amount of pollution. The compounds of petroleum origin is mainly a mixture of aliphatic hydrocarbons having different lengths. This design has an influence on the physical and chemical properties (Ahamed et al. 2010).

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Microbial decomposition of petroleum and petroleum products is of considerable importance. Petroleum is a rich, source of carbon and the hydrocarbon within it are readily oxidized aerobically with the release of carbon-dioxide by a variety of microorganisms in soil (Obire and Nwaubeta 2001). Maila and Cloete (2005) shown, that the increase of dehydrogenase (EC 1.1.1.x) and catalase (EC 1.11.1.6) activities in soil indicates the intensification of hydrocarbon decomposition. The o-diphenol oxidase (EC 1.10.3.1) catalyzes oxidation of phenol compounds, the importance of phenol acids and microbiological released during hydrolysis of naturally occurring substances (plant residues and organic) in soils and aromatic xenobiotics has been demonstrated in the formation of humic substances (Perucci et al. 2000).

The recent studies demonstrated that chemical oxidation can coexist with microbial aerobic metabolism in soils and that combination is more effective solution compared to natural bioremediation processes (Khodaweisi et al. 2011). Currently, research is being done to find the possibility of using other reagents in the processes of advanced oxidation. Compounds like peroxides are of high interest. Peroxides are a group of compounds that contain a peroxide group in its structure. During the degradation of these compounds, H_2O_2 is produced, which is the source of oxygen for microorganisms taking active part in the biodegradation of contaminants while at the same time being the source of free radicals capable of oxidizing organic contaminants (Małachowska-Jutsz and Niesler 2015).

The aim of study was to assess a chemical oxidant calcium peroxide effect on changes of soil oxidoreductase: dehydrogenases, catalase and *o*-diphenol oxidase activities affected by gasoline and diesel oil.

MATERIAL AND METHODS

Soil samples were collected from Agricultural Experimental Station in Lipnik (53°24’ N and 14°28’ E), located in West Pomeranian District, Poland. This field remained under conventional farming practices. The soil was classified as loamy sand. Some of its physical and chemical characteristics as follows: pH_{KCl} 6.36, organic C 8.71 g · kg^{-1} and total N 0.89 g · kg^{-1}. To consider the spatial heterogeneity, soil samples were taken in triplicates, and each replicate consisted of five 10-cm auger cores. Then, the soil samples were manually and gently crumbled, mixed thoroughly, air dried at room temperature, and sieved through a 2-mm mesh to remove stones and plant roots before being used. The soil material was divided into 0.5 kg samples. Calcium peroxide at the amounts of 100 and 200 mg · kg^{-1}, as aqueous solutions, and gasoline or diesel oil at the dosage of 50 g·kg^{-1} were added to the soil samples in appropriate combinations. Samples were adjusted to 60% maximum water holding capacity, and they were incubated for four weeks in tightly closed glass containers at temperature of 20°C. Each treatment was replicated three times at each sampling stage.

In soil treated with CaO_2 and petroleum products, soil oxidoreductase activities were measured on days 1, 7, 14 and 28. The activity of dehydrogenases [EC 1.1.1.x] was determined using the method described by Thalmann (1968) The *o*-diphenol oxidase [EC 1.10.3.1] activity was measured according to procedure of Perucci et al. (2000). The catalase [EC 1.11.1.6] was determined as described by Johnson and Temple (1964).

Based on the activity of assayed soil oxidoreductases, indices of CaO_2 effects were calculated using the following formulas (Kaczyńska et al. 2015):

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

where:

IF_{CP} – index of CaO_2 effect,

A_{CP} – activity of oxidoreductases in the soil treated with CaO_2,

A – activity of oxidoreductases in the soil untreated with CaO_2.

If IF = 1, there is no influence of the CaO_2 on oxidoreductases; IF < 1, there is inhibition of the oxidoreductases activity by CaO_2 and IF > 1, there is stimulation of the oxidoreductases activity by CaO_2 (Stręk and Telesiński 2016).

The results of the studies were determined statistically using a statistical software package Statistica v. 12.5 (Statsoft, Inc.). Based on the analysis of the effect measure η^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 $p < 0.05$.

RESULTS AND DISCUSSION

The activities of oxidoreductases in soil non-contaminated with petroleum products were 8.45–20.68 mg TPF · (kg DM · 16 h)$^{-1}$, 2.65–3.74 mmol H_2O_2 · (kg DM · min)$^{-1}$ and 2.04–3.25 mmol oxidated catechol · (kg DM · 10 min)$^{-1}$, for dehydrogenases, catalase and *o*-diphenol oxidase, respectively (Table 1).

Table 1. Changes of oxidoreductase activities in soil after treatments with petroleum products
Tabela 1. Aktywność oksydoreduktaz w glebie po wprowadzeniu substancji ropopochodnych

Type of petroleum product Rodzaj substancji ropopochodnych	DHA	CAT	*o*-DPO
Day 1 – Dzień 1.			
Control – Kontrola	8.45 ± 0.71 ^{b}	3.74 ± 0.23 ^{a}	3.25 ± 0.77 ^{b}
Gasoline – Benzyna	0.55 ± 0.12 ^{c}	2.26 ± 0.26 ^{c}	3.56 ± 0.17 ^{a}
Diesel oil – Olej napędowy	22.54 ± 2.09 ^{a}	2.87 ± 0.09 ^{b}	3.28 ± 0.30 ^{b}
Day 7 – Dzień 7.			
Control – Kontrola	8.93 ± 0.63 ^{b}	2.90 ± 0.09 ^{a}	2.04 ± 2.00 ^{a}
Gasoline – Benzyna	0.42 ± 0.02 ^{c}	1.42 ± 0.09 ^{b}	1.91 ± 0.06 ^{b}
Diesel oil – Olej napędowy	66.56 ± 1.03 ^{a}	2.90 ± 0.47 ^{a}	2.02 ± 0.15 ^{a}
Day 14 – Dzień 14.			
Control – Kontrola	11.13 ± 0.82 ^{b}	2.65 ± 0.10 ^{b}	2.14 ± 0.19 ^{a}
Gasoline – Benzyna	0.41 ± 0.01 ^{c}	1.24 ± 0.10 ^{c}	1.82 ± 0.04 ^{b}
Diesel oil – Olej napędowy	42.72 ± 1.21 ^{a}	3.04 ± 0.17 ^{a}	2.11 ± 0.16 ^{a}
Day 28 – Dzień 28.			
Control – kontrola	20.68 ± 0.36 ^{a}	2.78 ± 0.48 ^{a}	2.09 ± 0.09 ^{a}
Gasoline – Benzyna	0.61 ± 0.02 ^{b}	1.11 ± 0.10 ^{c}	1.54 ± 0.17 ^{b}
Diesel oil – Olej napędowy	21.48 ± 1.51 ^{a}	2.28 ± 0.25 ^{b}	2.01 ± 0.21 ^{a}

DHA – dehydrogenases [mg TPF · (kg d.m. · 16 h)$^{-1}$] – dehydrogenazy [mg TPF · (kg s.m. · 16 h)$^{-1}$].
CAT – catalase [mmol H_2O_2 · (kg d.m. · min)$^{-1}$] – katalaza [mmol H_2O_2 · (kg s.m. · min)$^{-1}$].
o-DPO – *o*-diphenol oxidase [mmol oxidized catechol · (kg d.m. · 10 min)$^{-1}$] – oksydaza o-difenolowa [mmol utlenionego katecholu · (kg s.m. · 10 min)$^{-1}$].
Values denoted by the same letters in the columns for an enzyme and day of experiment do not differ statistically at p < 0.05 – Wartości oznaczone tymi samymi literami w kolumnach dla enzymu i dnia doświadczenia nie różnią się istotnie na poziomie p < 0,05.

Treatments with gasoline caused of decrease in activities of dehydrogenases and catalase on every day of experiment. This inhibition ranged from 93.47 to 97.05% for dehydrogenases and from 39.73 to 60.07% for catalase. Activity of *o*-diphenol oxidase was decreased by gasoline on days: 7, 14, 28 (6.37, 14.95 and 36.32%, respectively), while on day 1 stimulation of this enzyme activity was observed. Earlier reports indicated that soil polluted with gasoline inhibited in activity of different oxidoreductases (Frankenberger and Johanson 1981; Kaczyńska et al. 2015; Stręk and Telesiński 2015). In soil treated with diesel oil activity of dehydrogenases was decreased on almost all test days (except day 28), and this stimulation ranged from 166.74 to 645.35%. This is similar with results obtained by Hawrot et al. (2005) and Achuba and Okoh (2014). Diesel oil also, increased in catalase activity on day 14 (14.72%), while on days 1 and 28 statistically significant inhibition on this enzyme was reported (23.26 and 17.99%, respectively). Treatments with diesel oil did not cause significant changes of *o*-diphenol oxidase in whole experiment.

Effect of CaO_2 on oxidoreductase activities in soil uncontaminated and contaminated with gasoline and diesel oil proved diversified, and depended on enzyme, incubation time, type of petroleum product and calcium peroxide. On the most days of experiment CaO_2 application affected dehydrogenase and catalase positive, but did not effect on *o*-diphenol oxidase activity (Table 2).

Table 2. Indices of the calcium peroxide effects (IF_{CP}) on the activity of oxidoreductases in soil uncontaminated and contaminated with gasoline and diesel oil
Tabela 2. Wskaźniki oddziaływania nadtlenku wapnia (IF_{CP}) na aktywność oksydoreduktaz w glebie niezanieczyszczonej oraz zanieczyszczonej benzyną i olejem napędowym

Type of petroleum product Rodzaj substancji ropopochodnych	Amount of CaO_2 Ilość CaO_2 [mg · kg^{-1}]	Day of experiment – Dzień doświadczenia			
		1	7	14	28
		DHA			
Control Kontrola	100 200	0.74 0.89	0.95 1.02	1.02 1.44	0.64 1.61
Gasoline Benzyna	100 200	1.75 1.12	0.82 1.00	1.17 0.67	0.68 1.02
Diesel oil Olej napędowy	100 200	1.06 1.06	1.03 1.13	1.09 1.17	1.32 1.61
		CAT			
Control Kontrola	100 200	1.03 1.03	1.04 0.89	1.13 1.17	1.12 1.06
Gasoline Benzyna	100 200	1.16 1.16	1.19 1.19	1.23 1.32	1.20 1.10
Diesel oil Olej napędowy	100 200	1.21 1.11	1.09 1.32	0.96 0.91	1.00 1.15
		o-DPO			
Control Kontrola	100 200	0.84 0.89	1.01 1.01	1.00 0.97	0.98 0.93
Gasoline Benzyna	100 200	0.83 0.87	1.04 1.06	0.98 0.97	0.97 1.00
Diesel oil Olej napędowy	100 200	0.95 0.93	0.95 0.93	0.94 0.92	1.04 0.97

DHA – dehydrogenases – dehydrogenazy, CAT – catalase – katalaza, *o*-DPO – *o*-diphenol oxidase – oksydaza *o*-difenolowa.

Mean values of index of calcium peroxide effect (IF_{CP}) in soil uncontaminated petroleum products showed negative effect of CaO_2 at the dosage of 100 $mg \cdot kg^{-1}$ on dehydrogenases (IF_{CP} = 0.84), while the dosage of 200 $mg \cdot kg^{-1}$ stimulated these enzymes (IF_{CP} = 1.24). Activity of other enzymes in soil without petroleum was slightly changed after application of CaO_2 at both dosages (IF_{CP} about 1).

In soil treated with gasoline addition of CaO_2 at the dosage of 100 $mg \cdot kg^{-1}$ activated dehydrogenases (IF_{CP} = 1.11) and catalase (IF_{CP} = 1.19). For catalase mean value of index of calcium peroxide effect was higher than 1, also after application of CaO_2 at the dosage of 200 $mg \cdot kg^{-1}$ (IF_{CP} = 1.19). Addition of CaO_2 into soil containing diesel oil caused positive effect on dehydrogenases, for both CaO_2 dosages (IF_{CP} = 1.14 and IF_{CP} = 1.24), and on catalase, for dosage of 200 $mg \cdot kg^{-1}$ (IF_{CP} = 1.12). In other cases the mean values of calcium peroxide effect were close to 1 – the changes do not exceed 0.10 (Fig. 1). Małachowska-Jutsz and Niesler (2015) reported that application of calcium peroxide at the dosage of 120 $mg \cdot kg^{-1}$ limited of fluoranthene impact on phenol oxidase and acid phosphatase in soil. Many authors found that the application of calcium peroxide to be one of the most efficient for removal petroleum hydrocarbons, polycyclic aromatic hydrocarbons, tetrachloroethylene, and 2,4,6-trinitrotoluene from soil (Bianchi-Mosquera et al. 1994; Arienzo 2000; Hanh et al. 2005; Goi et al. 2011; Khodaweisi et al. 2011). Low solubility of calcium peroxide allows continuous release of hydrogen and oxygen peroxide for a long period of time (Goi et al. 2011). Moreover, the addition of calcium peroxide couses decomposition of contaminations in a wide pH range (Arienzo 2000). Małachowska-Jutsz and Niesler (2015) showed also that the application of peroxides such as calcium peroxide allows shortening the duration of remediation of soils by increasing the content of oxygen available to the microorganisms and alkalization of the environment.

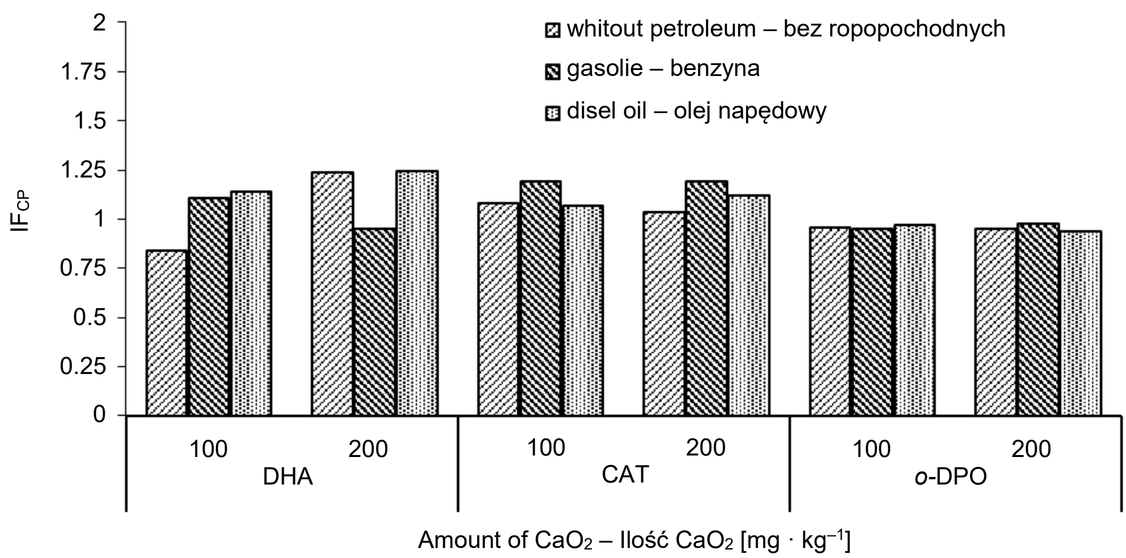

Fig. 1. Mean values of index of calcium peroxide effect (IF_{CP}) on the activity of dehydrogenases (DHA), catalase (CAT) and *o*-diphenol oxidase (o-DPO) in soil uncontaminated and contaminated with gasoline and diesel oil

Ryc. 1. Średnie wartości wskaźnika oddziaływania nadtlenku wapnia (IF_{CP}) na aktywność dehydrogenaz (DHA), katalazy (CAT) i oksydazy *o*-difenolowej (*o*-DPO) w glebie niezanieczyszczonej oraz zanieczyszczonej benzyną i olejem napędowym

The data presented in Table 3 indicate unequivocally that the activity of oxidoreductases varied over time. The activity of dehydrogenases and catalase were mostly determined by type of petroleum products, while the activity *o*-diohenol oxidase was mainly formed by day od experiment. On the other hand, the dosage of CaO_2 affected all oxidoreductases significantly.

Table 3. Participation of variable factors in the formation of soil oxidoreductase activities in percentage
Tabela 3. Procentowy udział czynników doświadczalnych w kształtowaniu aktywności oksydoreduktaz w glebie

Factor – Czynnik	DHA	CAT	*o*-DPO
Type of petroleum products Rodzaj substancji ropopochodnych (A)	83.33	77.17	3.25
Amount of CaO_2 Ilość CaO_2 (B)	0.97	2.33	1.87
Day of experiment Dzień doświadczenia (C)	6.37	16.52	90.03
A × B	0.37	0.23	0.14
A × C	8.58	2.69	2.96
B × C	0.20	0.08	1.03
A × B × C	0.15	0.63	0.20
Error – Błąd	0.02	0.35	0.52

CONCLUSIONS

1. Soil contamination with gasoline decreased in all oxidoreductase activities, while contamination with diesel oil increased in dehydrogenases and caused slight changes in catalase and *o*-diphenol oxidase activity.
2. Effect of CaO_2 on oxidoreductase activities in soil uncontaminated and contaminated with gasoline and diesel oil proved diversified, and depended on enzyme, incubation time, type of petroleum product and calcium peroxide dosage.
3. Application of CaO_2 in soil contaminated with petroleum products caused mainly stimulation of dehydrogenases and catalase.

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Abstract. This paper presents the study on assessment of the effect of calcium peroxide on activity of chosen enzymes: dehydrogenases, catalase and *o*-diphenol oxidase in soil contaminated gasoline or diesel oil. Laboratory experiment was carried out on loamy sang with C_{org} content 8.7 g · kg^{-1}. Into soil samples different combinations of gasoline or diesel oil (at the dosage of 50 g · kg^{-1}) and calcium peroxide (at the amounts of 100 and 200 mg · kg^{-1}) were added. Samples were adjusted to 60% maximum water holding capacity, and they were incubated for four weeks in tightly closed glass containers at temperature of 20°C. In soil treated with CaO_2 and petroleum products, soil oxidoreductase activities were measured on days 1, 7, 14 and 28. Soil contamination with gasoline decreased in all oxidoreductase activities, while contamination with diesel oil increased in dehydrogenases and caused slight changes in catalase and *o*-diphenol oxidase activity. Effect of CaO_2 on oxidoreductase activities in soil uncontaminated and contaminated with gasoline and diesel oil proved diversified, and depended on enzyme, incubation time, type of petroleum product and calcium peroxide dosage. Application of CaO_2 in soil contaminated with petroleum products caused mainly stimulation of dehydrogenases and catalase.